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EFFECT OF MONOUNSATURATED FAT IN THE DIET ON THE SERUM

CAROTENOID LEVELS

By

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Bachelors in Science (Nutrition), Diploma (Nutrition and Health Education) Graduate Diploma (Dietetics) Graduate Diploma (Human Nutrition)

SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF

MASTERS OF BIOMEDICAL SCIENCE (Research)

University of Tasmania

November, 2001

UNIVERSITY OF TASMANIA



CANDIDATE DECLARATION

I certify that the thesis entitled

"Effect of monounsaturated fat in the diet on the serum carotenoid levels"

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ABBREVIATIONS USED IN THIS THESIS

ATBC	Alpha Tocopherol Beta-carotene
BMI	Body Mass Index
BHT	Butylated Hydroxytoluene
CARET	Carotene and Retinol Efficacy Trial
CHD	Coronary Heart Disease
CI	Confidence Intervals
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVD	Cardiovascular Disease
EDTA	Ethylenediamine Tetraacetic Acid
GLM	General Linear Model
HDL	High Density Lipoprotein
HPLC	High Pressure Liquid Chromatography
LDL	Low Density Lipoprotein
MI	Myocardial Infarction
MJ	Mega Joule
MUFA	Modified fat monounsaturated fat rich diet
PBS	Phosphate Buffer Solution
PEG	Polyethylene Glycol
PUFA	Polyunsaturated Fatty Acid
RPM	Revolutions Per Minute
SD	Standard Deviation
SEM	Standard Error of Mean
SFA	Saturated Fatty Acid
SPSS	Statistical Packages for Social Scientists
TBARS	Thiobarbituric Acid Reactive Substances
VLDL	Very Low Density Lipoprotein
WHO	World Health Organisation

ABSTRACT

Epidemiological data suggest that populations with higher serum/ tissue levels of carotenoids have a lower risk of coronary heart disease (CHD), possibly due to the antioxidant capacity. Lycopene, a carotenoid mainly found in tomatoes, has been suggested to have the greatest antioxidant capacity of the carotenoids found in fruits and vegetables. Carotenoids are fat-soluble compounds and their absorption from the diet into the body may depend on the amount of dietary fat ingested.

For years there has been debate about what energy source should replace the saturated fat in the diet, to give the optimum serum lipid profile to reduce CHD risk. Studies have compared monounsaturated fat rich diets with high carbohydrate, low fat diets and have found that both diets decrease serum cholesterol and low-density lipoprotein (LDL) cholesterol levels. Results for high-density lipoprotein (HDL) cholesterol and triglycerides have been inconsistent. However, it is of interest to study the effects of different diets on lipid oxidation, as this may also influence CHD risk.

Studies have investigated the effect of different amounts of total fat on the serum levels of carotenoids especially beta-carotene and lutein, but to our knowledge no study has looked at the effect of different amounts of fats on the serum lycopene levels, and whether this could subsequently affect the oxidation of LDL *in vitro*.

Two separate randomised crossover dietary intervention studies were conducted; one in healthy men and the other in healthy women aged 20 to 70 years. The aim was to compare the effects of monounsaturated fat rich (MUFA) diet (38% of energy from fat) and high carbohydrate low fat (HCLF) diet (15% energy from fat) with controlled lycopene content, on serum lycopene levels. Main sources of lycopene in the diets were canned tomatoes and tomato soup for the study in women (lycopene content -15.9 mg/day) and tomato paste and tomato soup for the study in the study in men (lycopene content - 20.2 mg/day). Serum lipids and lipoproteins levels and *in vitro* oxidation of LDL particles were also measured.

Compared to the baseline levels there was a significant increase in the serum *trans* lycopene and total lycopene levels after MUFA diet for the study in women. Comparing the levels at the end of the two diets no difference was observed. In the study in men serum *trans, cis* and total lycopene levels increased after the MUFA and HCLF diet periods. There was no significant difference in *trans; cis* and total lycopene levels at the end of two diets.

Thus, high levels of monounsaturated fat in the diet do not appear to increase lycopene absorption and serum levels compared to very low fat diet. There was however a better serum lipid profile after MUFA diet compared to HCLF diet. Lag phase for the *in vitro* oxidation of LDL particles was also longer on the MUFA diet. The lack of difference in antioxidant levels would indicate that this was due to the different fatty acid component of the diet.

INTRODUCTION

Over the years, there has been considerable interest in the role of different components of diet in the causation, possible prevention and treatment of degenerative diseases including cardiovascular disease and cancer.

Dietary fat has been under investigation for its role in the pathogenesis, prevention and the treatment of atherosclerosis. Studies have confirmed an association between serum cholesterol and dietary saturated fat intake with high incidence of coronary heart disease (CHD) (Brown et al., 1984; Hu et al., 1999; Kromhout et al., 1995). Some researchers advocate a reduction in total fat consumption and replacement with carbohydrates, whereas others recommend replacing saturated animal fat with polyunsaturated fat and/or monounsaturated fat.

Oxidation of low-density lipoprotein (LDL) cholesterol is thought to be involved in the initiation and promotion of atherosclerosis (Witztum and Steinberg, 1991). Studies have favoured the replacement of saturated fat with monounsaturated fat rather than polyunsaturated fat, because diets enriched with polyunsaturated fat have been shown to increase the *in vitro* susceptibility of LDL to oxidation compared to monounsaturated fat. A few studies have investigated and reported increased resistance of LDL to oxidation *in vitro* on consumption of a monounsaturated fat enriched diet compared to a high carbohydrate low fat diet Diets of the Mediterranean populations have been a subject of interest for decades; however, recently, interest has focused on the evident health benefits of traditional Mediterranean diets, as such populations have a lower rate of chronic diseases and higher life expectancies compared to other western populations (Kushi et al., 1995). The usual diet of the traditional Mediterranean population includes large amounts of carotenoid rich fruits and vegetables, grain foods and olive oil - an oil consisting largely of monounsaturated fatty acids.

Carotenoids are a group of over 600 non-nitrogenous, fat-soluble, natural pigments found in plants and microorganisms. Studies have indicated that populations with increased risk and mortality of CHD tend to have lower levels of serum lycopene (Kristenson *et al.*, 1997), a hydrocarbon carotene present mainly in tomatoes and consumed in abundance by Mediterranean populations. It has been suggested that lycopene has a higher antioxidant capacity than other carotenoids (Di Mascio *et al.*, 1989, Tinkler *et al.*, 1994).

Carotenoids are fat-soluble compounds and their absorption in the human body may depend on the presence of dietary fat. Studies have looked at the effect of different amounts of dietary fat on the serum levels of some carotenoids - alphacarotene, beta-carotene and lutein, but not the effect of different amounts of dietary fat on the serum levels of lycopene, and subsequently on the oxidation of LDL cholesterol *in vitro*.

This thesis describes two studies which investigated the hypothesis that the combination of lycopene and monounsaturated fat may have additional benefits

by increasing the serum lycopene, improving serum lipid profile and increasing the resistance of LDL to oxidation compared to a high carbohydrate, low fat, lycopene enriched diet.

To test this hypothesis, two separate studies were conducted in healthy, nonsmoking women and men aged 20-70 years. The study results, which are presented in this thesis, will contribute to our knowledge about the role of different diets on the risk factors for CHD.

1.1 Coronary Heart Disease prevalence

Coronary heart disease is the major cause of mortality and morbidity in industrialised countries and is also the cause of many deaths in developing countries. In Australia, although the age-adjusted death rate from CHD has fallen by over 60% since 1968, it still is the largest single cause of death (Australian Facts, 1999). Australia ranks towards the middle of 17 countries compared for the death rate from CHD, with Asian and Mediterranean countries having the lowest and the Russian Federation the highest death rates (Australian Facts, 1999).

1.2 Serum lipids and CHD

Mediterranean countries have been in the limelight for their lower rates of mortality and morbidity for the last 50 years. As early as the 1950s, Keys (an epidemiologist) and his co-workers observed that people in Naples (Italy) had low rates of mortality and morbidity from CHD. This observation led to a number of epidemiological studies throughout the world and the findings demonstrated that serum cholesterol is etiologically related to the risk of atherosclerotic disease, particularly CHD (Castelli, 1984; Keys et al., 1986). Observational studies suggest a linear relationship and that a one percent increase in serum cholesterol level may increase CHD risk by two percent (LaRosa et al., 1990).

Further research revealed that in addition to the total serum cholesterol, high levels of LDL cholesterol (greater than 3.5 mmol/L) and low levels of high-density lipoprotein (HDL) cholesterol are closely related to the prevalence and

incidence of CHD (Castelli, 1984; Castelli et al., 1986; Roberts, 1989). HDL is a small component of total serum cholesterol but is strongly and inversely related to the risk of CHD. Any dietary change that reduces HDL (and hence total cholesterol) may have an adverse effect on CHD risk rather than the beneficial effect predicted by the overall change in total serum cholesterol. The relationship of plasma lipids to CHD risk is often summarised as the ratio of total (or LDL) cholesterol to HDL cholesterol (Castelli et al., 1983). Data from the Physicians Health Study suggested that a one unit decrease in this ratio decreases the risk of myocardial infarction (MI) by 53% (Stampfer et al., 1991). Further, evidence indicates that triglyceride rich lipoproteins may also play an important role in atherogenesis (Hodis and Mack, 1995). Elevated triglycerides, a crude marker for triglyceride rich lipoproteins, are associated with CHD, particularly in the presence of low HDL cholesterol levels (Austin et al., 1998; Castelli, 1986). Whether or not they represent an independent factor for a higher risk of CHD, after control for other lipids, remains unclear (Gaziano and Manson, 1996; Parks and Hellerstein, 2000).

1.2.1 Dietary fat and serum cholesterol

Observational studies have found a strong independent positive correlation between saturated fat consumption and increased serum cholesterol levels (Hegsted et al., 1965; Keys et al., 1957; Kromhout, et al., 1995; LaRosa, et al., 1990). A 25 year follow-up study of seven countries, reported dietary saturated fatty acids, *trans* fatty acids and dietary cholesterol as the important determinants of differences in the serum cholesterol levels and hence the rates of CHD death in different populations (Kromhout, et al., 1995). The information that saturated fat intake is related to the risk of CHD led to a number of clinical and dietary intervention studies using serum cholesterol as a substitute endpoint. Among volunteers in metabolic wards, where diets were tightly controlled, serum LDL cholesterol levels decreased when saturated fat in the diet was decreased, or when polyunsaturated fat was increased in the diet (Hegsted, et al., 1965). Equations were developed on the comparative decrease in serum cholesterol levels with dietary saturated fat decrease and polyunsaturated fat increase (Keys, et al., 1957). These observations led to a number of cholesterol lowering diet trials, which reported a seven to 50 percent fall in CHD. Though for some trials the change was not statistically significant, there was a minimum mean fall of 6.8%. A decrease of nine percent was reported when saturated fat was replaced with polyunsaturated fat in the diet in the Los Angeles Veteran Administrative Study and the Finnish Mental Hospital Study (Dayton and Pearce, 1969; Turpeinen et al., 1979). Four large trials – the Multiple Risk Factor Intervention Trial, the Oslo Study, the Gotenborg Primary Prevention Trial and the World Health Organisation (WHO) Collaborative Trial, showed similar results (Benfari, 1981; Hjermann, 1983; WHO, 1974; Wilhelmsen et al., 1986). Monounsaturated fat failed to show any effect when concentrates of oleic acid were consumed and so it was then thought that dietary monounsaturated fat had a neutral effect on serum cholesterol levels (Keys et al., 1958).

The Lyon Diet Heart Study, a randomised prospective trial of secondary prevention in a group of 605 patients, followed the patients for 27 and 46 months after the first MI. Patients were prescribed either an alpha linolenic acid rich Mediterranean diet (30.5% total fat, 8% saturated fatty acid, 4.6%

polyunsaturated fatty acid, and 13% monounsaturated fatty acid mainly as olive oil and canola margarine) or a prudent post-infarct diet (32.7% total fat, 12% saturated fatty acid, 6.1% polyunsaturated fatty acid, and 11% monounsaturated fatty acid) which was usually prescribed by the treating physician. After 27 months, the experimental group had a 70% decrease in recurrent MI, compared to those on the control diet (de Lorgeril et al., 1994). The protective effect persisted in the experimental group after the 46 month follow up, with a majority of the subjects still closely following the Mediterranean diet (de Lorgeril et al., 1999). The benefits revealed in this study were probably due to the further replacement of saturated fat in the study group versus control with olive oil and n-3 polyunsaturated fat alpha linolenic acid. Also the increase of vegetables, fruits, bread and grains in the diet may have contributed.

At the same time, based on interest on the fact that Mediterranean populations have lower death rates from CHD and have higher intakes of monounsaturated fat in their diets (**Table 1.1; pp 9**), smaller intervention studies were also conducted to compare the effect of polyunsaturated fat versus monounsaturated fat in the diet on the serum lipid profile (Bonanome et al., 1992; Grundy et al., 1988; Mata et al., 1996; Mata et al., 1997; Mensink and Katan, 1989). These small dietary intervention studies in free living individuals reported that replacing saturated fat with polyunsaturated fat or monounsaturated fat had similar effects on the blood lipids (Bonanome, et al., 1992; Grundy, et al., 1988; Mata, et al., 1996; Mata, et al., 1997; Mensink and Katan, 1989).

In a study on 31 healthy women and 27 healthy men, comparing the effect of a polyunsaturated fat diet and a monounsaturated fat diet on the serum LDL and HDL cholesterol, Mensink and Katan (1989) reported that the monounsaturated fat rich diet was equally effective as the polyunsaturated fat rich diet in lowering the total and LDL cholesterol. Both the diets reduced HDL cholesterol in men but not in women (Mensink and Katan, 1989).

Mata, et al. (1996) studied the effect of four diets consecutively (a saturated fat diet, a monounsaturated fat diet, a polyunsaturated fatty acid diet (n-6), a polyunsaturated fatty acid diet (n-3)) lasting five weeks each, on 42 individuals and reported increased total cholesterol, HDL cholesterol as well as LDL cholesterol, on the high saturated fat diet. Polyunsaturated fatty acid (n-3) and polyunsaturated fatty acid (n-6) diet reduced the total cholesterol, LDL cholesterol and HDL cholesterol levels in plasma to a greater extent than the other two diets. The monounsaturated fat diet reduced total cholesterol and LDL cholesterol but did not affect HDL cholesterol. In another randomised crossover study on eleven healthy individuals, after three weeks consumption of both the polyunsaturated fat and the monounsaturated fat diet, serum total cholesterol, LDL cholesterol and triglyceride levels were reduced. No change was reported for HDL cholesterol on either of the two diets (Bonanome, et al., 1992).

A meta analysis from 14 studies, in which the total fat, saturated fat, fibre and dietary cholesterol were similar, reported no difference in the serum total, LDL or HDL cholesterol when polyunsaturated fat versus monounsaturated fat diets were compared (Gardner and Kraemer, 1995). Triglycerides were slightly lower after the polyunsaturated fat diets.

1.2.2 Dietary carbohydrates and serum cholesterol

Though the seven-country study showed that Japanese have lower death rates from CHD and their diets were high in carbohydrates and very low in total fat (**Table 1.1**), no relationship was seen between death rates and percentages of energy obtained from carbohydrates (Kromhout, et al., 1995). Epidemiological studies found no association between the percentage of energy intake from carbohydrates and the CHD risk (Colditz et al., 1992; Esrey et al., 1996; Garcia-Palmieri et al., 1980). However, in a study on baseline nutrient intake of 8218 urban and rural Puerto Rican men aged 45 to 64 years, investigating the nutrient intake and the incidence of CHD in the subsequent six years, an inverse association between carbohydrate intake from legumes and CHD incidence was noted, suggesting a protective effect (Garcia-Palmieri, et al., 1980).

Dietary Characteristics	US	Greece	Japan
Fat (% of energy)	39	37	11
Saturated fat (% of energy)	18	8	3
Vegetables (gm/d)	171	191	198
Fruits (gm/d)	233	463	34
Legumes (gm/d)	1	30	91
Breads and cereals (gm/d)	123	453	481
Potatoes (gm/d)	124	170	65
Meat (gm/d)	273	35	8
Fish (gm/d)	3	39	150
Eggs (gm/)	40	15	29
Alcohol (gm/d)	6	23	22

Table 1.1 Dietary characteristics in the United States, Greece and Japan in the 1960s#n

gm/d - grams per day

Dietary information from the seven countries study.

η Source (Kromhout, 1989)

Cross-sectional studies have found an inverse association between the glycaemic index (Frost et al., 1999), simple carbohydrates in the diet (Starc et al., 1998) and HDL cholesterol.

Results from dietary interventions found that the consumption of high carbohydrate low fat diets lowered plasma total cholesterol, LDL cholesterol and HDL cholesterol (Schaefer et al., 1995; Turley et al., 1998) and significantly increased the triglyceride levels (Schaefer, et al., 1995). In an intervention study, the ability of a low fat, low cholesterol diet (American Heart Association phase three diet) to improve the risk factor profile of moderately hypercholesterolemic, premenopausal women was evaluated (Cole et al., 1992). After five months of intervention, the results showed a decrease in total, LDL and HDL cholesterol. Triglycerides were increased by about 30 percent (Cole, et al., 1992).

However Turley, et al. (1998) compared a typical western style diet with a high carbohydrate diet in a randomised trial on 38 subjects and reported a decrease in HDL levels but an improved LDL to HDL ratio on the high carbohydrate diet. No change in triglycerides was seen. It was concluded that when free-living individuals changed to a diet rich in fibre and low in fat with high carbohydrate foods, a modest decrease in weight and an improvement in the lipoprotein profile could be seen. Retzlaff et al. (1995), looked at the effect of four different levels of carbohydrates in the diet - less than 45% of energy from carbohydrates, 45-51.9% of energy from carbohydrates, 52-59.9% of energy from carbohydrates and more than 60% of energy from carbohydrates, in 372 hyperlipidemic subjects over a period of two years. Subjects consuming less than 60 percent of energy from

carbohydrates did not show any significant increase in the triglyceride levels, suggesting that moderately low fat, high carbohydrate diets can be used over the long term without showing any deleterious effect on the serum triglyceride levels.

Mensink and Katan (1992), in a meta-analysis of 27 trials, found that all types of fat (except *trans* fatty acids) tend to raise plasma HDL concentrations when examined relative to carbohydrates, whereas saturated fat increases plasma LDL concentration, and monounsaturated fats and polyunsaturated fats reduce plasma LDL concentrations. Thus, the ratio of total cholesterol to HDL cholesterol is lowest when the intake of monounsaturated fat is low (Mensink and Katan, 1992). In another meta-analysis applied to 17 population based prospective studies of triglyceride and cardiovascular disease (CVD), after adjusting for HDL and other risk factors, the increase in the disease risk factors was by 14% in men and 37% in women with increased triglycerides (Austin, et al., 1998). However, in a recent review, Parks and Hellerstein (2000) pointed out that although carbohydrates increase the serum triglycerides there is no proof that hypertriglyceridemia is associated with a high risk of CHD (Parks and Hellerstein, 2000). So, the position of carbohydrates in relation to CHD is still not clear.

Although there is general agreement that reducing the intake of saturated fat in the diet would be beneficial, controversy remains as to what energy source should replace these harmful fats. Some researchers advocate a reduction in total fat consumption with replacement by carbohydrates, whereas others recommend replacing saturated animal fat with polyunsaturated fat and/ or monounsaturated fat.

1.3 Oxidation of LDL

Polyunsaturated fat has the ability to reduce the total and LDL cholesterol, but evidence suggests that it increases the susceptibility of LDL to oxidation (Bonanome, et al., 1992; Mata, et al., 1997; Reaven et al., 1991; Reaven et al., 1994) which is thought to be the major step in the initiation and promotion of atherosclerosis (Steinberg et al., 1989; Witztum, 1991).

It is assumed that oxidative modification of LDL occurs in the arterial intima (Steinberg and Witztum, 1990). LDL is a complex of a large molecular-weight protein containing apolipoprotein B, neutral and polar lipids and lipophilic antioxidants mainly vitamin E and beta-carotene. It is hypothesised that lipid peroxidation starts in the polyunsaturated fatty acids on the LDL-surface phospholipids, and then propagates to core lipids, resulting in oxidative modification of polyunsaturated fatty acids and phospholipids which leads to the modification and degradation of apolipoprotein B. This leads to the formation of a number of aldehydes and ketones. Some of these aldehydes form covalent bonds with LDL apolipoprotein B, and generate an 'oxidatively modified LDL' (Ox LDL). Ox LDL is not recognised by the native LDL receptors, so are taken up by acetyl LDL receptors called the 'scavenger' receptors (Steinberg and Witztum, 1990). This stimulates the monocytes in vessel walls, which are transformed to tissue macrophages. The macrophages ingest the Ox LDL and are transformed into bodies called 'foam cells' (Jessup et al., 1990) and get loaded

with cholesterol and cholesteryl esters (Esterbauer et al., 1992; Steinberg, et al., 1989; Witztum, 1994; Witztum and Steinberg, 1991; Witztum, 1991). Ox LDL exerts a chemotactic effect on monocytes and contributes to its accumulation in the lesion area. Ox LDL also exerts an inhibitory effect on the motility of tissue macrophages, which leads to the 'trapping' of the macrophages within the intima (**Figure 1.1**). As the levels of LDL within the intima rise, some of it undergoes oxidative modification, induced by endothelial or smooth muscle cells or macrophages themselves which may lead to a vicious cycle generating more macrophages responsible for LDL oxidation, uptake and increasing the fatty streaks. Oxidised LDL can also stimulate monocytes to secrete interleukin-1, which in turn can stimulate smooth muscle cell proliferation.



Figure 1.1 Generation process of fatty streak lesion in atherosclerosis Reproduced from Steinberg and Witztum (1990)

Additionally, Ox LDL are highly toxic to cells and may be responsible for damage to the endothelial layer and destruction of smooth muscle cells. This endothelial cell injury may change the fatty streaks to the fibrous plaque - the advanced lesions by - inhibiting the endothelial cells to release relaxing factors in

response to stimulants (**Figure 1.2**) (Esterbauer, et al., 1992; Steinberg, et al., 1989; Witztum, 1994; Witztum and Steinberg, 1991; Witztum, 1991).



Figure 1.2 Link between lipid infiltration and endothelial injury Modified from Steinberg and Witztum (1990)

During the vicious cycle of uptake of modified LDL by macrophages and accumulation in the lesion area, superoxide anions from the endothelial or smooth muscle cell may be released. These superoxide anions are the free radicals which may further aggravate the lipid peroxidation and hence increase the formation of foam cells and advanced lesions. Also in the macrophages, enhanced

15-lipoxygenase activity can generate lipid hyroperoxides, which could be transferred to extracellular LDL, which may enhance the lipid peroxidation (Witztum, 1994).

Free radicals can be defined as any species that contain one or more unpaired electrons and are capable of independent existence. In the human body some free radicals are also produced during aerobic metabolism. Free radicals can join with other free radicals and form covalent bonds, or they can combine with nonradicals and can start a free radical chain reaction, resulting in the formation of more free radicals. Within the body, lipids or fat molecules are responsible for maintaining the structural integrity of the cell membranes. Free radicals like superoxides and hyroperoxides, can react with the polyunsaturated fatty acids in the presence of metal ions like copper or iron *in vivo*, producing a polyunsaturated fatty acid molecule devoid of one hydrogen atom. This polyunsaturated fatty acid then undergoes rearrangement, reacting with oxygen and forming a peroxyl radical. This peroxyl radical can react with the adjacent fatty acid, oxidise it and hence lead to a chain reaction. This chain reaction can oxidise a number of fatty acids to lipid peroxides, which can damage the cell membrane, make it leaky and eventually cause complete membrane breakdown (Halliwell, 1994).

1.3.1 Factors affecting the oxidation of LDL

A number of factors are thought to have an effect on the oxidation of LDL *in vivo*. These factors are broadly classified into two categories - factors intrinsic to LDL and factors extrinsic to LDL (**Table 1.2**).

Factors intrinsic to LDL

- Fatty acid composition (polyunsaturated fatty acid content in particular)
- Content of antioxidants: endogenous (e.g. beta-carotene, vitamin E, ubiquinol-10); exogenous (probucol)
- Phospholipase A₂ activity
- Others, including size of particle, inherent properties of apo B, location of fatty acids (e.g. on surface phospholipids or in core of triglycerides or cholesterol esters)

Factors extrinsic to LDL

- Potential variation in cellular proxidant activity
- Concentration of plasma and extracellular fluid proxidant components (e.g.trace metal concentration)
- Concentration of plasma and extracellular fluid antioxidant components (e.g. ascorbate, urate)
- Concentrations of other factors influencing oxidation of LDL (e.g. HDL)
- Factors influencing residence time of intermediate density lipoprotein in intima (e.g. factors that increase binding such as Lp(a); nonenzyme glycosylation of LDL or matrix; differences in localised matrix proteins that bind LDL)

Taken from Witztum and Steinberg (1991)

1.3.1.1 Fatty acid composition

As discussed earlier, the oxidation of the LDL starts with the degradation of polyunsaturated fatty acids in the LDL. The content of polyunsaturated fatty acids is one of the major factors that can influence the oxidation of LDL. Polyunsaturated fatty acids, the oxidisable component of LDL, are an integral component of most dietary fats. Saturated fatty acids such as palmitic and stearic acids are largely found in the solid fats from animal sources. Polyunsaturated fatty acids like linoleic, linolenic and arachidonic acid have two, three or four unsaturated bonds and occur predominantly in oil like safflower and sunflower. Monounsaturated fatty acids such as oleic acid occur in high proportion mainly in olive oil. **Table 1.3** shows the proportion of different fatty acids in some commonly used cooking oils in Australia.

Name	Saturates	Polyunsaturates	Monounsaturates
Canola oil	8	30	62
Sunola oil™	10	5	85
Safflower oil	9	77	14
Sunflower oil	11	66	23
Olive oil	14	10	76

 Table 1.3 Fatty acid composition of some commonly used cooking fats in

 Australia

Source- Fats and oils: The facts. Meadow Lea Foods, Australia (1995).

1.3.1.1.1 Dietary fat and oxidation of LDL

Polyunsaturated fat in the diet reduces the intake of saturated fat which is an independent risk factor for increasing serum total cholesterol, LDL cholesterol and hence the risk of CHD. However, polyunsaturated fatty acid diets rich in linoleic acid (18:2) increases the content of linoleate fatty acid in plasma LDL that contains 2 unsaturated bonds in their structure, which may make the LDL more susceptible to oxidative damage by free radical attack. Monounsaturated fatt diets rich in oleic acid (18:1) lower the content of linoleate in plasma LDL (Reaven, et al., 1994). Studies comparing the level of saturation of dietary fat and oxidation of LDL have suggested that altering the diet by increasing the monounsaturated fatty acid content has a two fold effect on the CHD risk (Abbey et al., 1993a; Bonanome, et al., 1992; Mata, et al., 1997; Reaven, et al., 1991;

Reaven, et al., 1994). Firstly it does not reduce HDL cholesterol and secondly it decreases the susceptibility of LDL to oxidative modification compared to polyunsaturated fatty acids. The susceptibility of LDL to oxidation is measured by lag phase (**Figure 2.4 pp 71**), which can be described as the time taken by the endogenous antioxidants of LDL to disappear, when exposed to the pro-oxidants like copper ions (Esterbauer, et al., 1992)

Abbey, et al. (1993a), compared the effect of oleic acid enriched and linoleate fatty acid enriched diets of 14 days duration each, on eight subjects and reported an increased tendency of LDL particles to oxidise and a higher formation of conjugated dienes after a linoleate diet compared to an oleate diet.

As discussed earlier, Mata, et al. (1996) studied the effects of four diets consecutively (saturated fat diet, monounsaturated fat diet, polyunsaturated fatty acid diet (n-6), polyunsaturated fatty acid diet (n-3)) on 42 individuals. After five weeks of diets resistance to *in vitro* copper induced oxidative modification of LDL was increased during the monounsaturated fat diet period with respect to all other diets. Consumption of monounsaturated fat increased the lag time of LDL oxidation relative to all other dietary periods.

Reaven, et al. (1994) compared the effects of a linoleate rich, an oleate rich and a normal diet on 12 subjects taking 1200 mg/day alpha tocopherol on the oxidation of LDL. The study reported increased susceptibility of isolated LDL to oxidation measured by the formation of conjugated diene, loss of peroxides, and loss of unsaturated fatty acids on the linoleate diet followed by the normal diet and the

oleate rich diet. This study suggested that increasing linoleic acid in the diet can actually overtake the antioxidant effects of alpha tocopherol, and decrease the resistance of LDL to oxidation.

1.3.1.2 Content of antioxidants

In addition to the dietary fat and its components, fruits and vegetables gained importance in relation to CHD when studies reported that populations consuming low saturated fat (from meat sources) and an abundance of fruits and vegetables have lower rates of CHD and higher life expectancies (Willett, 1994). Verlangieri et al. (1985) hypothesised that cardiovascular mortality in the US was declining because there was a greater availability of fruits and vegetables all year round. In the last few years, the focus of some research moved from foods as a whole to the specific nutrients like vitamin C, vitamin E (tocopherol) and carotenoids found in plant foods. These substances are also known as 'antioxidants'.

As discussed earlier, free radicals in the body can cause damage and enhance the oxidation of LDL by a series of chain reactions. Antioxidants are substances, which can inhibit these chain reactions by reacting rapidly with the free radicals, and generating by-products that will not propagate auto-oxidation. The core of the LDL particles contains lipophilic antioxidants like alpha tocopherol, carotenoids like beta-carotene, lycopene and cryptoxanthin, ubiquinol-10 and phytofluene (Esterbauer, et al., 1992). These lipophilic antioxidants can quench singlet oxygen, neutralise thioyl radicals, combine with and stabilise peroxyl radicals and hence prevent free radical oxidation of other molecules (Esterbauer, et al., 1992).

Though the exact mechanism of the activity of these antioxidants is not known, it is thought that carotenoids are efficient quenchers of singlet oxygen and peroxyl radicals. Singlet oxygen, an excited form of a partially reduced form of oxygen, is unstable and highly reactive. Singlet oxygen scavenging (**Figure 1.3**) can occur by the physical transfer of the excitation energy of singlet oxygen to the carotenoid, with subsequent dissipation of this energy as heat, without the concomitant loss of the carotenoid molecule (Krinsky, 1998a).

Sensitizer + light ॓	¹ Sensitizer
¹ Sensitizer \Longrightarrow	³ Sensitizer
³ Sensitizer + ${}^{3}O_{2}$ (ground state) \Longrightarrow Set	ensitizer + ${}^{1}O_{2}$ (singlet excited oxygen)
$^{1}O_{2}$ + Carotenoid \Longrightarrow	$^{3}O_{2}$ + $^{3}Carotenoid$
³ Carotenoid \Longrightarrow	Carotenoid + heat

Figure 1.3 Antioxidant action of carotenoids Adapted from Krinsky (1998a)

In cells, vitamin E is situated in the membranes, adjacent to unsaturated fatty acids that are vulnerable to free radical attack. Free radicals extract hydrogen from a membrane polyunsaturated fatty acid and convert it to a fatty acid free radical, which takes up oxygen from the adjacent double bond and forms a peroxyl fatty acid radical. This peroxyl fatty acid is stabilised by abstracting a hydrogen atom from another membrane polyunsaturated fatty acid, which leads to initial free radical damage along the membrane by isomerisation and further oxygen uptake. Vitamin E arrests this propagation of the free radical damage by converting the peroxyl fatty acid free radical to a hydroperoxyl fatty acid. The resulting vitamin E radical can be regenerated to vitamin E by oxidation of

vitamin C. The resulting vitamin E radical can stabilise another peroxyl fatty acid radical to form the quinone form of vitamin E (Skeaff, 1998). **Figure 1.4** represents the antioxidant function of vitamin E.



Figure 1.4 Antioxidant action of vitamin E Reproduced from Skeaff (1998)

1.3.1.2.1 Dietary antioxidants and oxidation of LDL

Oxidation of polyunsaturated fatty acids proceeds as the antioxidants in LDL are depleted in the sequence of alpha tocopherol, lycopene and beta-carotene (Esterbauer et al., 1989). It is thought that supplementing the carotenoids and vitamin E in the diet may provide an additional protection against the oxidation of LDL. Clinical studies were performed to investigate the effect of the increased intake of carotenoids and vitamin E on the oxidation of LDL (Abbey et al., 1993b; Jialal and Grundy, 1993; Princen et al., 1992). Increased resistance of LDL to oxidation was observed, especially with the supplementation of vitamin E (Abbey, et al., 1993b; Jialal and Grundy, 1993; Princen, et al., 1992).
Twenty subjects were given a daily dose of vitamin supplement that contained beta-carotene, vitamin C and alpha tocopherol for six months, compared to 23 control subjects who took no supplements. In addition to the increase in the plasma levels of beta-carotene, vitamin C and alpha tocopherol, a significant increase in the LDL lag phase in the experimental group, measured by in vitro formation of conjugated diene was observed, compared to the control group. Alpha tocopherol was directly correlated to the longer lag phase where as betacarotene was correlated only in the presence of alpha tocopherol (Abbey, et al., 1993b). Similar results were shown by Jialal and Grundy (1993) while investigating the safety and antioxidant effect of a combination of alpha tocopherol, vitamin C and beta-carotene. Similarly, in a study on six healthy nonsmoking subjects ingesting 1000 international units/ day of D-L-alpha tocopherylacetate for seven days showed a significant increase in the plasma and LDL levels of alpha tocopherol. The change in LDL levels of alpha tocopherol was positively related to the increase in the *in vitro* resistance to oxidation of LDL (Princen, et al., 1992).

For carotenoids, the role of beta-carotene is quite controversial, as some studies have reported inhibition in the oxidation of LDL with supplementation of betacarotene (Jialal et al., 1991; Lavy et al., 1993), whereas others reported that though the LDL levels of beta-carotene increase with beta-carotene intake, it does not protect the oxidation of LDL (Gaziano et al., 1995a; Reaven et al., 1993; van het Hof et al., 1999a). Similarly, the results from the supplementation of lycopene in diets and oxidation of LDL have been inconsistent (Lee et al., 2000; Rao and Agarwal, 1998; Sutherland et al., 1999).

1.4 Carotenoids, vitamin E and CVD

Several epidemiological and case control studies have looked at the association of antioxidants to CVD, some of which are discussed below.

1.4.1 Dietary intake of carotenoids, vitamin E and CVD

Epidemiological studies (**Table 1.4**) of dietary intake have primarily investigated the total provitamin-A carotenoids, predominantly beta-carotene, because the dietary values of individual carotenoids were not available. Gaziano et al. (1995b); Knekt et al. (1994); Rimm et al. (1993) and Sahyoun et al. (1996) found the relative risk of a coronary event to be lower in people consuming greater amounts of carotenoid rich foods. Gaziano, et al. (1995b) examined the combined servings of food categories high in carotenoids in a cohort of 1,299 elderly Massachusetts residents for about 5 years and found that the people who were in the upper 25^{th} percentile of consumption

			(as relative risk)
Rimm <i>et al</i> (1993) Male Health Professionals Study	39,910 male health professionals 40 to 75 years of age initially free from heart disease.	Carotene* Vitamin E	Inversely related to CHD
Knekt <i>et al</i> (1994) Finnish Mobile Clinic Study	5,133 Finnish men and women aged 30 to 69 years of age initially free from heart disease followed for 14 years.	Carotenoids Vitamin E	Inversely related to CHD death
Gaziano <i>et al</i> (1995)	1,299 elderly Massachusetts residents	Carotenoids	Inversely related to fatal MI
Pandey <i>et al</i> (1995) Western Electric Study	1,556 employed, middle aged men followed for 24 years	Beta-carotene	No effect
Sahyoun <i>et al</i> (1996)	747 normal subjects over 60 years followed for 9-12 years.	Carotenoids	Inversely related to CHD death
Kushi <i>et al</i> (1996)	34,486 postmenopausal women with no	Carotenoids	No effect
、 <i>/</i>	cardiovascular disease followed for 7 years	Vitamin E	Inversely related to CHD death
Klipstein-Grobusch <i>et</i> <i>al</i> (1999) Rotterdam Study	4802 subjects free from MI 55 to 95 years of age	Beta-carotene	Inversely related to MI incidence
5		Vitamin E	No association

Table 1.4 Epidemiological and case control studies of intake of antioxidar	its
(carotenoids and vitamin E) and CVD	

POPULATION

CHD is coronary heart disease MI is Myocardial Infarction * Significant for smokers

STUDY

PARAMETER OUTCOME

of carotene containing fruits and vegetables had a significantly lower risk of fatal MI. The study also reported an inverse association between the intake of one or more servings of carrots and/or squash, salads and green leafy vegetables and CVD death risk adjusted for age and sex. In the Finnish mobile clinic 14-year follow up study, a stronger association was detected between high fruit and vegetable intake and less deaths from CHD in the men than the women. Dietary carotenoids seemed to have a less protective effect against CHD deaths when analysed separately, though they strengthened the effect when combined with vitamin E (Knekt, et al., 1994). Vitamin E was significantly inversely related to coronary mortality in both men and women, even after adjustment for age, smoking, serum cholesterol, hypertension, body mass index (BMI) and energy intake (Knekt, et al., 1994).

In the Male Health Professionals Study, 39,910 males 40-75 years of age, free of diagnosed CHD, diabetes and hypercholesterolmia, were followed for four years and their dietary intake of vitamin C, carotene and vitamin E from food and supplements was assessed (Rimm, et al., 1993). The results showed a significant inverse association between provitamin A carotenoid intake and coronary disease risk among current and former smokers, but not in non-smokers. Dietary vitamin E was inversely associated with the risk of CHD among men who did not take any vitamin supplements (Rimm, et al., 1993). A study by Sahyoun, et al. (1996) supported the hypothesis that antioxidant vitamins protect against CHD, but the inverse association was stronger with the consumption of all vegetables, rather than dark green and orange vegetables, which are better sources of carotenoids, implying that vegetables may contain other constituents that provide protection.

Pandey et al. (1995) reported a small but statistically non-significant association between the intake of beta-carotene and the risk of coronary disease. Kushi et al. (1996) found no association between provitamin-A carotenoids and a risk of coronary disease in postmenopausal women. However, the intake of vitamin E from food was negatively associated with the risk of death from CHD (Kushi, et al., 1996). In a four year follow-up study on older men and women (1856 men and 2946 women, \geq 55 years of age), Klipstein Grobusch et al. (1999) detected a reduced risk of MI with a high intake of beta-carotene, after adjustment for age and sex, with energy intake. The results were more pronounced in current and former smokers. No association was seen between dietary vitamin E intake and the risk of MI (Klipstein Grobusch, et al., 1999).

1.4.2 Serum and/ or tissue carotenoids, vitamin E and CVD

In the majority of studies (**Table 1.5**), beta-carotene and /or other carotenoids such as alpha-carotene, lycopene, lutein and zeaxanthin, were found to be protective against CHD, angina or MI (Gey et al., 1991; Gey et al., 1993; Kardinaal et al., 1993; Kohlmeier et al., 1997; Riemersma et al., 1991). Two studies found cigarette smoking to be an important risk modifier with the strong association found only in smokers (Kardinaal, et al., 1993; Street et al., 1994). Kardinaal, et al. (1993) found that a high beta-carotene concentration in adipose tissue had a greater protective effect against first MI, and current smokers with low adipose beta-carotene levels were at greater risk of MI. In the 12 year follow-up of the Basel Prospective Study, significantly increased relative risk of CHD and stroke

STUDY	POPULATION	PARAMETER	OUTCOME (as relative risk)
Riemersma <i>et</i> al (1991)	110 patients with symptoms of angina and 394 normal subjects, 35-54 years of age	Carotene (plasma) Vitamin E (plasma)	Inverse association with angina
Gey <i>et al</i> (1991)	16 European regions	Alpha tocopherol (plasma)	Inverse association with cardiovascular mortality rate
Kardinaal <i>et al</i> (1993)	683 people with acute MI and 727 control, less than 70 years of	Beta-carotene (adipose tissue)	Inverse association with nonfatal MI*
	age	Alpha tocopherol	No association
Gey <i>et al</i> (1993)	Males 12 years follow up	Alpha and Beta-carotene (plasma)	Inverse association with CHD and stroke
Morris <i>et al</i> (1994)	1,899 men with type 2 hyperlipidemia aged 40 to 59 years	Total carotenoids (serum)	Inverse association with CHD**
Street <i>et al</i> (1994)	123 subjects with first diagnosis of MI, 23-58 years of age	Beta-carotene (serum) Lycopene (serum) Lutein (serum) Zeaxanthin (serum)	Inversely related to the relative risk of MI*
		Alpha tocopherol (serum)	Reduced in hypercholesterolemic
Sahyoun <i>et al</i> (1996)	747 normal subjects over 60 years	Carotenoids (serum) Vitamin E	Insignificant result for mortality from heart disease
Kohlmeir <i>et al</i> (1997)	1,387 men of first acute MI under 70 years of age	Alpha-carotene (adipose tissue) Beta-carotene (adipose tissue) Lycopene (adipose tissue)	Inversely related to nonfatal MI
Evans <i>et al</i> (1998)	734 samples analysed from MRFIT study	Total carotenoids (serum) Tocopherol	Insignificant results for CHD death & nonfatal MI

Table 1.5 Epidemiological and case control studies of blood/ tissue antioxidants (carotenoids and vitamin E) and coronary disease

CHD is coronary heart disease *Significant only for smokers MI is Myocardial Infarction **Stronger association for men who never smoked

was revealed in the subjects with initially low plasma levels of alpha and betacarotene (Gey, et al., 1993).

Three studies that looked at the total carotenoids and not at the individual carotenoids are those by (Evans et al., 1998; Morris et al., 1994; Sahyoun, et al., 1996). Morris, et al. (1994), reported a significant protective effect of the carotenoids on CHD, especially in hyperlipidemic men who had never smoked, whereas for the other two studies (Evans, et al., 1998; Sahyoun, et al., 1996) the relationship disappeared after accounting for confounding factors, like existing diseases, disabilities affecting shopping, and nutrients like vitamin C and vitamin E. Kohlmeier, et al. (1997) and Street, et al. (1994) looked at the individual incidence of nonfatal MI and found an inverse relation with carotenoids. But the results for alpha-carotene and beta-carotene were insignificant when confounders like blood pressure and BMI were taken into account. Lycopene remained the independent predictor of the outcome (Kohlmeier, et al., 1997; Street, et al., 1994). However, Street, et al. (1994) found a stronger association between carotenoid levels and reduced risk of MI in current smokers, than in non-smokers. Alpha tocopherol was found to be protective against MI only in people with high serum cholesterol levels.

In a cross sectional study of men aged 50 years in Sweden and Lithuania, it was reported that beta-carotene, lycopene and lipid adjusted gamma tocopherol levels were significantly higher in Swedish men and total mortality from CHD was lower, suggesting a possible role for antioxidant status (Kristenson et al., 1997). In a case control study by Klipstein Grobusch et al. (2000), age and sex adjusted serum lycopene was found to be inversely associated with the risk of atherosclerosis. The association was most pronounced for current and former smokers. No relation was detected between the risk of atherosclerosis and serum concentrations of alpha-carotene, beta-carotene, lutein and zeaxanthin.

In a prospective cross sectional study on a randomly selected population sample of 392 men and women aged 45-65 years, D'Odorico et al. (2000) reported an inverse association between plasma alpha-carotene, beta-carotene levels and the prevalence of atherosclerosis in carotid and femoral arteries, after adjustment for other cardiovascular risk factors like age, sex, LDL concentration, smoking and alcohol consumption. Serum levels of dietary vitamin E and other carotenoids lutein, lycopene and cryptoxanthin, were not related to the risk of atherosclerosis.

Recently, Rissanen et al. (2001) examined 725 men aged 46 to 64 years, from Kupoio Ischaemic Heart Disease Risk Factor Study, and reported an inverse relationship between serum levels of lycopene and risk of atherosclerotic vascular events in men previously free of CHD and stroke.

1.4.3 Supplemental carotenoids, vitamin E and CVD

In the Finnish alpha-tocopherol beta-carotene (ATBC) prevention study (ATBC, 1994), the supplementation of beta-carotene (20 mg/ day for five to eight years years) was found to be harmful for male smokers in the age group of 50 to 69 years. There was an increased incidence of total deaths due to lung cancer and CHD in men who received beta-carotene supplements compared to those who did not. The trial raised the possibility of harmful effects of supplementation of beta-

carotene. Alpha tocopherol did not show any apparent effect on total mortality, although more deaths from haemorrhagic stroke were observed among the men who received supplements (50 mg/day) of tocopherol than those who did not (ATBC, 1994). Later, 1862 men were studied by Rapola et al. (1998) from the ATBC study group for frequency of major coronary events and deaths from coronary heart disease. The study reported no difference in the occurrence of coronary events in the supplemental or placebo group. Surprisingly, there were more deaths from CHD in beta-carotene and combined alpha tocopherol and beta-carotene supplementation groups, than in the placebo group.

Likewise in the beta-Carotene And Retinol Efficacy Trial (CARET), 18,000 men and women at high risk of lung cancer due to a history of cigarette smoking or occupational exposure to asbestos, were treated with supplemental beta-carotene and retinol for a period of about four years (Omenn et al., 1996). The results reported a higher rate of CVD or cancer among participants taking beta-carotene.

Hennekens et al. (1996) suggested that long-term studies were required to determine if the possible benefit became evident after longer treatment and follow-up. Twenty two thousand and seventy one healthy male physicians, 40 to 84 years old, were studied in a random, double blind, placebo controlled trial. In the beta-carotene group, subjects were treated with 50 mg beta-carotene every alternate day. After 12 years of supplementation, no beneficial or harmful effect of beta-carotene was noticed with respect to the number of MI, stroke and deaths due to CVD or deaths from all causes.

Stampfer et al. (1993) showed a negative association between vitamin E supplementation and CHD in females 34 to 54 years old, with a 41% reduction in the risk of coronary disease in women who were taking supplements for two or more years. Although a relationship between dietary vitamin E and the risk of MI was not found in the Euramic study by Kardinaal, et al. (1993), vitamin E supplements were found to be inversely related to the risk of MI in the people taking supplements.

Similarly, a double blind placebo Cambridge Heart Antioxidant Study (CHAOS) reported that supplementation of 800 IU (546 patients) or 400 IU (981 patients) of vitamin E per day compared to placebo (967 patients) reduced the risk of non fatal MI after about one year in the angiographically proven coronary arteriosclerosis patients but no significant change in the cardiovascular deaths (Stephens et al., 1996). In another randomized placebo controlled trial, supplementation of 800 IU of vitamin E per day reduced MI in 196 patients on haemodialysis, but no differences in total and cardiovascular disease mortality were observed (Boaz et al., 2000). Similarly, The Heart Outcomes Prevention Evaluation (HOPE) study did not show any effect of vitamin E supplementation (400 IU from natural sources per day for 4.5 years) compared to placebo on the cardiovascular outcomes in 2545 women and 6996 men of age > 55 years, who were at a high risk of cardiovascular event because they had cardiovascular disease or diabetes with one more risk factor (Yusuf et al., 2000). Another study compared the effects of supplementing n-3 polyunsaturated fatty acids with or without vitamin E in 11324 patients with MI and reported that the combined and the n-3 polyunsaturated group had reduced incidence of non-fatal MI and death

compared to the vitamin E supplementation group after 2 years of supplementation, suggesting that vitamin E does not provide a protective effect from death in MI patients (GISSI, 1999).

In summary, the role of antioxidant carotenoids and vitamin E in relation to cardiovascular diseases is controversial. While higher consumption and high tissue levels of carotenoids and vitamin E are positively related to a decreased risk of cardiovascular mortality, the supplementation of these carotenoids and vitamin E show a different side. Therefore, it is essential to learn more about the characteristic properties of these compounds, their absorption and their metabolism in a biological system, which may assist in understanding the actions of these compounds and their possible relationship with chronic diseases.

1.5 Carotenoids: Structure and sources

Carotenoids (Figure 1.5) are a group of over 600 non-nitrogenous, fat-soluble pigments, widely distributed in nature. Carotenoids are found mainly in plants and micro-organisms but are not synthesised by humans. They are classified as carotenes, xanthophylls and acyclic carotenoids (Olson, 1989). The carotenes (alpha, beta and gamma) consist of 18-carbon chain, conjugated double bonds with a six carbon ring at each end. Xanthophylls have one or more oxygen atoms added and include zeaxanthin, lutein, astaxanthin, cryptoxanthin and canthaxanthin. Lycopene, an acyclic carotenoid, consists of an open chain 40 carbon atom containing 11 conjugated and two non conjugated double bonds



Figure 1.5 Chemical structure of carotenoids

In nature, carotenoids are responsible for the characteristic colour in leaves, fruit and vegetables. The red colour of the tomato is attributable to lycopene, the acyclic carotenoid. The orange colour of carrots is due to beta-carotene, the hydrocarbon carotenoid. Spinach is a rich source of lutein and zeaxanthin - the xanthophylls, also called oxygenated carotenoids. **Table 1.6** presents some carotenoid rich foods.

CAROTENOID	FOOD SOURCE
Beta-carotene	Carrots, cantaloupe, broccoli, spinach
Alpha-carotene	Carrots
Lycopene	Tomato and tomato products
Beta cryptoxanthin	Orange, orange juice, tangerines, peaches
Lutein and	Spinach and other greens, broccoli, corn, green
zeaxanthin	peas, green beans

Table 1.6 The major contributors of carotenoids in the human diet

Some carotenoids also exist in different geometric forms as *cis* and *trans* isomers, which are inter-converted by light, thermal energy and chemical reactions (Stahl and Sies, 1992). In nature, carotenoids are predominantly present in *all-trans* configuration, which is thought to be more stable (Krinsky, 1998b). Also the *trans* isomers are readily absorbed in the body compared to the *cis* form (Rock et al., 1996). However processing may induce the formation of *cis* isomers (Stahl and Sies, 1992). Lessin et al. (1997) reported that canning fresh tomatoes increases beta-carotene *cis* isomer content from 12.9% to 31.2%. Also, heating tomato juice (Stahl and Sies, 1992) and bench top preparation of spaghetti sauce from canned tomatoes (Schierle et al., 1997) has been reported to increase the lycopene *cis* isomer levels. However, in a study by Khachik et al. (1992), no change was found in *cis/trans* isomerisation of lutein between cooked and raw broccoli, spinach and green peas. The cooking procedure included steaming for three to five minutes, microwaving for 1.5 to three minutes, and boiling for 9 minutes. There is a possibility that the degree of isomerisation is related to the

intensity and duration of cooking, because (Stahl and Sies, 1992) heated the tomato juice for one hour at 100°C, which is not the normal or the standard process of cooking. Similarly, Schierle, et al. (1997) had cooked the canned and peeled tomatoes for 45 to 60 minutes at 85-110°C, which they themselves described as 'long term' cooked spaghetti sauce. If regular cooking procedures caused this effect in the isomer form, then it may affect the absorption and hence influence the body content.

In addition, the medium of dispersion for carotenoids may have an effect on the isomerisation of carotenoids in cooked foods. In an experiment by Schierle, et al. (1997), tomato paste was dispersed in water or oil and heated for three hours at 75°C. Heat treatment increased the *cis* lycopene content. Heating in oil had a stronger effect than heating in water and especially favoured the rise of 9-*cis* lycopene and 5-*cis* lycopene. However, Nguyen and Schwartz (1998) reported no shift in the distribution of *cis* lycopene isomer in tomato products of varying moisture content, fat content, container type and thermal treatment during usual food preparation or commercial processing. Also, changes (loss/increase) in the total carotenoid content of vegetables on cooking appeared to be minimal (Khachik, et al., 1992; Nguyen and Schwartz, 1998).

1.5.1 Absorption of carotenoids and nutritional factors affecting absorption

Not much is known about the absorption of carotenoids. Beta-carotene has been the most studied carotenoid, and it is assumed that other carotenoids are also absorbed and metabolised in a similar manner to beta-carotene, under defined conditions (Erdman et al., 1993; Parker, 1996). Absorption is defined as the movement of dietary carotenoids or metabolites of carotenoids to the lymphatic or portal circulation. In human beings, an appreciable amount can be absorbed by the mucosal cells and is incorporated and secreted unchanged in the chylomicra (Erdman, et al., 1993). The duodenal mucosal cells absorb carotenoids by mechanisms involving passive diffusion, similar to that of cholesterol and the products of triglycerides lipolysis (Parker, 1996). **Figure 1.6** represents the steps in the carotenoid absorption and the factors that may affect the absorption of carotenoids.

The first step in the absorption of carotenoids is the release of the carotenoids from the food ingested, which depends on the physical disposition of carotenoids, the achieved particle size after mastication, stomach action and the efficiency of the digestive enzymes (Parker, 1996). A number of studies have looked at the effect of different physical matrices of the food on the bioavailability of carotenoids in humans (Castenmiller et al., 1999; Gartner et al., 1997; Rock et al., 1998). Bioavailability is the fraction of an ingested nutrient that is available for utilisation in normal physiological functions and for storage (Gartner, et al., 1997). In a study on the food matrix of spinach and serum concentration of betacarotene and lutein after three weeks of dietary intervention, it was reported that the beta-carotene concentration was highest in serum on intake of liquefied spinach followed by liquefied spinach plus added fibre, minced spinach and whole leaf (Castenmiller, et al., 1999). However, no effect of homogenisation was noticed for the bioavailability of lutein. The intake of spinach increased the concentration of all carotenoids except lycopene. Lutein bioavailability was higher than that of the beta-carotene.

In addition, the bioavailability of the carotenoids may also depend on the extent of processing which includes homogenisation as well as cooking by heat treatment. Carotenoids are found in protein complexes in the food sources, which may prevent the digestion and absorption of these compounds. Mild thermal processing and/or mild heating may denature these protein complexes and increase the bioavailability of the carotenoids (Erdman, et al., 1993). In a crossover study on healthy females (23-36 years of age), Rock, et al. (1998) looked at the effect of daily consumption of 9.3 mg of beta-carotene from raw or thermally processed, pureed carrots and spinach for a period of four weeks. Serum beta-carotene concentration was approximately three times higher after the processed spinach and carrot intake period compared to the raw spinach and carrot intake period.

Studies have shown an increased lycopene response in plasma or triglyceride-rich lipoproteins after consumption of tomato paste compared to the same amount of lycopene consumed as fresh tomatoes (Gartner, et al., 1997; Porrini, et al., 1998). Giovannucci and Clinton (1998) observed that the association between the consumption of various tomato products and the risk of prostate cancer depends on the bioavailability of lycopene. Stahl and Sies (1992) demonstrated that preheating of tomato juice at 100°C for one hour with one percent corn oil increases the concentration of lycopene in blood compared to when it is ingested with one percent oil without heating.



Figure 1.6 Steps of carotenoid absorption and dietary factors that affect carotenoid absorption

Modified from van Het Hof et al. (2000)

The amount of dietary fat present with the carotenoids may also affect the absorption of carotenoids (Dimitrov et al., 1988; Erdman, et al., 1993; Parker, 1996; Parker, 1997; Williams et al., 1998). Dietary fat stimulates bile flow from the gall bladder, which facilitates the emulsification of fat and fat-soluble vitamins into lipid micelles within the small intestine (Erdman, et al., 1993). Some studies have looked at the effect of dietary fat on the bioavailability of carotenoids (Dimitrov, et al., 1988; Jalal et al., 1998; Jayarajan et al., 1980; Roodenburg et al., 2000). A study on the plasma response to beta-carotene supplementation (45mg for five days), where ten subjects consumed a controlled high or low fat breakfast (18-24 gram fat on high fat diet, fat free on low fat diet) and mid day meal (45 gram fat on high fat diet and six gram fat on low fat diet) showed a significant increase in plasma beta-carotene on the high fat diet compared to the low fat diet (Dimitrov, et al., 1988).

Roodenburg, et al. (2000) reported that the amount of dietary fat required for the optimal uptake of lutein esters in the plasma is higher than the amount required for the uptake of vitamin E, alpha-carotene or beta-carotene. In this study, no change was observed in the plasma concentration of vitamin E, alpha-carotene and beta-carotene after the consumption of a hot meal containing three or 36gram fat for a period of seven days by 14 subjects. But the lutein levels in plasma were higher after the consumption of a 36 gram fat meal rather than a three gram fat meal. There is a possibility that the low fat diet may have limited the solublisation of lutein esters from the diet in the fat phase and /or the release and activity of esterase and lipase. These enzymes are required to hydrolyse the lutein

esters, a step very likely to be crucial for their absorption (van Het Hof, et al., 2000).

Some forms of dietary fibre may also inhibit carotenoid utilisation, perhaps by reducing the lipid micelle formation (Rock and Swendseid, 1992). Pectin, a type of dietary fibre, has been shown to reduce the absorption of beta-carotene (Erdman, et al., 1993; Rock and Swendseid, 1992; Williams, et al., 1998). Any substance that affects the bile acid formation e.g. vitamin C deficiency, biliary disease with obstructive bile duct or chronic liver disease can inhibit the absorption of carotenoids. Non-digested lipids such as sucrose polymer (olestra), interfere with the carotenoid absorption in the intestinal lumen. Sucrose polymers probably serve as a hydrophobic sink, and the magnitude of effect is likely to be a function of the partition coefficient of the carotenoid between droplet and mixed lipid micelles (Parker, 1996). A decrease (about 30% to 50%) in the levels of most lipophilic carotenoids - beta-carotene and lycopene from baseline have been reported in subjects consuming 12 grams of olestra for four weeks (Schlagheck et al., 1997; Williams, et al., 1998).

Uptake of the micelles by the intestinal mucosal cells is thought to take place by a passive process, although this may not ensure absorption (Erdman, et al., 1993). Mucosal uptake requires the incorporation of the carotenoids into mixed micelles, which consist of bile acids, free fatty acids, monoglycerides and phospholipids. The amount of carotenoid incorporated into micelles is likely to depend on the polarity of the carotenoid, and on micellar fatty acid composition and saturation (Erdman, et al., 1993). However, beta-carotene appears at the same time in lymph

as newly absorbed fat from a meal, suggesting that these fat soluble compounds and lipids move together across the plasma membrane within the mucosal cell (Erdman, et al., 1993).

It is suspected that due to the slightly different structures of different carotenoids, there may be competition for uptake into mucosal cells and for absorption. A study by Wahlqvist et al. (1994) reported increased plasma lycopene and alphacarotene in 224 people with colorectal adenomas after supplementation of 20 mg of beta-carotene for two years. Johnson et al. (1998) reported that a combined dose of beta-carotene and lycopene, 60 mg each in oil in gelatine capsules, had little effect on the absorption of beta-carotene but improved that of lycopene in ten males. No significant change was seen for lutein and zeaxanthin levels. However, Micozzi et al. (1992) reported a decline in plasma lutein levels with chronic beta-carotene supplementation. Kostic et al. (1995) showed reduced serum lutein on ingestion of a combined dose of lutein and beta-carotene, 0.5 microgram/kg body weight, for weeks plus a diet low in carotenoids. These results suggest that beta-carotene may have an effect on the metabolism of other carotenoids.

1.5.2 Postabsorptive transport and distribution of carotenoids

Lipoproteins are the main transport carriers of carotenoids in humans, as they are for cholesterol. Carotenoids are transported from the intestinal mucosa to the bloodstream via the lymphatics by chylomicrons. Plasma lipids are the main determinants of plasma carotenoid concentrations. Carotenoids are distributed between very low-density lipoprotein (VLDL), LDL and HDL. Hydrocarbon carotenoids are mainly transported in LDL, and polar carotenoids in LDL and HDL. Beta-carotene, alpha-carotene and lycopene are distributed similarly amongst LDL (58-73%), HDL (17-26%), and VLDL (10-16%) (Forman et al., 1998; Parker, 1996). In contrast, lutein and zeaxanthin are predominantly found in HDL (53%), followed by LDL (31%) and VLDL (16%). LDL and HDL carry about 80% of beta cryptoxanthin together and the rest is found in VLDL (Parker, 1996).

In women, the concentration of lipoproteins fluctuates with the phase of the menstrual cycle, so it is thought that the carotenoid concentration changes with the change in lipoprotein concentration because carotenoids are transported in the human plasma by the circulating lipoproteins (Forman et al., 1996; Heber, 1996). However, a study by Forman, et al. (1996) reported the lowest carotenoid levels during menses and the levels increased after that and reached a peak in the luteal phase. These changes were not in accordance with the change in lipoproteins as LDL was at a peak in the early follicular phase and HDL in the late follicular phase. The authors suggested further research was needed to find the difference and indicated the reason for carotenoid not matching the lipoprotein concentration could have been the small size of the study group (n=12).



Figure 1.7 Postabsorption transport of beta-carotene to liver and extrahepatic tissues Modified from Erdman, et al. (1993)

Carotenoids are stored in different human tissues and the concentration varies from tissue to tissue. Human organs such as adrenal, liver and testes have a large number of LDL receptors, therefore it is suggested that lipoprotein uptake accumulates carotenoids passively in these organs (Schmitz et al., 1991). Beta-carotene is predominantly stored in liver and adrenal glands whereas testes are a storage depot for lycopene (Schmitz, et al., 1991). Among the different geometric isomers, *trans* beta-carotene is primarily found in circulation whereas *cis* beta-carotene is mainly found in peripheral tissues.

Whereas plasma carotenoid levels indicate the immediate intake of fruits and vegetables rich in carotenoids, tissue levels reflect the long-term consumption of

fruits and vegetables. The predominant carotenoids are lutein, beta cryptoxanthin, lycopene, alpha-carotene and beta-carotene, which account for 90% or more of circulating carotenoids in human beings. Food habits of Asian people are different from those of western people and hence it is expected that their plasma carotenoid profile also differ. Yeum et al. (1999) compared the serum carotenoid and tocopherol levels of caucasian American, Chinese and Korean adults and found considerable variation in the serum carotenoid profile among the three groups. Lycopene was the major carotenoid present in the American population where as lutein and zeaxanthin was in Chinese population. **Table 1.7** presents the comparison of serum concentrations of carotenoids in caucasian Americans (from Boston) and Chinese (from Shanghai) as observed by Yeum, et al. (1999).

Antioxidant	American N=56 μgm/dL	Chinese N=25 μgm/dL
Alpha tocopherol Gamma tocopherol Alpha-carotene Beta-carotene Lycopene Lutein + Zeaxanthin	947.1 ± 37.5 145.9 ± 37.5 4.8 ± 0.6 17.1 ± 1.5 32.2 ± 1.8 15.7 ± 1.0	690.4 ± 51.6 399.5 ± 63.6 0.9 ± 0.1 15.6 ± 2.6 4.8 ± 1.6 21.0 ± 2.7

 Table 1.7 Plasma content of lipid soluble antioxidants

Compiled from Yeum, et al. (1999)

1.5.3 Association of carotenoids with physiological and lifestyle factors

In addition to dietary factors, a number of physiological and lifestyle factors may influence the bioavailability of carotenoids. The health of an individual can affect carotenoid absorption and utilisation. Poor iron, zinc, and protein status has been shown to reduce the absorption, transportation and/or metabolism of the carotenoids (Williams, et al., 1998). In addition, intestinal parasites, malabsorption and liver and kidney diseases can reduce the absorption of carotenoids. Lower non-HDL cholesterol is associated with a low serum concentration of carotenoids (Brady et al., 1996; Erdman, et al., 1993), probably because carotenoids, especially hydrocarbon carotenoids, are transported in LDL.

Age, BMI, sex and lifestyle factors like smoking and alcohol intake also affect the concentration of carotenoids in human plasma. Lower alpha-carotene, betacarotene, beta cryptoxanthin, lutein and zeaxanthin levels are associated with younger age, male gender, high BMI, increased ethanol consumption and smoking (Brady, et al., 1996; Olmedilla et al., 1994; Scott et al., 1996). Interestingly, in a study by Yeum et al. (1996) at Boston, USA, older men had higher serum levels of carotenoids than older women in the 60 to 80 year age group.

Low levels of alpha-carotene, beta-carotene, beta cryptoxanthin, lutein and zeaxanthin in smokers and people drinking alcohol have been attributed to differences in eating habits rather than differential absorption and utilisation mechanisms between smokers and non-smokers and alcohol drinkers and non drinkers (Brady, et al., 1996). In contrast, Forman et al. (1995), reported an increase in alpha and beta-carotene concentrations in premenopausal women taking 30 gram alcohol per day for three menstrual cycles. Lutein and zeaxanthin levels were comparable in the two studies (Brady, et al., 1996; Forman, et al., 1995). Lycopene concentrations differ from other carotenoids in respect of the physiological factors that have an effect. Low concentrations of lycopene in older men have been reported by different studies (Brady, et al., 1996; Yeum, et al., 1996). There is no evidence of reduced serum response with ageing, which could be explained by the passive nature of the absorption (Castenmiller and West, 1998).

Seasonal changes may also have an impact on the concentration of carotenoids in human plasma. Scott, et al. (1996) reported increased plasma concentrations of lycopene, beta-carotene and lutein in summer and autumn in 50 to 65 years old women in the UK. Olmedilla, et al. (1994) did not find any significant change in zeaxanthin and lycopene levels in different seasons in either males or females. An increased proportion of alpha and beta-carotene in summer was reported in both the studies. Both sexes had an increased concentration of beta cryptoxanthin in winter (Olmedilla, et al., 1994). Different results for lutein were reported; Olmedilla, et al. (1994) observed increased concentrations in men in summer and women in spring, whereas Scott, et al. (1996) reported higher concentrations of lutein in women in the summer season.

1.5.4 Lycopene

Lycopene, an acyclic carotenoid, is thought to be the most potent antioxidant (Di Mascio et al., 1989; Mortensen et al., 1997) compared to other carotenoids. The overall quenching capacity of lycopene is about three times that of beta-carotene in the human plasma (Di Mascio, et al., 1989). According to Tinkler, et al. (1994), all carotenoids bound to membrane have a protective effect, with highest protection given by lycopene followed by beta-carotene. Sies and Stahl (1998) suggested that the increased reactivity of lycopene is related to the structure of the two additional non-conjugated double bonds compared with other C40 carotenoids. Mortensen, et al. (1997) on the other hand, argued that the rate of scavenging is dependent on the nature of the oxidising radical species, e.g. nitrogen dioxide NO⁻², thioyl RS⁻ sulphonyl RSO⁻ rather than the carotenoid structure.

Tomatoes and related tomato products are the major sources of lycopene in the human diet. Other sources of lycopene (**Table 1.8**) include watermelon, pink grapefruit juice and guava.

Food Product	Lycopene (µgm/100 gm)
Tomato- red, ripe, raw, year round	3,025
average	
Tomato-red, ripe, canned,	9,708
Whole, regular pack	
Tomato sauce, canned	15,916
Tomato Puree, canned salt added	16,670
Tomato paste- Canned, salt added	29,330
Tomato Juice- canned without salt	9,318
Grape fruit, raw, pink and red all	1,462
areas	

 Table 1.8 Lycopene concentration in different tomato products.

Compiled from USDA (1998)

The intake of lycopene in the population ranges from three mg/day to about 25 mg/day. In a ten-year follow-up population study of 46924 men and 77283 women aged 40 to 75 years, a food frequency questionnaire was used to calculate the average carotenoid intake of the subjects. Lycopene intake ranged from 3.7 mg/day to 18.2 mg/day in men and 4.4 mg/day to 14.7 mg/day in women in Ireland (Michaud et al., 2000). Carroll et al. (1999) reported an intake of 3.1 mg/day in men and 2.6 mg/day in women aged 24 - 45 years in the United States. In older men and women aged 54 - 65 years, the average lycopene intake was 2.2 mg/day and 2.3 mg/day respectively (Carroll, et al., 1999). However, Canadians have been shown to have higher lycopene intake from tomatoes and tomato products (Rao et al., 1998). In a study of the lycopene content of tomatoes and tomato products and their contribution to dietary lycopene, the lycopene intake of the study population was reported to be 25.2 mg/day/subject, with processed tomato products accounting for 50% of the total intake (Rao, et al., 1998). Lycopene from tomato products appears to be more bioavailable than from raw tomatoes (Gartner, et al., 1997; Stahl and Sies, 1992). The processing, presence of dietary fat and heat-induced transformation of trans isomer to cis isomer (Figure 1.8) of lycopene may enhance the lycopene bioavailability (Agarwal and Rao, 2000). The presence of other carotenoids may also have an effect on the bioavailability of lycopene (Johnson et al., 1997).



Figure 1.8 Structure of *trans* and *cis* isomers of lycopene

Several biological and lifestyle factors may affect the plasma levels of lycopene which is the most predominant carotenoid in the human plasma. Fasting serum lycopene levels have been found to be higher and more reproducible compared to the postprandial levels, indicating that the diet induces 'metabolic stress' (Rao and Agarwal, 1998). Metabolic stress in this context of the postprandial state presumably refers to the state of increased metabolism, which leads to increased production of free fatty acid radicals and hence more utilisation of antioxidants to prevent the chain reaction and further formation of free radicals. Men and women have similar serum lycopene levels, though the serum levels seem to decrease in the elderly (Yeum, et al., 1996). In women, the lycopene levels are found to be affected by the phase of the menstrual cycle with the peak during mid-lute phase (Forman, et al., 1996). Controversial results are available for the effect of smoking on serum lycopene levels (Mayne et al., 1999; Pamuk et al., 1994; Ross et al., 1995). Alcohol consumption has been found to alter the lycopene levels (Forman, et al., 1995).

Cardiovascular diseases are one of the major causes of mortality in countries like the United States and Australia. As discussed earlier in the document, lower levels of plasma and /or tissue lycopene are found to be associated with increased risk of CHD (Kohlmeier, et al., 1997; Kristenson, et al., 1997). The scientific evidence suggests that oxidation of LDL plays a key role in the causation of atherosclerosis, which is thought to be the major disorder leading to heart attacks and ischemic strokes (Steinberg and Witztum, 1990; Witztum, 1991). Antioxidant nutrients, with their ability to inhibit the oxidative modification of LDL, may slow the progression of atherosclerosis (Morris, et al., 1994; Parthasarathy et al., 1998).

A number of studies have been undertaken to investigate the effect of lycopene on the oxidation of LDL. Agarwal and Rao (1998) studied the effect of dietary lycopene from tomato juice, spaghetti sauce and tomato oleoresin for a period of one week each in 19 healthy individuals (25-40 years age). The consumption of tomato products was one or two serves a day. The LDL oxidation was measured by thiobarbituric acid reactive substance (TBRAS) and the conjugated diene method. The results showed decreased LDL oxidation, suggesting the role lycopene may play in the prevention of heart disease (Agarwal and Rao, 1998). In another study on smokers, Rao and Agarwal found that lycopene concentrations in the plasma decreased in people taking a restricted low lycopene diet, compared to the subjects adhering to their normal diet. The LDL oxidation increased in subjects on the restricted diet (Rao and Agarwal, 1998). In a short-term intervention study, a dietary intake of > 40 mg/day of lycopene by a group of non-smoking individuals significantly reduced the susceptibility of LDL to oxidation, whereas an equivalent increase in lycopene in a group of smokers showed no such effect (Chopra et al., 2000).

A crossover study by Sutherland, et al. (1999), in 15 renal transplant recipients, found no change in the susceptibility of LDL to oxidation after four weeks of supplementation with tomato juice (400ml per day), even though there was an increase in the plasma lycopene levels after 4 week supplementation, suggesting no effect of this increase in plasma lycopene on the oxidation of LDL. A study by

Dugas et al. (1999) compared the effect of supplementation of beta-carotene (15mg) and lycopene (34mg) on the endothelial cell mediated oxidation of LDL. After four weeks of supplementation with beta-carotene, the results exhibited an increase in plasma beta-carotene levels and a marked inhibition of oxidation of LDL *in vivo*, whereas supplementation with lycopene increased the plasma lycopene levels but no change was noted for LDL oxidation.

Though these results are not entirely convincing, there is substantial evidence that populations with higher serum/ tissue levels of lycopene reduces the risk of MI and angina. Also there is evidence that Mediterranean populations, who have a higher intake of lycopene, have higher life expectancies and lower rates of CHD (Kushi et al., 1985).

1.6 Vitamin E: Structure and sources

Vitamin E (**Figure 1.9**) is a generic term used to describe a group of 8 components that exhibit the biological activity of alpha tocopherol. These compounds are alpha, beta, gamma & delta tocopherol and alpha, beta, gamma & delta tocotrienol. All these compounds occur in a variety of isomers, and alpha tocopherol is thought to be the most active form of vitamin E. Alpha tocopherol has the superior antioxidant activity followed by beta tocopherol, gamma tocopherol and delta tocopherol as observed by Sies and Stahl (1995) in a study on quenching of singlet oxygen.

Vegetable oil is the primary source of vitamin E and the tocopherol content of the oil varies with the source. Corn and soybean oil have large amounts of gamma

tocopherol, whereas olive, canola, safflower and sunflower oils are rich in alpha tocopherol. Other sources of vitamin E include fruits, vegetables, nuts, eggs and peanut butter.



Figure 1.9 Structure of vitamin E

1.6.1 Absorption and metabolism of vitamin E

The absorption of tocopherols is inefficient, with only 20 - 40% of alpha tocopherol being absorbed (Rock, et al., 1996). The percentage of absorption decreases with the increase in dose (Rock, et al., 1996). Dietary fat may facilitate the absorption of vitamin E, because bile salts and pancreatic juices are thought to be necessary for its absorption. A lack of bile acids, due to biliary disease with obstruction to bile duct, chronic liver disease or lack fat digestive enzymes, damage to the gastrointestinal lining, or an inability to synthesise chylomicrons can decrease vitamin E absorption. The vitamin is incorporated into chylomicrons, which is then taken by liver. It is then released back into the circulation in association with VLDL. Like carotenoids, it seems there is

competition for absorption, as well as a relationship of different forms of tocopherol. Increased intake of alpha tocopherol decreases the tissue retention of alpha tocopherol (Handelman et al., 1994). Cystic fibrosis, biliary obstruction, and coeliac disease may reduce the absorption of vitamin E. Faecal elimination is thought to be the main excretion route of vitamin E as it is metabolised in very small amounts.

1.6.2 Tissue distribution, transport of vitamin E

Like carotenoids, vitamin E circulates in association with lipoproteins, mostly in LDL. The uptake of tocopherol by the tissues is dependent on the tocopherol intake and absorption. About 80 to 90% of alpha tocopherol is stored in adipose tissue. Handelman, et al. (1994) reported increased tissue storage of vitamin E with the supplementation of 800 mg of vitamin E per day for one year. However, the increase was a modest 10 - 20%. Adipose tissue vitamin E is not freely metabolised when plasma vitamin E is low. Movement of excess vitamin E from adipose tissue to plasma can occur, but is the consequence of the breakdown of adipose triglycerides, as would occur during energy restriction, rather than a response to low plasma levels of vitamin.

1.7 Research Aims

For years, nutritionists and dieticians have been promoting low fat high carbohydrate diets to reduce the risk of CHD. The dietary pyramid of the American Heart Association and National Heart Foundation (Australia) have suggested less than 30% of energy should come from dietary fat. Mediterranean populations, who have been found to have higher life expectancies and lower rates of CHD, follow a different diet. In the Mediterranean diet, more than 30% of energy is provided by dietary fat, the source of which is monounsaturated fat mainly from olive oil. There is substantial evidence that monounsaturated fat reduces the susceptibility of LDL to oxidation, hence potentially reducing the risk of atherosclerosis.

In addition, lycopene - an acyclic carotene (from tomatoes) - consumed in abundance by populations in Mediterranean countries, exhibits superior antioxidant capability to quench singlet oxygen compared to other carotenoids like beta-carotene (Di Mascio, et al., 1989; Krinsky, 1998a). Some studies have also suggested that lycopene decreases the susceptibility of LDL to oxidation.

With all this information discussed available, we had some questions which needed to be addressed - Is it possible that the combination of lycopene and monounsaturated fat will have an improved effect on three parameters - better serum lipid profile, increased serum lycopene, and increased resistance to LDL oxidation compared to the high carbohydrate diets? If high levels of lycopene in serum are associated with the reduced risk of CHD and its availability is dependent on the fat content in the diet, then what different effect does a high carbohydrate diet have on the serum levels of lycopene? How much lycopene is required in the diet to alter the serum levels of lycopene? How do monounsaturated fat rich and high carbohydrate, low fat diets affect LDL oxidation and where does vitamin E fit into this equation - does it double the effect of monounsaturated fats?

To find answers to some of these questions, separate studies in men and women were conducted given the hypothesis 'the combination of lycopene and monounsaturated fat diet (MUFA) may have an additional benefit by increasing serum lycopene levels, improving the serum lipid profile and subsequently increasing the resistance of LDL to oxidation compared to a high carbohydrate low fat (HCLF) lycopene enriched diet'.

The primary aim of the two studies was to:

- Investigate the effect of a MUFA diet enriched in lycopene on the serum lycopene and other carotenoid levels;
- Investigate the effect of a HCLF diet enriched in lycopene on the serum lycopene and other carotenoid levels;
- Compare the effects of a MUFA and a HCLF diet on the serum lycopene and other carotenoid levels.

The secondary aim of the thesis was to compare the effects of the two diets on the serum lipid and lipoprotein profile, and on the susceptibility of LDL to oxidation *in vitro*.

2.1 Study sample

Eighteen women and 14 men aged from 20 years to 70 years were recruited through personal contacts, advertisements in university newsletters, university notice boards and local newspapers. All the subjects who participated in the research project fulfilled the following criteria:

- Non-smokers;
- No history of cardiovascular, hepatic, gastrointestinal or renal disease;
- No antibiotic or supplemental vitamin and /or mineral use for ≥ four weeks before the start of the study; and
- Not following a weight loss regime.

Women were specifically excluded from the study if they were pregnant, were lactating, using hormonal replacement therapy, or had a history of menstrual irregularities. Women in the peri-menopausal age group (40 to 55 years) were also excluded from the study.

The protocol of the study was approved by Deakin University Ethics Committee, Melbourne, Australia. The study protocol was explained to all the participants and they gave their written, informed consent.
2.2 Study Design

This was a within subject randomised crossover dietary intervention study, to compare the effects of two different diets. The study was randomised to reduce any carry over or order effect. **Figure 2.1** presents the dietary protocol of the study for the female subjects. The dietary protocol of the study for the male subjects is shown in **Figure 2.2**.

2.2.1 Weighed food records

All the subjects were asked to record their food intake on their habitual diet at the start of the study for four days. These four-day records (habitual) were used to calculate the average daily energy intake of the subjects. The study diets were then based on each particular subject's average daily energy intake. Subjects were provided with both written and verbal instructions on how to complete the food record and were shown how to use the electronic food scales (Bosco, model 312, AND Medical Products, Kensington, Australia) that were provided. Where there was the possibility of subjects being unable to weigh or measure food items, the subjects were instructed to estimate quantities using household measures, or to select portion sizes from the photographs, which were included in the diet record book (Appendix A). The four day dietary records were assessed using Food Works version 2.01 using NUTTAB 95 (Xyris software, Brisbane, Australia). When foods were not present in the database (e.g. some oils and margarine, health foods), the nutrient compositions supplied by the manufacturer were manually added to the database, or foods with the closest composition were selected.

In addition to the four day weighed food records, the subjects were asked to complete a food frequency questionnaire (**Appendix B**) for their lycopene rich food intake to calculate the usual intake of lycopene on their habitual diets.



Figure 2.1 Dietary protocol for women

LCD – low carotenoid diet; MUFA-modified fat monounsaturated fat diet; HCLF- High carbohydrate low fat diet Wt – weight; Ht- height ; W circ- waist circumference; H circ- hip circumference;



Figure 2.2 Dietary protocol for men

2.2.2 Diets

The subjects received the modified fat, monounsaturated fat enriched (MUFA) diet and high carbohydrate low fat (HCLF) diet with a 'washout' period of 6 weeks in between. The order of the diets was randomly allocated. The diets for women were each of 16 days duration and for men of 14 days duration. A set menu plan was designed for each person for the two dietary periods (MUFA and HCLF), which were isocaloric to the habitual diets and contained the same basic foods but different amounts of fats. An example of the menu is shown in Appendix E. All the subjects were asked to take a low carotenoid diet (LCD) for two to three days prior to starting the two dietary periods. This LCD was to avoid the acute peaks of carotenoids, which may occur after 10-12 hours of the intake of carotenoid rich food (Porrini, et al., 1998). A list of acceptable and non-acceptable foods was given to the subjects (**Appendix C**).

Both the MUFA and the HCLF diets were designed to have a similar carotenoid content, especially of lycopene. As fruits and vegetables are the major sources of carotenoids, in order to control their intake, a limited amount and variety of fruits and vegetables were allowed in the two diets. The lycopene source was in the form of tomato soup (300 gram) every alternate day, with 125 gram of guava nectar in breakfast and 100 gram of cooked canned tomatoes in dinner for women. For men the lycopene intake was in the form of one 300 gram ready to serve tomato soup, and 60 gram of tomato paste every day. To avoid seasonal and processing differences that may affect the carotenoid content of foods, tomato soup, canned tomatoes and tomato paste from the same batch provided by H. J. Heinz Australia (Melbourne, Australia) were used for the study. Meadowlea

Foods Ltd (Mascot, Australia) provided the cooking oil and margarine (one batch) for the duration of the study. The subjects were asked to buy frozen vegetables (peas and corn mix) of the same brand for the two dietary periods. For the study in women, canned tomatoes were cooked in the university food laboratory, portioned, frozen at -20°C and were given to the subjects for the dietary periods. A single batch of guava nectar was bought for the study in women. The male subjects were given detailed instructions and recipes (**Appendix D**) on the cooking of tomato paste and heating of tomato soup. Fruit intake was restricted to one piece a day, either an apple or a banana in both the studies.

An oleate enriched variant of sunflower oil (SunolaTM oil) was used for the MUFA diet. The composition of monounsaturated fat in SunolaTM oil is 77.8%, compared to 19.5% in conventional sunflower oil (e.g. Crisco oil), the oil used for the HCLF dietary period. The diets were designed to provide 36% to 38% of energy from fat in the MUFA diet (approximately 68% of fat from monounsaturated fat) and 16% to 18% of energy from fat in the HCLF diet. The carbohydrate content of the two diets was designed to be 42% and 64% of energy in the MUFA and the HCLF diets, respectively. The protein content of both the diets was designed to be 15% to 18% of the total energy. The diets were designed to have similar amounts of cholesterol, fibre, and vitamin C content. Intake of alcoholic drinks was limited and subjects were asked to avoid the intake of red wine.

The energy balance was maintained by using non-carotenoid containing foods such as bread, carbonated beverages and polyjoule (Nutricia, Australia), a glucose polymer, in the HCLF diet. Polyjoule contains maltodextrin and provides 1615kJ per 100g. It is a sucrose free, fat free source of carbohydrate. Toasted muesli and biscuits made with Sunola[™] oil were given to the subjects on the MUFA diet.

2.2.3 Blood specimen collection

The women had fasting venous blood samples taken on the day one and day 17 of the two dietary periods. For men, the fasting venous blood samples were taken on day one and day 15 of the two dietary periods. All subjects were asked to fast for ten to 12 hours, during which only drinking water was allowed. Venous blood samples were collected between 7.30 a.m. and 9.30 a.m. Subjects were rested for ten minutes on arriving at the university before the blood sample was taken to reduce stress. Blood was always collected from the arm with the subject in the lying position, using a 21-gauge needle and minimal constriction of the vessels by a tourniquet. Blood samples were collected in three different types of tubes; a) one containing anticoagulant lithium heparin; b) one containing anticoagulant ethylenediamine tetraacetic acid (EDTA) and; c) plain tubes without any anticoagulant.

Plasma was separated from the blood collected into lithium heparin and EDTA tubes by immediate centrifugation at 3000 revolutions per minute (rpm) at 4°C for 20 minutes in a GS-6R Centrifuge, (Beckman Instruments Inc. USA). For serum separation, the blood was allowed to coagulate for one hour and then

centrifuged at 3000 rpm at 4°C for 20 minutes. Both plasma and serum were aliquotted and stored at -80°C for later analysis. All biochemical analyses were subsequently carried out in the same run to reduce inter-assay variability.

2.2.4 Anthropometric measurements

Weight, height, waist and hip measurements were taken at the start of the first dietary period. During the study, it was emphasised that the subjects were to maintain their baseline weight and it was measured on every visit to the university. Weight was measured in minimal light clothing and no shoes on a Seca electronic balance (Model 708 Mannheim, Germany) to the nearest 0.1kg. Height was recorded by using a wall hanging stadiometer (Seca, Mannheim, Germany) to the nearest 0.1cm. BMI was calculated as weight(kg)/ height (m²). Waist and hip measurements were taken using a flexible metric tape measure in light clothing to the nearest 0.1cm. Waist measurement was determined as the smallest circumference between the bottom of the 12th rib and top of the iliac crest. Hip measurement was determined as the maximum circumference around the top of the great trochanters according to the recommendations of WHO (Document, 1988). Waist to hip ratio was then calculated. These measurements were always taken by the same person to minimise measuring errors.

2.2.5 Dietary Compliance

To assess the dietary compliance of the subjects, diets were discussed with them over the telephone at least once a week and at the time of their visit to the university for fasting blood samples in each dietary period. The subjects were asked to report if they felt hungry, too full or satisfied on the diets, and changes in the diets were made accordingly. Energy was also adjusted if body weight had significantly changed. At the end of the two dietary periods, the subjects were asked to record their diets for four days for analysis purposes, and this also provided information on compliance.

2.2.6 Geelong osteoporosis study (GOS)

Data from the food frequency questionnaire used in GOS, a population study conducted on women in Geelong (Victoria, Australia), was analysed on Diet 4 (version 4.22) software using NUTTAB 95 (Xyris software, Brisbane, Australia), to calculate the intake of serves lycopene rich foods, which were then analysed by using USDA carotenoid database to calculate the usual (habitual) intake of lycopene of the women in different age groups. The data was used to compare the lycopene intake between women from the general population and the women who participated in the present study.

2.3 Biochemical analysis

2.3.1 Carotenoids

A high pressure liquid chromatography (HPLC) method, developed by Dr. Su Qing (Su et al., 1999), which is a modification of the method previously published (Khachik, et al., 1992), was used for the analysis of serum carotenoids and lycopene content in tomato products. The assay was performed by Su Qing who was blinded to the nature of the specimen. The assay was performed in a dark room under red light.

Internal standard

Tocopherol acetate was used as the internal standard for carotenoids and tocopherol, while retinyl acetate was used as the internal standard for retinol. The individual internal standard stock solutions were kept in brown bottles at -20°C and mixed before the assay of serum samples.

Stock solutions

The stock solutions were prepared using alpha-carotene, beta-carotene, *trans* lycopene, lutein + zeaxanthin and cryptoxanthin. The dissolution of alphacarotene, beta-carotene and *trans* lycopene was made in one ml of dichloromethane, which was then diluted with n-hexane containing 0.01% butylated hydroxytoluene (BHT) into brown 15ml bottles. For the stock solution of alpha-carotene, beta-carotene, lutein + zeaxanthin and cryptoxanthin, n-hexane (0.01% BHT) was used as the solvent. Retinol, retinol acetate, alpha tocopherol, gamma tocopherol and tocopherol acetate were dissolved in 95% ethanol. All stock solutions were prepared under red light and stored under nitrogen at -20°C. Working standards of each compound were prepared from the stock solution and the standard dilutions were made by diluting with hexane or methanol.

Two ml of every antioxidant standard was pipetted into a 20ml brown, glass bottle. Duplicate aliquots of 200 μ L, 400 μ L, 800 μ L, 1600 μ L, 2000 μ L of this mixed standard solution were placed in 13 x 100mm borosilicate glass tubes and evaporated under a stream of nitrogen at room temperature. The duplicate dry tubes were vortexed for one minute with 200 μ L of 95% ethanol containing alpha tocopherol acetate 200 μ g/ml) and retinyl acetate (5.7 ng/ml) as the internal standard. The standard solutions were then extracted with hexane (one ml) containing 0.01% BHT dried under nitrogen and reconstituted as described for serum samples in the next section. Standard curves were established by using known concentrations of the working solutions of the antioxidants.

Serum carotenoids

Serum samples which had been stored at -80°C were thawed to room temperature in the dark. All the samples were analysed in the dark under red light. 200 μ L of internal standard containing alpha tocopherol acetate and retinyl acetate in 95% ethanol solution was added to 200 μ L of the serum sample and vortexed for one minute, to denature the proteins. Then one ml of hexane was added and the sample was vortexed for one minute. To separate the phase containing all the carotenoids and tocopherols, the sample was centrifuged at 2500 rpm for ten minutes, and the supernatant was separated and dried under nitrogen stream. The sample was reconstituted with 40 μ L of chloroform, vortexed for one minute and then 80 μ L of acetonitrile: methanol was added and vortexed again for one minute. The sample was then transferred to amber coloured vials for analysis by HPLC.

The solvents in mobile phase were acetonitrile/methanol/chloroform (45:45:10, v/v/v) containing 0.05% ammonium acetate in methanol, and 0.1% trietylamine in the acetonitrile at the flow rate of one ml/min. A 50µL injection of the sample was used for the carotenoid and tocopherol analysis. Carotenoids were monitored at 450 nm, and tocopherol and retinols were monitored at 292 nm. The results

generated were then put on the standard curves to calculate the concentration of the carotenoids in the serum.

LDL isolated for the oxidation assay was frozen at -80°C and then used to analyse the carotenoid content of the LDL by the same method as used for serum.

Chromatography conditions

The chromatographic system used for carotenoid identification included the Waters Alliance 2690 separations module, Waters 996 photodiode array detector and the Millennium Chromatography Manager PDA software (Waters, Melbourne, Australia). A UV-visible spectrophotometer (Hewlett-Packard) was used for the determination of the concentration of standard solutions. All mobile phase solvents were prefiltered and degassed through 0.45um millipore filter discs and vacuum millipore filters. **Figure 2.3** shows a chromatograph of serum carotenoids obtained for a male subject on the usual diet.



Figure 2.3 Serum carotenoid chromatograph

Carotenoid analysis of tomato products.

Tomato soup, tomato paste and canned tomatoes were analysed for their carotenoid content by Su Qing, using the method developed by her (Su, et al., 1999). One gram of food sample was homogenised in a food processor (Magimix, compact 3100, Australia) and one ml of internal standard, prepared by dissolving β -apo-8-carotenal in hexane with 0.01% of BHT, was added followed by magnesium carbonate and chloroform: methanol (2:1; 10ml) containing 0.01% BHT. The solution was kept in the dark for thirty minutes. The extract was then filtered under suction and the solid material was extracted repeatedly with chloroform: methanol (2:1, 10ml) until the resulting filtrate was colourless. The solution obtained was then transferred to a separation funnel and washed with water (2 x 20ml) to remove the water soluble material. Organic phase was collected, dried over anhydrous sodium sulphate and then the solution was dried under nitrogen. The residue was reconstituted with 200µL of solvent acetonitrile/methanol/chloroform (70µL: 70µL: 60µL). The reconstituted sample was then transferred to amber vials and read through HPLC. In HPLC 50µL and 10µL of each sample was injected and the results obtained with the higher resolution were used for analysis.

2.3.2 Lipids and Lipoprotein

Total Cholesterol and Triglycerides

Fasting serum total cholesterol and triglycerides were measured by an enzymatic colourimetric test on a centrifugal autoanalyser (Hitachi Boehringer Mannheim Automatic Analyser 704, Japan), using commercially available enzymatic calorimetric kits (Boehringer Mannheim, Melbourne, Australia). CHOD-PAP

reagent was used for total cholesterol analysis and GPO-PAP reagent for the triglycerides. Calibration of the auto analyser was undertaken using a calibrator for automated systems (CAS). Precinorm L and precipath L were used as normal and pathological quality controls respectively (Boehringer Mannheim, Melbourne, Australia).

Serum stored at -80°C was thawed and 200 μ L of sample was used for total cholesterol and triglycerides. Samples and 200 μ L of control were each placed into the autoanalyser cups and absorbencies were spectrophotometerically read by the autoanalyser at 540 nm. The autoanalyser combines 5 μ L of sample with one ml of cholesterol and triacylglycerol reagent, the mixture then undergoes a colour reaction. The red colour formed is read spectrophotometerically.

HDL Cholesterol

After precipitating all serum lipoproteins except high density lipoprotein (HDL) cholesterol with polyethylene glycol 6000 (PEG 6000), HDL cholesterol was determined by enzymatic colourimetric reaction and read on the Hitachi autoanalyser, using a cholesterol reagent. The standard for calibration and high and low controls were from QChem HDL - cholesterol kits (Special Diagnostics, Melbourne, Australia). Two hundred μ L of sample and control were thawed, mixed with PEG 6000 and allowed to stand for ten minutes, then spun at 15000 rpm for three minutes in a Hernle z-229 centrifuge (MEDOS Company Pty Ltd, Melbourne, Australia) to form a pellet. The supernatant containing HDL-C was transferred to the autoanalyser cup and read spectrophotometerically.

LDL cholesterol was calculated using the Friedewald equation (Friedewald et al., 1972):

LDL cholesterol (mmol/L) = Total cholesterol – HDL cholesterol – $\frac{\text{triglycerides}}{2.3}$ Triglycerides / 2.3 represents the concentration of very low density lipoprotein in the serum. The ratio of LDL to HDL cholesterol was also calculated.

2.3.3 Oxidation of LDL

Plasma separated from the blood collected in EDTA tubes was used for the LDL oxidation assay. The assay was performed with the help of Dr. Emma Ashton, who had set up this method for her PhD thesis (Ashton, 2000). Four ml of thawed plasma was added to 1.526g of potassium bromide and mixed gently until dissolved, then 1.7ml of plasma was added to an ultracentrifuge tube, overlaid with 3.3ml of buffer (8.76g/L sodium chloride and 1.0g/L EDTA). Ultracentrifuge tubes were heat sealed and centrifuged at 100,000 rpm at 15°C for two hours in a Beckman TLX-100 ultracentrifuge.

After centrifugation, distinct bands of lipoproteins were visible and the LDL band was extracted through the side of the tube using a 25-gauge needle and syringe. To remove the EDTA, the isolated LDL was transferred to dialysis tubing and immersed in phosphate buffer (2.271g of di-Sodium hydrogen orthophosphate (Na₂HPO₄), 0.544g potassium dihydrogen orthophosphate (KH₂PO₄) and 8.766g of NaCl to one litre of distilled water and pH at 7.4 and purged with nitrogen for 20 minutes). The sample was then dialysed with two changes of buffer for the maximum removal of EDTA.

Sigma Bicinchoninic Acid protein assay kits, using the Lowry method (Lowry, 1985), were used to analyse the protein content of the LDL sample. The amount of sample required to supply 50µg of protein was then calculated. The calculated amount of LDL sample was added to oxygenated phosphate buffer solution (purged with oxygen for 20 min) to make a final volume of two ml and then 32μ L of 1mM CuCl₂ was added to make the final copper concentration of 16 μ M (Sattler et al., 1991). The oxidation reaction was then followed at 234nm at 37°C on a UV-1601 Shimadzu spectrophotometer with a six cell Shimadzu CPS - controller multisampler, with absorbance recorded at two minute intervals until the products reached maximum concentration and started decomposing. A graph (**Figure 2.4**) was plotted from these readings and the lag phase was calculated.



Figure 2.4 LDL oxidation kinetics graph

Maximum diene formation and rate of oxidation were calculated using the following equations:

Maximum diene concentration (nmol diene/ mg LDL protein) = $\Delta Abs \ge 10^9$ 2.95 $\ge 10^4 \ge 50$

where 50 refers to the protein concentration.

Oxidation rate (nmol diene/ mg LDL protein/ min) = $\frac{\Delta \text{ Abs} / \Delta \text{ time x } 10^9}{2.95 \text{ x } 10^4 \text{ x } 50}$

where the Δ time refers to the difference in time from the start of the propagation phase and the end of the propagation phase.

2.4 Statistical Analysis

Statistical package for social scientists (SPSS, version 9, 1999, Chicago, USA) was used for the analysis of all the data obtained. The data was first assessed for normality of distribution. If the data was not normally distributed, log transformation was performed. Non-parametric tests were performed if the data was not normally distributed even after log transformation. All data are presented as mean \pm standard deviations (SD), except for carotenoids where data are presented as mean \pm standard error of mean (SEM) as well as standard deviation (SD). Though SD is a better way of presenting results but for comparison SEM was used for carotenoids, as most of the data in literature have used SEM rather than SD. The p value of < 0.05 was taken as statistically significant. General linear model (GLM) was used to investigate the overall carry over and order effect of the two diets (Fliess, 1986). Pearson's correlation coefficient for non-normally distributed data were used to identify any relationship between different

variables. Mean differences with 95% confidence intervals of the means were calculated for values in the two diets in both the studies. Bivariate correlation was checked for serum lipids and serum carotenoids, lycopene intake and serum lycopene levels.

3.1 Baseline Characteristics

One of the fourteen males commencing the study withdrew for personal reasons, and thirteen completed the study. For the study in women, all eighteen women completed the study. It was difficult to compare the post and pre-menopausal group separately because the number of subjects in the postmenopausal group was small; hence the results are presented as one study group. The descriptive characteristics of the subjects are given in **Table 3.1**

Variable	Men N=13	Women N=18	Premenopausal N=12	Post- menopausal N=6
Age (years)	39.0 ± 11.0	40.4 ± 16.6	30.0 ± 7.0	61.1 ± 7.0
Weight (kg)	82.5 ± 8.6	$60.2\pm~9.9$	57.0 ± 8.0	66.7 ± 10.5
BMI (kg/m ²)	$25.5\pm~2.4$	23.2 ± 4.1	$21.8\pm~2.6$	$26.1\pm~5.2$
Total cholesterol (mmol/L)	5.5 ± 0.6	5.0 ± 1.3	$4.5\pm\ 0.9$	6.1 ± 1.2
HDL cholesterol (mmol/L)	1.1 ± 0.2	1.5 ± 0.7	1.4 ± 0.5	1.8 ± 0.9
LDL cholesterol (mmol/L)	3.8 ± 0.8	3.1 ± 1.0	2.7 ± 0.8	4.1 ± 1.0
Triglycerides (mmol/L)	1.8 ± 1.0	1.0 ± 0.3	0.9 ± 0.4	1.0 ± 0.1
LDL:HDL	3.6 ± 0.8	2.3 ± 1.0	2.2 ± 0.9	2.6 ± 1.1

Table 3.1 Baseline characteristics of the study sample¹

¹ Mean \pm SD

3.2 Diets

Seven of the 13 men and nine of the 18 women began the study with the MUFA diet. The body weight of the women at the baseline of the two diets was similar. A significant decrease in their body weight was observed on the HCLF diet whereas no change was noted on the MUFA diet. For the men, the body weight of the group was similar at the baseline of the two diets and there was no significant change on either diet. No significant change in BMI or waist to hip ratio was noted in either of the groups between the two dietary periods.

Table 3.2 and **Table 3.3** present the mean \pm SD nutrient intake in women and men respectively. The nutrient intake was calculated from the last four day dietary records of the subjects on both the HCLF and the MUFA diets.

3.2.1 Women

The energy intake on the HCLF diet was lower than on the MUFA diet. There was no significant difference in the protein intake on the two diets. The total fat intake was statistically higher and carbohydrate intake was lower on the MUFA diet compared to the HCLF diet, as designed to test the hypothesis. The monounsaturated fat intake was 5.8 times higher on the MUFA diet compared to the HCLF diet. The intake of saturated fat was higher on the MUFA diet compared to the HCLF diet. There was no statistically significant difference in the total amount of polyunsaturated fat intake on the two diets.

The total sugar, starch and fibre intake was higher on the HCLF diet compared to the MUFA diet. There was no difference in alcohol, cholesterol and vitamin C intake on the two diets.

3.2.2 Men

Energy intake on the MUFA diet was significantly higher than on the HCLF diet. There was no difference in the grams of protein consumed, but the percentage of energy provided by protein was significantly higher on the HCLF diet compared to the MUFA diet. As designed, the carbohydrate intake was higher and total fat intake was lower on the HCLF diet. The total sugar and starch intake was significantly higher on the HCLF diet compared to the MUFA diet.

The total fat intake on the MUFA diet was 2.6 times higher than on the HCLF diet. The polyunsaturated to saturated fat ratio was 0.93 on both the diets, though the grams of polyunsaturated fat and saturated fat were higher on the MUFA diet compared to the HCLF diet. Monounsaturated fat intake was significantly higher (about 6 times) on the MUFA diet compared to the HCLF diet. The intake of alcohol, fibre, dietary cholesterol and vitamin C were similar on the two diets in men.

Nutrient N = 18	HCLF Mean ± SD	MUFA Mean ± SD
Energy (MJ)	7.6 ± 1.4	8.4 ± 1.3*
Protein(gm) % energy	89.1 ± 17.5 19.8 ± 1.5	85.5 ± 17.2 17.4 ± 1.8
Total fat (gm) % of energy	33.6 ± 4.7 16.6 ± 1.9	$86.0 \pm 11.8^{*}$ $38.2 \pm 2.1^{*}$
Carbohydrate (gm) % of energy	$286.6 \pm 56.8 \\ 60.0 \pm 2.9$	219.7 ± 37.5 $42.0 \pm 2.2*$
Saturated Fat (gm) % of total fat	9.0 ± 2.0 30.4 ± 3.2	$14.3 \pm 2.4*$ $17.1 \pm 1.3*$
Monounsaturated fat (gm) % of total fat	$\begin{array}{rrr} 10.3 \pm \ 1.76 \\ 35.2 \pm \ 2.3 \end{array}$	$59.3 \pm 8.2*$ $71.9 \pm 1.4*$
Polyunsaturated Fat (gm) % of total fat	10.1 ± 0.9 34.4 ± 4.3	9.1 ± 1.2 $11.0 \pm 0.6*$
Fibre (gm)	$27.8\pm~5.6$	$24.7 \pm 4.3*$
Cholesterol (mg)	201.4 ± 76.7	206.7 ± 70.9
Vitamin C (mg)	81.8 ± 18.9	82.2 ± 17.0
Total sugars (gm)	143.1 ± 41.2	97.7 ± 22.4*
Starch	140.4 ± 28.1	119.2 ± 19.3*

Table 3.2 Average nutrient intake in women on HCLF and MUFA diets¹

¹Based on the last 4 days weighed food intake records of each diet. *p<0.05, significantly different from HCLF diet

Nutrient N=13	HCLF Mean ± SD	MUFA Mean ± SD
Energy (MJ)	10.2 ± 1.2	11.4 ± 1.9*
Protein (gm) % energy from protein	109.6 ± 19.7 18.1 ± 1.6	106.3 ± 20.8 $15.9 \pm 1.2*$
Total fat (gm) % energy from fat	39.2 ± 8.0 14.2 ± 2.8	$116.4 \pm 18.1*$ $37.9 \pm 2.2*$
Carbohydrate (gm) % energy from carbohydrates	412.9 ± 50.9 64.6 ± 3.3	$303.8 \pm 57.4*$ $42.6 \pm 2.7*$
Saturated Fat (gm) % of total fat	12.1 ± 3.4 34.3 ± 4.6	$19.4 \pm 2.9*$ $17.4 \pm 1.7*$
Monounsaturated fat (gm) % of total fat	11.6 ± 2.6 33.4 ± 1.8	$74.9 \pm 12.8^{*}$ $66.6 \pm 2.0^{*}$
Polyunsaturated Fat (gm) % of total fat	11.1 ± 2.4 32.3 ± 4.7	$18.0 \pm 2.5^*$ $16.1 \pm 2.2^*$
Fibre (gm)	$32.2\pm~3.8$	$32.0\pm~5.6$
Cholesterol (mg)	174.6 ± 42.0	206.9 ± 56.1
Alcohol (% of energy)	0.32 ± 0.6	1.5 ± 2.2
Total sugars (gm)	179.9 ± 35.0	131.6 ± 31.3*
Starch (gm)	215.7 ± 43.8	169.7 ± 31.4*

Table 3.3 Average nutrient intake in men on HCLF and MUFA diet¹

¹Based on the last 4 days weighed food intake records of each diet. *p<0.05, significantly different from HCLF diet

3.3 Usual diet and serum retinol, tocopherol and carotenoids

The serum carotenoid levels of men and women on their habitual diet are presented in **Table 3.4**

Parameter	Women	Men
(µmol/L)	N=16	N=8
Lutein + zeaxanthin	0.37 ± 0.03	0.28 ± 0.02
Cryptoxanthin	0.17 ± 0.03	0.17 ± 0.03
Total Lycopene	0.52 ± 0.07	0.41 ± 0.01
Alpha-carotene	0.13 ± 0.02	0.12 ± 0.01
Beta-carotene	0.39 ± 0.04	0.37 ± 0.03

Table 3.4 Serum carotenoid levels on usual diet¹

¹values are (Mean \pm SEM)

The habitual diet serum lycopene levels in men (r = 0.74, p = 0.03) and premenopausal women (r = 0.62, p = 0.04) were correlated (Spearman's correlation) to the usual lycopene intake. Though the correlation was seen for the dietary lycopene intake and usual serum lycopene concentrations in the postmenopausal women (r = 0.81) but it did not reach significance (p = 0.10). This may be because of the smaller group (n = 6). **Table 3.5** presents the calculated lycopene intake from the food frequency questionnaire and serum lycopene levels of the subjects. The lycopene levels were calculated using the food carotenoid database developed by Chug Ahuja et al. (1993) and Mangels et al. (1993) and updated by USDA (1998).

	Lycopene intake	Serum lycopene
	mg/day	μmol/L
Premenopausal women (n=11)	4.71 ± 1.15 (3.83)	$0.55 \pm 0.09 \; (0.32)$
Range	0.50 - 14.12	0.11 – 1.35
Postmenopausal women (n=5)	4.65 ± 0.28 (0.62)	$0.46 \pm 0.06 \; (0.15)$
Range	4.09 - 5.64	0.33 - 0.69
Men (n=8)	$9.99 \pm 0.59 \ (1.67)$	0.41 ± 0.02 (0.07)
Range	8.01 - 12.85	0.34 - 0.52

Table 3.5 Calculated lycopene intake and serum lycopene levels on usual diet¹

¹ mean \pm SEM (SD)

Table 3.6 presents the results of lycopene intake calculated from the food frequency questionnaire in women from the GOS study and the present study subject group.

Study group	Lycopene	Range
Mean ± SEM	(mg/day)	
30-39 years ¹ (n=76)	5.66 ± 0.59	0.07 - 35.41
40-49 years ¹ (n=71)	3.97 ± 0.43	0.15 - 18.91
50-59 years ¹ (n=72)	4.70 ± 0.49	0.03 - 19.72
60-69 years ¹ (n=69)	3.50 ± 0.33	0.00 - 11.88
Premenopausal ² (n=11)	4.71 ± 1.15	0.50 - 14.42
Postmenopausal ² (n=5)	4.65 ± 0.28	4.09 - 5.64
$Men^2(n=8)$	9.99 ± 0.59	8.01 - 12.85

Table 3.6 Lycopene intake on usual diet

¹ data from the GOS study

² results from the present study group.

Due to the unavailability of the Australian database on the carotenoid content of the different fruits and vegetables, the usual carotenoid intake (except lycopene) of the study group was not assessed. At present, American database is available for this purpose (USDA). However the carotenoid content of the foods depends on the season, variety, cultivator, soil etc and it is not possible to use data from another country for this kind of information. USDA database was used for the calculation of lycopene, for the reason that lycopene is present in very small number of foods - only tomatoes and tomato products. However, to check the difference, the lycopene content of the food products (tomato paste, tomato soup, canned tomatoes) used in the present investigation (analysed in duplicate by HPLC) and the results available from USDA are presented in **Table 3.8** to show a comparison.

3.3.1 Lycopene intake during dietary intervention periods

The average lycopene intake by the women in the present study intervention period was 15.9 mg/day. This was achieved by ingesting 125 gram of guava nectar and 100 gram of canned tomatoes every day. In addition, 300 gram of tomato soup was taken every alternate day. In men, the average daily intake of lycopene was 20.2 mg/day, which was taken in the form of 60 gram of tomato paste and 300 gram of tomato soup every day. **Table 3.7** shows the daily distribution of lycopene rich food intake during the intervention period in both women and men. **Table 3.8** shows the analysed lycopene content of the foods used in the present study, compared to the lycopene content of the foods available from USDA (1998) database.

Product	Serving	Wa	omen	Men
	size	Day	Day	Everyday
		1,3,5,7,9,11,	2,4,6,8,10,12,	
		13,15	14,16	
Guava nectar	125 gm	1.5	1.5	
Tomato soup	300 gm	15.5		15.5
Canned tomato	100 gm	6.7	6.7	
Tomato paste	60 gm			4.7
Total (mg)		23.7	8.2	20.2

Table 3.7 Lycopene content of the study diets in women and men¹

¹HPLC analysis – all samples were analysed in duplicate

|--|

Product	USDA	Present study ¹
	µgm/100 gm	μgm/100 gm
Tomato Soup	$10,920^2$	5,179 ³
Canned Tomato	9,708	6,723
Tomato Paste	29,330	7,895
Guava Nectar	NA	1,541

¹All samples were analysed in duplicate by HPLC ² values are for condensed soup ³ values are for ready to serve tomato soup

3.3.2 Effect of MUFA and HCLF diets on serum retinol, tocopherol and carotenoids

Serum carotenoid and alpha tocopherol concentrations may be associated be associated with serum lipid concentrations as these antioxidants are carried predominantly in the lipid fractions like LDL and HDL in variable amounts. Conclusive data is not available if the corrections of the carotenoids should be made with the total serum cholesterol levels or other fractions or should be left uncorrected. For this thesis work, serum carotenoids were adjusted for cholesterol for both the study groups. Adjusting these variables did not significantly affect results or correlations compared to the analysis without correcting; hence the uncorrected data is presented.

3.3.2.1 Women

The baseline values of serum retinol, alpha tocopherol, gamma tocopherol and carotenoids were not statistically different on the two diets (Table 3.9). There was no significant change in retinol, cis-lycopene and lutein + zeaxanthin from baseline to day 17 on the HCLF or the MUFA diet. Serum levels of gammatocopherol, cryptoxanthin, alpha-carotene and beta-carotene decreased significantly from baseline to day 17 on both the diets. The percentage drop in gamma-tocopherol was 34.5% on the HCLF and 38.1% on the MUFA diet. Serum cryptoxanthin levels decreased by 23.8% on the HCLF diet and by 16.7% on the MUFA diet. Alpha-carotene was reduced by 26.6% on the HCLF and by 20% the MUFA diets, respectively. Beta-carotene decreased by 17.7% on the HCLF diet and by 13.6% on the MUFA diet. Alpha-tocopherol reduced significantly by 10.8% on the HCLF diet. No change in alpha tocopherol was observed on the MUFA diet.

No significant change was observed in the serum levels of *trans*, *cis* and total lycopene levels on the HCLF diets. Compared to the baseline, serum *trans* and total lycopene increased significantly on the MUFA diet. The increase in the *trans* lycopene was by 47.1% and in total lycopene by 40.7% on the MUFA diet compared to the baseline. No change was noted in the *cis* lycopene levels after the MUFA diet.

Comparing the day 17 results of the two diets, alpha tocopherol and lutein + zeaxanthin were significantly higher at the end of the MUFA diet compared to the HCLF diet.

There was no significant difference in the serum retinol, gamma-tocopherol, cryptoxanthin, alpha-carotene and beta-carotene levels. No significant difference was seen in the serum *trans*, *cis* and total lycopene levels at the end of the two diets

The carotenoid content of the isolated LDL was also compared at the end of the two dietary periods. No significant difference was seen in any of the carotenoid or tocopherol levels.

N=18	Baseline	HCLF Day 17	mean	Baseline	MUFA Day 17	mean
(µmol/L)	(Mean	\pm SEM)	difference	(Mear	n ± SEM)	difference
Retinol (SD)	$\begin{array}{c} 2.15\pm0.12\\ 0.51\end{array}$	$\begin{array}{c} 2.05\pm0.12\\ 0.50\end{array}$	-0.10 ± 0.06	$\begin{array}{c} 2.07\pm0.13\\ 0.54\end{array}$	$1.98 \pm 0.12 \\ 0.51$	-0.08 ± 0.09
Gamma tocopherol (SD)	$\begin{array}{c} 1.68\pm0.18\\ 0.75\end{array}$	$1.10 \pm 0.13 * $ 0.55	-0.58 ± 0.13	$\begin{array}{c} 1.60 \pm 0.15 \\ 0.65 \end{array}$	$0.99 \pm 0.13* \\ 0.54$	-0.60 ± 0.16
Alpha tocopherol (SD)	$\begin{array}{c} 22.29\pm1.61\\ 6.84\end{array}$	$\begin{array}{c} 19.85 \pm 1.70 * \\ 7.17 \end{array}$	-2.44 ± 0.76	$\begin{array}{c} 21.89 \pm 1.57 \\ 6.65 \end{array}$	$22.33 \pm 1.74^{*}$ 7.40	0.44 ± 0.91
Lutein + zeaxanthin (SD)	$\begin{array}{c} 0.30\pm0.02\\ 0.06\end{array}$	$\begin{array}{c} 0.30 \pm 0.02 \\ 0.07 \end{array}$	-0.01 ± 0.01	$\begin{array}{c} 0.33 \pm 0.03 \\ 0.12 \end{array}$	$0.32 \pm 0.02^{*}$ 0.09	-0.01 ± 0.02
Cryptoxanthin (SD)	$0.21 \pm 0.03 \\ 0.13$	$0.16 \pm 0.05*$ 0.20	-0.04 ± 0.02	$\begin{array}{c} 0.18\pm0.03\\ 0.15\end{array}$	$0.16 \pm 0.04*$ 0.18	-0.03 ± 0.02
<i>Trans</i> lycopene (SD)	$\begin{array}{c} 0.18\pm0.03\\ 0.11\end{array}$	$0.21 \pm 0.03 \\ 0.11$	0.03 ± 0.02	$\begin{array}{c} 0.17 \pm 0.02 \\ 0.09 \end{array}$	$0.25 \pm 0.03 * $ 0.12	0.08 ± 0.03
Cis-Lycopene (SD)	$\begin{array}{c} 0.11 \pm 0.02 \\ 0.08 \end{array}$	$\begin{array}{c} 0.12\pm0.02\\ 0.07\end{array}$	0.01 ± 0.01	$\begin{array}{c} 0.10 \pm 0.02 \\ 0.07 \end{array}$	$0.13 \pm 0.01 \\ 0.05$	0.02 ± 0.1
Total lycopene (SD)	$\begin{array}{c} 0.29 \pm 0.04 \\ 0.18 \end{array}$	$\begin{array}{c} 0.33 \pm 0.04 \\ 0.17 \end{array}$	0.04 ± 0.03	$\begin{array}{c} 0.27 \pm 0.04 \\ 0.15 \end{array}$	$0.38 \pm 0.04*$ 0.18	0.11 ± 0.04
Alpha-carotene (SD)	$\begin{array}{c} 0.15\pm0.02\\ 0.10\end{array}$	$0.11 \pm 0.03*$ 0.11	-0.04 ± 0.01	$\begin{array}{c} 0.15\pm0.03\\ 0.12\end{array}$	$0.12 \pm 0.02* \\ 0.12$	-0.04 ± 0.01
Beta-carotene (SD)	$\begin{array}{c} 0.45\pm0.08\\ 0.35\end{array}$	$0.37 \pm 0.09 * $ 0.38	-0.08 ± 0.03	$\begin{array}{c} 0.44 \pm 0.09 \\ 0.40 \end{array}$	$0.38 \pm 0.08* \\ 0.35$	-0.07 ± 0.02

Table 3.9 Serum concentration of retinol, alpha-tocopherol and carotenoids in women

*p<0.05 significantly different from the baseline of the same diet, based on Wilcoxon signed rank test *p<0.05 significantly different from the day 17 of HCLF diet, based on Wilcoxon signed rank test

3.3.2.2 Men

Baseline serum retinol, and alpha-tocopherol levels at the start of the two diets were similar (**Table 3.10**). Serum gamma-tocopherol was significantly higher at the start of the MUFA diet compared to the HCLF diet because of one subject, whose serum gamma-tocopherol level was high after the washout period. The baseline serum carotenoid concentrations measured at the beginning of the two fourteen day dietary periods were not significantly different.

Serum retinol, alpha tocopherol, lutein + zeaxanthin and beta-carotene did not change significantly from the baseline to day 15 on both of the diets. There was no change in gamma tocopherol on the HCLF diet but there was a significant reduction by 51.8% on the MUFA diet compared to the baseline values. Cryptoxanthin and alpha-carotene reduced on both the HCLF and the MUFA diets. The percentage drop of cryptoxanthin was 25% on the HCLF diet and 26.6% on the MUFA diet. Alpha-carotene decreased by 25% on the HCLF diet and by 42.9% on the MUFA diet.

Both the diets showed a significant increase in the serum *trans*, *cis* and total lycopene levels compared to the baseline. The increase in the serum *trans* and *cis* lycopene from baseline to day 17 on the HCLF was 114.3% and 105.9%. On the MUFA diet, the increase in the serum *trans* and *cis* lycopene levels was 118.5% and 141.2%. Total lycopene increased by 106.5% on the HCLF diet and 125% on the MUFA diet.

		HCLF			MUFA	
N=13	Baseline (Mea	Day 15 n + SEM)	mean difference	Baseline (Me	Day 15 an + SEM)	mean difference
(µ11101/L)	(11200			(1120)		
Retinol	2.96 ± 0.20	3.25 ± 0.32	0.30 ± 0.34	3.00 ± 0.21	3.14 ± 0.24	0.15 ± 0.20
(SD)	0.69	0.10		0.74	0.84	
Gamma tocopherol	1.93 ± 0.27	1.36 ± 0.20	-0.57 ± 0.22	$2.80 \pm 0.63^{\bullet}$	$1.35 \pm 0.33*$	-1.46 ± 0.37
(SD)	0.95	0.68		2.21	1.14	
Alpha tocopherol	31.63 ± 2.31	29.04 ± 2.04	-2.59 ± 2.39	30.87 ± 2.59	30.20 ± 2.76	-0.66 ± 2.68
(SD)	8.01	7.07		8.97	9.40	
Lutein+ zeaxanthin	0.28 ± 0.04	0.27 ± 0.03	0.01 ± 0.03	0.26 ± 0.03	0.27 ± 0.04	0.01 ± 0.02
(SD)	0.13	0.11		0.10	0.13	
Cryptoxanthin	0.20 ± 0.04	$0.15 \pm 0.30^{*}$	-0.05 ± 0.03	0.30 ± 0.11	$0.22 \pm 0.09*$	0.07 ± 0.03
(SD)	0.14	0.10		0.39	0.32	
Trans lycopene	0.28 ± 0.03	$0.60 \pm 0.04*$	0.32 ± 0.03	0.27 ± 0.03	$0.59\pm0.07*$	0.31 ± 0.07
(SD)	0.12	0.13		0.12	0.25	
Cis-Lycopene	0.17 ± 0.03	$0.35 \pm 0.04*$	0.17 ± 0.04	0.17 ± 0.03	$0.41 \pm 0.07*$	0.24 ± 0.07
(SD)	0.09	0.14		0.10	0.24	
Total Lycopene	0.46 ± 0.06	$0.95\pm0.07*$	0.49 ± 0.07	0.44 ± 0.06	$0.99 \pm 0.11*$	0.55 ± 0.11
(SD)	0.20	0.26		0.21	0.40	
Alpha-carotene	0.12 ± 0.02	$0.09\pm0.01*$	-0.03 ± 0.01	0.14 ± 0.03	$0.08\pm0.02*$	-0.06 ± 0.01
(SD)	0.06	0.04		0.09	0.07	
Beta-carotene	0.55 ± 0.09	0.49 ± 0.06	-0.06 ± 0.06	0.49 ± 0.07	0.44 ± 0.06	-0.05 ± 0.05
(SD)	0.31	0.20		0.26	0.21	

Table 3.10 Serum concentration of retinol, alpha-tocopherol and carotenoids in men

*p<0.05 significantly different from the baseline of the same diet, based on Wilcoxon signed rank test *p<0.05 significantly different from the baseline of HCLF diet, based on Wilcoxon signed rank test

Comparing the end of the two diets, there was no significant difference in any of the carotenoid levels. The change in the carotenoid concentration in the two dietary periods was also examined, but no statistically significant difference was detected. As carotenoids are thought to be dependent on the serum lipid and lipoprotein levels, serum carotenoid values were adjusted for the change in serum cholesterol and LDL cholesterol, but no difference was observed.

3.4 Serum lipids and lipoproteins

3.4.1 Women

Total serum cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and LDL to HDL ratio were similar at the start of the two dietary periods (**Table 3.11**). Total cholesterol and LDL cholesterol reduced significantly from the baseline to day 17 on both of the diets. The fall in the total cholesterol concentration was 11.3% and 14.9% on the HCLF and the MUFA diets respectively. The LDL cholesterol reduced by 11.6% and 19.6% on the HCLF and the MUFA diet respectively. There was no significant change in the LDL to HDL cholesterol ratio on either diet. HDL cholesterol was reduced significantly by 16.4% on the HCLF diet, whereas no significant change was seen on the MUFA diet but no significant change was noted on the HCLF diet.

Comparing the results on day 17 of the two diets, there was no significant difference in total cholesterol, LDL cholesterol and LDL to HDL cholesterol ratio. HDL cholesterol was significantly higher (mean difference 0.14 mmol/L, 95% confidence interval 0.03; 0.25) on day 17 of the MUFA diet compared to day 17

of the HCLF diet. Triglycerides were significantly lower (mean difference -0.17 mmol/L, 95% confidence interval –0.31; -0.03) on the MUFA diet compared to the HCLF diet.

3.4.2 Men

Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and LDL to HDL ratio were not statistically different at the start (baseline) of the two dietary periods (**Table 3.11**). Two weeks of intervention diets resulted in a significant decrease in the total cholesterol (12.6% on HCLF and 13.6% on MUFA) and LDL cholesterol (18.3% on HCLF and 17.1% on MUFA) from baseline. HDL cholesterol reduced significantly by 10.3% on the HCLF diet, whereas no statistically significant difference was observed on the MUFA diet. Serum triglyceride levels increased on the HCLF diet by 18.7%, whereas no change was observed on the MUFA diet. There was no significant change in LDL to HDL cholesterol ratio from baseline to day 15 of the two diets.

Comparing the serum lipid concentrations on day 17 of the two diets, there was no significant difference between total cholesterol or LDL cholesterol. HDL cholesterol was significantly higher (mean difference 0.17 mmol/L, 95% confidence interval 0.24; 0.10) at the end of the MUFA diet compared to the HCLF diet. Triglycerides were significantly lower (mean difference –0.40 mmol/L, 95% confidence interval –0.18; -0.62) at the end of the MUFA diet compared to the HCLF diet. The LDL to HDL cholesterol ratio was significantly lower (mean difference –0.42, 95% confidence interval –0.11; -0.73) at the end of the MUFA diet compared to the HCLF diet.

	WOMEN					MEN			
Parameter (Mean ± SD)	He Baseline N	CLF Day 17 =18	M Baseline M	IUFA Day 17 N=18	Baseline	HCLF Day 15 N=13	Baseline	MUFA Day 15 N=13	
Total cholesterol (mmol/L)	4.89 ± 1.13	4.34 ± 1.12*	5.02 ± 1.17	$4.27 \pm 1.04*$	5.42 ± 0.75	$4.86 \pm 0.63*$	5.66 ± 0.94	$4.89 \pm 0.77*$	
HDL (mmol/L)	1.46 ± 0.39	$1.22 \pm 0.38*$	1.44 ± 0.49	1.36 ± 0.44*	1.07 ± 0.22	$0.96 \pm 0.20*$	1.07 ± 0.22	1.13 ± 0.23 [‡]	
LDL (mmol/L)	3.03 ± 1.05	$2.68 \pm 1.08*$	3.17 ± 1.03	$2.55 \pm 0.83*$	3.83 ± 0.76	$3.13 \pm 0.63*$	3.81 ± 0.94	$3.16 \pm 0.68*$	
LDL:HDL	2.18 ± 0.82	2.36 ± 1.15	2.37 ± 0.90	1.99 ± <u>0.68</u>	3.71 ± 0.95	3.39 ± 0.85	3.60 ± 0.80	$2.96\pm0.94\texttt{\ddagger}$	
Triglycerides (mmol/L)	0.94 ± 0.35	1.01 ± 0.40	0.93 ± 0.25	0.84 ± 0.23**	1.50 ± 0.98	$1.78 \pm 0.77*$	1.80 ± 1.14	$1.37\pm0.50\texttt{\ddagger}$	

Table 3.11 Serum lipids and lipoproteins in women and $men^{\#}$

mean ± SD, *p<0.05 different from baseline of same diet
* p<0.05 different from day 17 of HCLF diet in women
‡ p<0.05 different from day 15 of HCLF in men

3.5 Oxidation of LDL cholesterol

The lag phase *in vitro* LDL oxidation was significantly longer on the MUFA diet compared to the HCLF diet in both women and men (**Table 3.12**). The mean difference in LDL oxidation lag phase was 7.41 minutes (95% confidence interval –9.40; -5.42) in women and 7.31 minutes (95% confidence interval – 9.87; -4.75) in men. No significant difference was noted for maximum diene formation or rate of oxidation between the two diets.

Table 3.12 Oxidisability of LDL, conjugated diene formation and rate of oxidation at the end of HCLF and MUFA diets

Parameter	Women		Men	
	HCLF	MUFA	HCLF	MUFA
	N = 18		N = 13	
Lag Phase (min)	33.72 ± 6.00	41.13± 6.09*	37.16 ± 4.62	$44.67 \pm 6.07*$
Max. Diene (nmol diene/mg LDL)	248.30 ± 55.57	229.24 ± 41.73	259.48 ± 39.77	269.33 ± 34.45
Rate of oxidation (nmol diene/mg LDL/min)	10.82 ± 3.03	10.15 ± 2.46	9.15 ± 1.39	9.14 ± 1.46

* p < 0.001 between the two diets based on Wilcoxon signed rank test

DISCUSSION

The main objective of the two studies was to elucidate the effect of a monounsaturated fat enriched diet and a high carbohydrate low fat diet on the serum lycopene and other carotenoid levels. Epidemiological studies (Gaziano et al., 1992; Gey, et al., 1993; Morris, et al., 1994) have suggested that people with a higher risk of CHD have lower serum carotenoid levels. Kristenson, et al. (1997) found that lower levels of serum lycopene were associated with increased risk and mortality from CHD in a cross sectional study comparing Lithuanian and Swedish populations showing diverging mortality rates from CHD.

4.1 Discussion of methodology

4.1.1 Subjects

Healthy subjects were recruited for the study to avoid any effect of disease or medication on any of the parameters measured. Poor iron, zinc and protein status have been correlated with reduced absorption, transport and/or metabolism of the carotenoids (White et al., 1993). Also, intestinal parasites, malabsorption diseases and liver or kidney disease may result in reduced carotenoid absorption (Williams, et al., 1998). Similarly, drugs that affect cholesterol absorption have been shown to inhibit carotenoid absorption (Elinder et al., 1995).

People taking any supplements of carotenoids were excluded from the present study to avoid any interaction between the carotenoids (Johnson, et al., 1997; Micozzi, et al., 1992). Johnson, et al. (1997) found that administering crystalline lycopene and beta-carotene (60 mg each) together in oil in gelatine capsules to humans did not increase the serum beta-carotene levels. However, serum lycopene levels were significantly higher with the combined dose than when it was administered alone (Johnson, et al., 1997).

Lifestyle and physiological factors may influence the status of serum carotenoids (Albanes et al., 1997; Bolton-Smith et al., 1991; Brady, et al., 1996; Roidt et al., 1988; Scott, et al., 1996). People who smoked were excluded from the study because smoking may have an effect on the serum carotenoid levels. A few studies have found a strong inverse relationship between carotene or betacarotene intake and serum levels of carotenoids in non-smokers compared to smokers (Albanes, et al., 1997; Bolton-Smith, et al., 1991; Roidt, et al., 1988). In the ATBC prevention study, at the same level of dietary intake of carotenoids, subjects who smoked the most (≥ 20 cigarettes/day) generally had lower serum carotenoid concentrations than did those who had quit smoking or smoked < 20cigarettes/ day (Albanes, et al., 1997). In an observational study on African-American women, plasma lycopene levels were found to be 20-30% lower in smokers compared to non-smokers, after adjustment for dietary intake of lycopene and other confounders (Pamuk, et al., 1994). Similarly, in a study by Handelman et al. (1996), plasma directly exposed to gas-phase cigarette smoke for nine hours showed an 80% decline in lycopene concentration. However, some studies have shown no effect of smoking on serum lycopene levels (Brady, et al., 1996; Mayne, et al., 1999; Peng et al., 1995; Ross, et al., 1995). Peng, et al. (1995) found that the plasma concentration of lutein, zeaxanthin, cryptoxanthin and *cis* beta-carotene, but not lycopene, were significantly lower in smokers than non-smokers, aged 26-82 years, even at the same level of intake of carotenoids in
the two groups. Similarly, Mayne, et al. (1999) found no difference in lycopene levels in smoking and non-smoking patients curatively treated for early stages of cancer of the oral cavity, pharynx or larynx. In an observational study on Scottish males, aged 50-59 years, Ross, et al. (1995) found lower levels of plasma alphacarotene, beta-carotene and cryptoxanthin in smokers compared to non-smokers, whereas plasma levels of lycopene, lutein + zeaxanthin and vitamin E were similar in the two groups. Due to these inconsistent results from different studies, smokers were excluded from the present investigation.

People trying to lose or gain weight were excluded from the study, because evidence indicates that BMI may have an effect on serum carotenoid levels (Casso et al., 2000; Roidt, et al., 1988). Roidt, et al. (1988) found a strong inverse association between serum alpha and beta-carotene with the BMI in a population study of 302 subjects, while looking at the association of food frequency questionnaire estimates of vitamin A intake with serum vitamin A levels. Similarly, in a population study of 400 individuals aged 50 years and above, the relation between physiological and lifestyle factors with serum carotenoid levels was determined (Brady, et al., 1996). The results found that lower serum concentration of carotenoids except lycopene were associated with high BMI (Brady, et al., 1996), whereas in a population study of 946 post-menopausal women in the United States, Casso, et al. (2000) reported an inverse association of BMI with serum lycopene levels. Due to this inconsistency of the effects of BMI on serum carotenoid levels, people who were on weight loss/ gain regimes were not included in the study.

People with a high intake of alcohol were excluded from the study as ethanol intake is reported to be inversely related to serum concentrations of the carotenoids (Brady, et al., 1996; Forman, et al., 1995). In a crossover study by Forman, et al. (1996), healthy, non-smoking premenopausal women on a controlled diet with 30gram of alcohol daily for three months showed an increase in alpha and beta-carotene, decrease in lutein + zeaxanthin and no change in lycopene concentrations compared to when they were on a controlled diet for three months with no alcohol intake. However, Casso, et al. (2000) reported a significant increase in serum lycopene levels with increasing intake of alcohol in an observational study on older women. Hence, to avoid the undue effect of excessive alcohol intake on the serum carotenoid levels, people with high alcohol intake were not included in the study.

Women with a history of menstrual irregularity, women in the peri-menopausal age group, and pregnant and lactating women as well as women undergoing hormonal replacement therapy were excluded from the present study. Fluctuations in plasma carotenoid and lipoprotein concentrations in premenopausal women with the phase of the menstrual cycle were reported by Forman, et al. (1995) and Heber (1996), indicating a relationship between carotenoid and hormone levels. To make sure that the premenopausal women were in the same phase of the menstrual cycle on the two dietary periods and to nullify the effect of the phase of the menstrual cycle on serum carotenoid or lipoprotein levels, a six-week washout period was given between the two dietary periods.

4.2 Study design

The randomised crossover design (Figure 2.1 for women, Figure 2.2 for men) was used for the present investigation because the aim of the study was to compare the effects of two diets, which were similar in all the foods except oil/ fat and carbohydrate content, on the serum lycopene and other carotenoid concentrations. This study design was used to minimise the individual differences between subjects. Factors like body composition, lifestyle pattern, eating habits, way of cooking and exercise regime, which may have an effect on the outcome of the study, were minimised because each subject acted as his/her own control. The design decreases the demand for a large sample size and eliminates genetic variations. The subjects were randomised to the order of the diets to reduce any effects of order or carryover from the first dietary period.

4.3 Dietary design

4.3.1 Diet records

Subjects were asked to weigh and write the amount of food intake for four days before starting the first dietary intervention period. These records were used to analyse the subject's energy intake on their usual (habitual) diets. The diets for the intervention periods were designed to be isocaloric to the usual diets. The four days weighed food intake method was used because studies have indicated that three to four day records are suitable to determine the energy supply (Luhrmann et al., 1999). Jula et al. (1999) reported an increased intake of meat, meat products and alcohol on the weekend, when assessing the influence of the days of the week on reported food, macro nutrient and alcohol intake in men and women aged 35 to 54 years. As this may have an effect on the energy intake of the subjects, the subjects were asked to write the four days weighed food intake, with three weekdays and one weekend day to take account of their energy intake during the weekend.

Bonifacj et al. (1997), while comparing different methods of dietary assessment in a sample size of 150 males and females in a population in the south of France, reported that food frequency questionnaires provide a reliable measure of macronutrient intake in larger populations. In the present study a food frequency questionnaire (**Appendix B**) was used to calculate the usual intake of lycopene in the subjects as data was needed for a longer period than four day and this may make it easier for the subjects to estimate how often they eat lycopene rich foods which is present in few foods like tomatoes, tomato products, watermelon, pink grapefruit and guava.

4.3.2 Low carotenoid diet

To avoid any acute peaks in the serum carotenoid concentrations, which may occur due to a high carotenoid intake ten to12 hours before the blood sample, subjects were asked to eat a low carotenoid diet two to three days before starting the dietary intervention periods. Porrini, et al. (1998) looked at the effect of a single portion of tomato puree and raw tomatoes on ten subjects and reported a rapid increase in serum levels of lycopene with the first peak at six hours (individual range four to eight hours) for raw tomatoes and at six to eight hours for tomato puree and a further peak at 12 hours. After that, there was a significant decline in plasma lycopene concentrations at 24, 36 and 72 hours compared to the concentrations at 12 hours.

4.3.3 Duration of the dietary periods

Dietary periods of 16 days duration for women and 14 days duration for men were designed in the protocol for the present study. Studies have suggested that measurable carotenoids of human plasma can be changed by moderate alterations in the diet within a short period of time (Lee, et al., 2000; Rock et al., 1992; Yeum, et al., 1996). In a study on younger and older men and women consuming basic high carotenoid diets, Yeum, et al. (1996) observed a significant increase in plasma carotenoid concentrations by day six to16 in younger and older women and younger men. Rock, et al. (1992), reported a significant decrease in serum carotenoid levels by day 14 - 15 for the subjects on low carotenoid diets. For the present study, the diets of the men were of two days shorter duration than those of the women, and the dose of lycopene was higher in the study in men. This different lycopene intake was by design to gain information about the effect of amount and different sources of lycopene in two different groups of people. The diet periods may not have been long enough to see the maximum effect of lipids, but this was of secondary interest in the study.

4.3.4 Why 38% and 15% of energy from fat in the diets

According to (WHO, 1990) - nutrient goals for populations, the limits for average fat intake should be lowest as 15% of energy from fat and highest as 30% of energy from fat. Fat providing less than 15% of the energy signifies a bulky low energy dense diet leading to an inadequate intake for young active adults (WHO, 1990). It is also difficult to design a palatable diet for studies with less than 15% of energy from fat. According to the present study protocol, in the MUFA diet fat provided 38% of the energy, because the knowledge that the total fat intake in the traditional diets of people in Mediterranean countries like Greece and Crete, was

about 40% and they had a low rate of CHD (Willett, 1994). The intakes were also chosen to provide a good comparison of the two extremes of fat in the diet on the different parameters of blood.

4.3.5 Tomatoes and tomato products

Single batches of canned tomatoes, tomato soup and tomato paste were used for the study to avoid any changes in lycopene concentrations which may occur due to processing and/or effect of seasons (Lessin, et al., 1997; Schierle, et al., 1997; Stahl and Sies, 1992). Heinonen (1989) while comparing the carotenoid content of foods in different seasons, reported that the lycopene concentration of tomatoes was higher in summer compared to winter. The intensity and duration of cooking may have an effect on the isomerisation of lycopene and other carotenoids (Khachik, et al., 1992; Lessin, et al., 1997; Schierle, et al., 1997; Stahl and Sies, 1992). Lessin, et al. (1997) reported an increase in beta-carotene cis isomer content from 12.9% to 31.2% upon canning of fresh tomatoes. Also, heating tomato juice for one hour at 100°C (Stahl and Sies, 1992) and bench top preparation of spaghetti sauce from canned tomatoes cooked for 45 - 60 minutes at 85°C - 110°C (Schierle, et al., 1997) have been shown to increase the lycopene cis isomer levels. However, in a study by Khachik, et al. (1992), no change was found in cis/trans isomerisation of lutein between cooked and raw broccoli, spinach and green peas. The cooking procedure included steaming for three to five minutes, microwaving for 1.5 to three minutes and boiling for nine minutes. Heating lycopene rich food with fat may increase the bioavailabity of lycopene in the human body. Stahl and Sies (1992) showed that the consumption of tomato juice heated with one percent corn oil for one hour resulted in a two to three fold

increase in serum lycopene concentrations, whereas no change was observed after ingestion of unprocessed tomato juice. Hence, to control the effect of cooking and to make the diets more practical, for the present study in women, the tomato dishes were cooked in the university's food laboratory under hygienic conditions, for the same amount of time at the same heat setting, using the same cooking appliances for both of the dietary periods. A batch of these dishes was cooked for one person at a time, as each subject was his/her own control for the study. The dishes were portioned and frozen at -20°C for the dietary intervention periods. For men, portions of tomato paste were given to the subjects with recipes and instructions on cooking. Subjects were asked to cook batches of dishes for the duration of the dietary period, portion and freeze them at -20°C. Special instructions were given on the time of cooking the paste. Subjects were given instructions to remove one portion of tomato dish from the freezer and leave in the fridge the night before the intake and microwave for two minutes before consumption. In both the studies, subjects were asked to buy the same brand of frozen peas and corn mix for the duration of the study and cook them for same amount of time in both the dietary periods before consumption. Subjects were also instructed to preferably use the same appliances and bowls for cooking and heating foods in the two dietary periods. This design was to ensure that each subject consumed comparable amount of lycopene and other carotenoids on the two diets.

4.4.1 Body weight and diets

The diets for subjects in the MUFA and the HCLF dietary periods were designed to be isocaloric to their habitual diets. On calculation from the four-day dietary records at the end of the two dietary periods, in women, as well as in men, the mean energy intake was higher on the MUFA diet compared to the HCLF diet, even though the diets were designed to be isocaloric. This was mainly due to two or three subjects who had particularly high intakes on the MUFA diet. On the MUFA diet, the body weight in women was higher compared to the HCLF diet, but this difference in weight was less than two percent, hence it is unlikely that the changes in the serum carotenoid levels, serum lipid or lipoprotein levels were due to the change in the body weight. No significant weight change was observed in men.

Studies have indicated that BMI may have an effect on the serum carotenoid (Casso, et al., 2000; Roidt, et al., 1988) and serum cholesterol levels (Clifton and Abbey, 1997; Clifton and Nestel, 1992). Compared to the baseline, no change in BMI was observed at the end of the two dietary periods or between the two dietary periods. Hence, it is presumed that the changes in the serum carotenoid, lipid and lipoprotein levels in the present study were not due to BMI.

The protein intake in both of the studies was within the recommended range of 15-18% of total energy intake given by National Health and Medical Research Council (NHMRC, 1991). The small difference (less than two percent) in protein intake in men is unlikely to have affected the parameters measured. In

confirmation with the protocol of the study, the carbohydrate intake on the HCLF diet was higher than the MUFA diet in both the study groups.

Using the equation (Δ plasma cholesterol mmol/L = 0.035 (2 Δ SFA- Δ PUFA)), where SFA is represents saturated fat and PUFA represents polyunsaturated fat, developed by Keys, et al. (1957), minor differences in the saturated fat (SFA) intake and the polyunsaturated fat (PUFA) intake (SFA: 1.2% in women, 0.3% in men; PUFA: 0.8% in women and 0.5% in men) observed, could have altered the total serum cholesterol in women by 0.056 mmol/L and 0.0035 mmol/L in men. These differences are unlikely to have influenced the study findings. The average monounsaturated fat intake calculated from the four-day diet records was 4.9% of the energy and 4.1% of the energy on the HCLF diet in women and men, respectively. On the MUFA diet, the monounsaturated fat intake was 25.4% of the energy in women and 23.7% of the energy in men. It is assumed that the high intake of monounsaturated fat in the MUFA diet, and low amounts of total dietary fat with high carbohydrates in the HCLF diet were the major influencing factors on the observed changes in the serum cholesterol levels from the usual diet in the two studies.

In both men and women, the dietary cholesterol and vitamin C intake were similar on the two diets, suggesting that the differences observed in the serum lipid and lipoprotein levels, in the present study, were not because of these factors. Total sugar and starch intake were significantly higher on the HCLF diet compared to the MUFA diet in both the studies. The fibre intake was higher on the HCLF diet in the women, mainly due to the higher intake of white bread. In men, there was no difference in the dietary fibre intake in the two dietary periods. The vitamin E content of the two diets was different because of the different types and amounts of the oils used. It was difficult to control these differences between the two diets unless supplements of different nutrients were allowed, which would have changed the protocol of the present study.

Alcohol intake in both of the dietary periods was similar, as calculated from the four day dietary records, hence it is unlikely that alcohol could have had a differential effect on the serum carotenoid or serum lipid levels observed in the present investigation.

4.4.2 Carotenoids

The main aim of the project was to elucidate the effect of the HCLF and the MUFA diets with controlled lycopene and other carotenoid content on the serum lycopene and other serum carotenoid levels.

The serum carotenoid levels on the habitual diet of the two study groups were similar to the levels shown by other studies for the people in similar age groups in the United States and Ireland (Carroll, et al., 1999; Michaud, et al., 2000). Lycopene was the predominant serum carotenoid in both women and men groups in this present study, whereas beta-carotene was the predominant one in others (Carroll, et al., 1999; Yeum, et al., 1996). In the present study, the serum carotenoid concentrations were ranked in the order: lycopene > beta-carotene >lutein + zeaxanthin >cryptoxanthin > alpha-carotene in both women and men.

On the habitual diets, the dietary intake of lycopene in the present study group was at the lower end of the range of dietary intake observed by population studies in the United States and Ireland (Carroll, et al., 1999; Michaud, et al., 2000). The range of lycopene intake in women was larger (0.50 to 14.12 mg per day) than in men (8.01 to 12.85 mg per day). The main source of lycopene was raw tomatoes in women and raw tomatoes and tomato sauce in men. Also, the dietary lycopene intake of the women study group was similar to that of the GOS study, indicating that the women study group was actually representative of the female population sampled in the GOS study, from the same geographical area of Australia. Studies have reported a strong positive correlation between dietary intake of lycopene and serum lycopene concentrations (Brady, et al., 1996; Carroll, et al., 1999; Scott, et al., 1996), which was confirmed in the present study.

Serum lycopene levels were significantly lower at the baseline of the intervention study, after the three day LCD, (where subjects were asked not to eat any tomatoes, tomato products, water melon, pink grapefruit or guava, but were allowed to have small amounts of other fruit and vegetables) than on the habitual diet in women, suggesting serum carotenoid levels can be used as markers of recent fruit and vegetable intake (Bowen et al., 1993; Martini et al., 1995; Yeum, et al., 1996). However, no significant change was seen in men after two days of LCD.

The baseline serum carotenoid levels in both of the studies were similar at the start of the two dietary periods, confirming with other studies that a 42 day

washout period was sufficient to allow carotenoids to return to baseline concentrations (Bowen, et al., 1993; Brown et al., 1989; Yeum, et al., 1996).

4.4.3 Effect of diets on serum lycopene and other carotenoid levels In women, the average daily lycopene intake during the intervention dietary periods was 15.9 mg/day, as described in the methods chapter (page 70). Fasting blood samples were taken on day 17 after 16 days of the dietary period. The last soup meal was taken on day 15, about 40 hours before the blood sample to avoid the acute peaks (Porrini, et al., 1998). No change was observed in the serum *cis* lycopene levels from the baseline to the end of the two dietary periods in women. Compared to the baseline, there was a significant increase in the serum trans lycopene and serum total lycopene concentrations after the MUFA diet. However, no difference was observed between the serum *cis, trans* and total lycopene levels at the end of the MUFA and the HCLF dietary periods. In men, the daily intake of lycopene was 20.2 mg, which mainly comprised of 60 gram of tomato paste and 300 gram of canned tomato soup every day. Compared to the baseline, serum *cis*, trans and total lycopene levels increased significantly after the MUFA as well as the HCLF dietary periods. However, there was no significant difference between the plasma trans, cis and total lycopene levels at the end of the two dietary periods.

These results suggest that changes in serum lycopene levels were not dependent on the amount of fat in the diet. There is a possibility that the dose of lycopene in the men was enough to bring about the change in the serum lycopene levels from baseline regardless of the dose of fat in the diet, whereas in women the lower lycopene dose might not have been enough to show any significant change from baseline in the serum lycopene levels on the HCLF diet. Studies have suggested that lycopene rich food cooked with fat increases the bioavailability of lycopene (Stahl and Sies, 1992). In the present investigation, tomato dishes in both the dietary periods were cooked with oil, although the amount of cooking oil was different in the two diets.

Studies have investigated the effect of different amounts of fat on the absorption of beta-carotene and other carotenoids. A study on the plasma response to betacarotene supplementation (45 mg for five days), where ten subjects consumed a controlled high or low fat breakfast (18-24 gram fat on high fat diet, fat free on low fat diet) and mid day meal (45 gram fat on high fat diet and six gram fat on low fat diet) showed a significant increase in plasma beta-carotene on the high fat diet compared to the low fat diet (Dimitrov, et al., 1988). However, in another study Roodenburg, et al. (2000) reported that the dietary fat required for the optimal uptake of lutein esters in the plasma is higher than the amount required for the uptake of vitamin E, alpha-carotene or beta-carotene. In this study serum lutein levels were higher after the high fat diet compared to the low fat diet but no difference was observed in the plasma concentration of vitamin E, alpha-carotene and beta-carotene with the consumption of three or 36 gram fat in a hot meal, for a period of seven days by 14 subjects. Jayarajan, et al. (1980) found that five grams of fat in a meal was sufficient to ensure carotenoid uptake in children. Three groups of children in the age range of two to six years were fed meals of spinach with no fat, five gram fat or ten gram fat for a period of four weeks. Compared to the baseline serum vitamin A levels increased in all the three groups at the end of the supplementation period. Though the increase in serum vitamin A

in the five and ten grams dietary fat group was higher compared to zero gram fat, no difference was seen between the groups getting five grams or ten grams fat (Jayarajan, et al., 1980). Another study, showed serum retinol concentrations increased similarly in two groups of five year (\pm six months) old children, when beta-carotene rich meals from sweet potato cooked without fat (providing 6.6% of energy from fat) or cooked with 15 gram of fat (providing 34.6% energy from fat) were given for a period of three weeks (Jalal, et al., 1998). The present study suggests that 15% of energy from dietary fat or 38% of energy from dietary fat does not have any differential effect on the serum lycopene levels and it is possible that 15% of energy from fat is enough to ensure absorption of lycopene and adding further fat in the diet does not increase the absorption and hence the serum lycopene levels.

Studies have suggested that the carotenoid change in serum after a high dose of carotenoid rich food also depends on the matrix of the food in which it is consumed (Castenmiller, et al., 1999; Gartner, et al., 1997; Stahl and Sies, 1992; van het Hof et al., 2000; van het Hof et al., 1999b). Gartner, et al. (1997) reported that the lycopene bioavailability from tomato paste and processed tomato juice was significantly higher than from unprocessed fresh tomatoes. In a study comparing the lycopene content of canned tomatoes homogenised to different degrees, it was reported that the lycopene content of the tomatoes increased with the increase in homogenisation (van het Hof, et al., 2000). Also the lycopene content of the canned tomatoes was increased if they were given additional heat treatment especially in non homogenised and mildly homogenised tomatoes (van het Hof, et al., 2000). Hence, there is a possibility that lycopene from the canned

tomatoes, that were used in the present study with women, did not leech out enough, compared to the lycopene in the tomato paste that was used for the study in men.

Serum lutein + zeaxanthin levels were significantly higher at the end of the MUFA diet compared to the HCLF diet in women. This was in confirmation with Roodenburg, et al. (2000), who showed a significantly larger increase in the plasma lutein levels after the high fat meals (36 gram fat) compared to low fat meals (three gram fat). In men, no difference was observed in the serum concentrations of lutein + zeaxanthin at the end of the two dietary periods. On closer observation of the data, 11 out of the 18 women showed no change or increase in lutein + zeaxanthin on the HCLF diet, compared to seven out of the 18 on the MUFA diet. In men, seven out of the 13 men showed no change or increase in lutein + zeaxanthin levels on both the MUFA and the HCLF diets. There is a possibility that the bioavailability of lutein + zeaxanthin is dependent on the amount of dietary fat, but the dietary period with the amount of dose that was given in the present diet was not long enough to show a significant change in men which was seen in the women. Though Roodenburg, et al. (2000) showed the difference after a seven day dietary period but, dose of lutein was 7.6 mg per day in the form of lutein ester, compared to the present study in which the dose was 0.97 mg per day in the form of free lutein as calculated from USDA (1998).

Serum cryptoxanthin and alpha-carotene levels decreased significantly in the two dietary periods in both of the study groups. No difference was observed at the end of the MUFA and the HCLF diets. On calculation from the USDA database on carotenoids (USDA, 1998), the average daily intake of cryptoxanthin, alphacarotene and lutein + zeaxanthin in the HCLF and the MUFA dietary periods for both the groups were 60 μ gm, 18 μ gm and 970 μ gm respectively. The daily intake of beta-carotene by women was 366 μ gm on the days when soup was not taken and 900 μ gm on the days when soup was taken. The beta-carotene content of the diet for the men was 1204 μ gm per day.

It is presumed that the dietary intake of cryptoxanthin, alpha-carotene and betacarotene in women and cryptoxanthin and alpha-carotene in men for the present study was lower than the intake on the group's usual (habitual) diets and hence, a decrease was observed after 14-16 days of the dietary periods. The decrease in the serum levels of cryptoxanthin, alpha-carotene and beta-carotene were in confirmation with Rock, et al. (1992), who reported a significant decrease in plasma lycopene, lutein + zeaxanthin, cryptoxanthin, alpha-carotene and betacarotene levels in 12 subjects who were fed low carotenoid diets for 13 weeks. The most significant differences in the plasma levels were observed between day 2-3 and day 14-15 (Rock, et al., 1992). These and the results from the present study then confirm that the dietary intake of carotenoids can alter the serum carotenoid levels and these levels further indicate the immediate dietary carotenoid intake (Lee, et al., 2000; Rock, et al., 1992; Yeum, et al., 1996).

Compared to the baseline, serum beta-carotene levels decreased significantly in the two dietary periods in the women but not in men. No significant difference was noted in serum beta-carotene levels after the two dietary periods in both women and men. The dietary intake of beta-carotene in was higher compared to the women in the intervention period. It is possible that the intake of betacarotene in men during the intervention period was similar to their habitual diet whereas in women the intake was lower during the intervention period hence the decrease in serum beta-carotene levels (Rock, et al., 1992).

4.4.4 Effects of diets on serum tocopherol levels

The baseline levels of serum alpha tocopherol and gamma tocopherol in the present study were similar to the subjects in other studies (Olmedilla et al., 2001; Pamuk, et al., 1994) in the similar age group. Compared to the baseline, gamma tocopherol decreased in both the dietary periods in women. In the case of men, no change was observed from the baseline to the end of the HCLF diet but there was a significant decrease in gamma tocopherol from the baseline to the end of the MUFA dietary period. This change may have been due to one subject, who had a high baseline level of serum gamma tocopherol after the washout period. No difference was noted for serum gamma tocopherol levels between the end of the MUFA and HCLF dietary periods.

On the HCLF diet serum alpha tocopherol levels reduced significantly from the baseline to day 17 in women, but no change was seen after MUFA diet. At the end of the two diets alpha tocopherol was higher after MUFA diet compared to the HCLF diet. It is possible that HCLF diet contained less alpha tocopherol content compared to the habitual diet of the women, and hence a decrease was seen after 16 days of diet. However, no other study has shown similar result and the reason for the difference is not known. In men, no significant change was observed from baseline to the end of the two dietary periods. The vitamin E content of the MUFA diet (alpha tocopherol – 26.98 mg/day, gamma tocopherol

-0.24 mg/day) was higher compared to the HCLF diet (alpha tocopherol -2.62 mg/day, gamma tocopherol -0.03 mg/day). In a study comparing the effect of olive oil versus sunflower oil with a controlled lycopene content, no difference was observed in the alpha tocopherol levels after the two diets of 7 days (Lee, et al., 2000). Oil was the main source of vitamin E on the two diets, with sunflower oil providing larger amounts than olive oil (Lee, et al., 2000). In a study investigating the effect of low fat and high fat diets with similar alpha tocopherol content, no difference was observed in the serum alpha tocopherol levels after 7 days diet (Roodenburg, et al., 2000), suggesting no differential effect of diets with different amounts of fat on serum alpha tocopherol levels.

4.4.5 Lipids and lipoproteins

A secondary point of the study was to document the effect of dietary monounsaturated fat on serum lipid concentrations. Studies have compared the effects of a monounsaturated fat rich diet with a high carbohydrate low fat diet on serum lipids and lipoproteins (Ashton, 2000; Colquhoun et al., 1992; Garg et al., 1988; Grundy, 1986). In agreement with some studies (Colquhoun, et al., 1992; Garg, et al., 1988; Grundy, 1986), both the HCLF and the MUFA diet in the present investigation reduced the serum total cholesterol compared to the baseline. In confirmation with Grundy (1986), the present study showed a decrease in LDL cholesterol after both HCLF and MUFA diet periods compared to other studies which showed no effect on LDL cholesterol (Ashton, 2000; Colquhoun, et al., 1992). The significant decrease in total and LDL cholesterol compared to the baselines after both of the dietary periods suggests that both of the

diets may be equally effective in reducing the risk of CHD compared to the habitual diets.

The present study showed a decrease in HDL cholesterol from baseline after the HCLF dietary period but not after the MUFA diet. These results are in confirmation with other studies (Ashton, 2000; Berry et al., 1992; Garg, et al., 1988; Grundy, 1986; Grundy, et al., 1988; Mensink et al., 1989; Mensink and Katan, 1987) which showed a significantly larger decrease of HDL cholesterol after a high carbohydrate low fat diet compared to a monounsaturated fat rich diet. The present study showed a significantly higher HDL after the MUFA diet than after the HCLF diet, suggesting a possible benefit of a MUFA diet (Colquhoun et al., 1998; Grundy, 1986). Analysis of data from the Framingham study over ten years of follow-up suggested that people with high levels of HDL had an appreciably lower rate of CHD than those with lower HDL (Gordon et al., 1981). Similarly, an analysis of four American prospective studies by Gordon et al. (1989) indicated that an increment of 0.026 mmol/L in HDL is associated with a two to three percent reduction in CHD.

The LDL cholesterol to HDL cholesterol ratio was higher after the HCLF diet compared to the MUFA diet. Similar results were reported by (Grundy, 1986). As higher LDL to HDL ratio depict higher risk of CHD (LaRosa, et al., 1990), the present investigation suggest that MUFA diet may reduce the risk of CHD compared to a HCLF diet. However, there is little data on whether the LDL:HDL ratio depicts a higher risk of CHD when low fat diets are replaced with high fat diets. Compared to the baselines, an increase in triglycerides after the HCLF diet was seen in the men, whereas in the women there was no statistically significant change observed after the HCLF diet. After the MUFA diet, a decrease in triglycerides was seen in women. In confirmation with other studies (Berry, et al., 1992; Garg, et al., 1988; Grundy, 1986; Grundy, et al., 1988; Mensink, et al., 1989; Mensink and Katan, 1987), higher serum triglycerides levels after the HCLF diet compared to the MUFA diet were observed. This may be because of differences in the dietary fibre, total sugar and starch intake on the two diets. In the present investigation, total sugar and starch intake was higher on the HCLF diet compared to the MUFA diet, which may have increased the serum triglyceride levels by increased hepatic synthesis. Dietary fibre was significantly higher on the HCLF diet compared to the MUFA diet in women. This was primarily from white bread which contains soluble fibre that may have an effect on the serum cholesterol levels, but according to a meta-analysis of 67 controlled trials, within the practical range of intake, soluble fibre makes very little contribution to lowering the serum cholesterol (Brown et al., 1999) and wheat fibre has been shown to have no effect (Jenkins et al., 1999). Hence it is unlikely that fibre would have had any differential effect on cholesterol.

The total serum cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and LDL to HDL ratio were similar at the baseline of the two dietary periods in both men and women. This suggests that the six-week washout period after the prescribed diet was enough to bring the serum lipid and lipoprotein levels to the usual values. In the present study, a six week washout period was given, though this was longer than others (Ashton, 2000; Berry, et al., 1992; Grundy, 1986), to control the effect of the phase of menstrual cycle on the serum carotenoid as well as lipid levels, which tend to change with the phase of menstrual cycle (Forman, et al., 1998; Heber, 1996).

Some studies have suggested that a high dietary intake of lycopene decreases the serum cholesterol levels (Fuhrman et al., 1997). In this present investigation, lycopene intake was the same on the two dietary periods and the only difference in the diets was of the type and amount of fat and carbohydrate. After the dietary periods, a change in the serum cholesterol levels were seen compared to baseline. There is a possibility that the serum cholesterol decreased due to the combined effect of lycopene and monounsaturated fat or lycopene and high carbohydrate low fat diets, but it cannot be confirmed from this study.

4.4.6 Effect of diets on oxidation of LDL

The present investigation and other studies have shown that replacing saturated fat with monounsaturated fat or carbohydrates in the diet can influence the plasma levels of LDL cholesterol. Some studies have compared the effects of monounsaturated fat and high carbohydrate low fat diets on the susceptibility of LDL to oxidise *in vitro* (Ashton, 2000; Berry, et al., 1992; O'Bryne et al., 1998), and as a secondary aim the present study also investigated the effect of the two different diets on the oxidation of LDL. The lag phase after the MUFA diet was about 7.4 minutes (women) and 7.3 minutes (men) longer than after the HCLF diet in the present study. The longer lag phase after the MUFA diet compared to

the HCLF diet was also reported by (Ashton, 2000; Berry, et al., 1992; O'Bryne, et al., 1998), and confirms the result of the present study that the oxidation of LDL is resisted for a longer time on the consumption of monounsaturated fat rich diet rather than a high carbohydrate low fat diet. This may have a biological significance in reducing the risk of atherosclerosis, which is the major cause of CHD.

Despite the fact that the same method was used for the *in vitro* oxidation of LDL cholesterol as (Ashton, 2000), no significant change in the maximum diene formation or rate of oxidation was seen in the present investigation between the HCLF and the MUFA diets, whereas Ashton (2000) showed a significantly lower formation of maximum dienes as well as rate of oxidation after the MUFA diet compared to the HCLF diet. This difference might be attributed to the fact that the dietary periods for the present investigation were shorter (16 days for women and 14 days for men) than in (Ashton, 2000) study which were 28 days, and there was less time to replace the polyunsaturated fatty acids in the LDL with monounsaturated fatty acids. To confirm this the LDL fatty acid profile should have been measured, but that was beyond the scope of this thesis work.

4.4.7 Effect of lycopene on oxidation of LDL

Studies have suggested that carotenoids may have an effect on the oxidation of LDL (Abbey, et al., 1993b; Agarwal and Rao, 1998; Jialal and Fuller, 1995; Lee, et al., 2000; Packer, 1993; Rao and Agarwal, 1999), and lycopene is thought to have the highest antioxidant activity compared to all other carotenoids (Di Mascio, et al., 1989; Mortensen, et al., 1997; Tinkler, et al., 1994).

In a study on 19 healthy subjects, LDL oxidation was significantly reduced after supplementation of the diet with lycopene rich foods for a period of one week (Agarwal and Rao, 1998). Comparing the effect of supplementation of lycopene on the oxidation of LDL in smokers and non-smokers, Chopra, et al. (2000) reported a significantly reduced susceptibility of LDL to oxidation in smokers after an intake of > 40 mg of lycopene per day for seven days. Similarly, in a study of 57 patients with well-controlled type two diabetes, Upritchard et al. (2000) showed a decrease in the oxidation of LDL cholesterol after supplementation of 500 ml of tomato juice for a period of four weeks.

However, in the present investigation, no association between lycopene and *in vitro* oxidation of LDL was observed, in men or in women. This was in confirmation with the study of Sutherland, et al. (1999) in 15 renal transplant recipients, which showed no change in the susceptibility of LDL to oxidation after four weeks of supplementation with tomato juice (400ml per day). Also a study by Dugas, et al. (1999) compared the effect of supplementation of beta-carotene (15mg/day) and lycopene (34 mg/day) on the endothelial cell mediated oxidation of LDL. After four weeks of supplementation of LDL in *vivo*, whereas no change was seen with the supplementation of lycopene. Similarly, in a study by Hininger et al. (2001), 175 male volunteers divided in four groups received 15 mg of either beta-carotene, lutein, lycopene or placebo supplements for three months. The results indicated an increase in the plasma as well as LDL concentrations in each of the caroteneoids supplemented without any modification

in other carotenoid levels in plasma or LDL. However, no effect was observed on the resistance of LDL to oxidation (copper induced) by the increased plasma levels of the different carotenoids - beta-carotene, lutein or lycopene.

Lee, et al. (2000) compared the effect of extra virgin olive oil and sunflower oil on the serum lycopene levels and the antioxidant activity of plasma on 8 healthy subjects. The lycopene content of the diets was 46 mg/ day. A similar increase in serum lycopene levels was observed after the seven-day dietary periods on both the diets, but the antioxidant activity of plasma was significantly higher after the olive oil dietary period compared to the sunflower oil dietary period. The difference in the antioxidant activity of plasma was attributed from the oleic acid and/or the presence of phenols and other polyphenolic compounds rather than the lycopene.

4.4.8 Effect of vitamin E on oxidation of LDL

Studies have suggested that vitamin E reduces the susceptibility of LDL to oxidation (Abbey, et al., 1993b; Jialal and Grundy, 1993; Princen, et al., 1992). In the present investigation, the susceptibility of LDL to oxidise was reduced after the MUFA diet, but it is difficult to suggest if this was due to the effect of just the oleic acid content of the diet or the combination of oleic acid and vitamin E. Vitamin E content of the MUFA diet was higher compared to the HCLF diet, but no difference was observed in the serum vitamin E levels after the two dietary periods.

Conflicting data is available on the effects of vitamin E and lycopene on the oxidation susceptibility of LDL. In the present investigation the susceptibility of LDL to oxidation was reduced on MUFA diet compared to the HCLF diet. Data suggests that vitamin E content of the diet and serum levels of vitamin E may play a role in reducing this susceptibility. Also some studies have suggested that lycopene may reduce the susceptibility in presence of other antioxidants like vitamin E. It is not possible to confirm if lycopene was one of the influencing factors in the present investigation or not because the lycopene content of the two diets and the serum lycopene levels on both the diets were similar. However, it is possible that lycopene in addition to monounsaturated fat and vitamin E on the MUFA diet showed this change in the oxidation of LDL compared to the HCLF diet. To confirm this, studies with similar amount of vitamin E on the two diets need to be conducted. In addition studies have shown that lycopene reduces the susceptibility of LDL to oxidation in smokers than non-smokers. Also some unknown factors in the diets e.g. unknown polyphenols in oil and/ or presence of other carotenoids in the diet e.g. beta-carotene, may affect the oxidation of LDL. For conclusive results, further studies are required in this area.

4.5 Conclusion and future directions

The primary aim of the present investigation was to compare the effects of a high monounsaturated fat diet and a high carbohydrate low fat diet, with the same dietary lycopene and other carotenoid content, on the serum lycopene and other carotenoid levels in healthy individuals. In the present investigation, the diets were designed to provide the two extremes of fat that were feasible as well as endurable. The results of the studies did not show any differential effect of the two diets on the serum lycopene and most other serum carotenoid levels. The studies suggested that 15% of energy from fat in the diet was sufficient, and a greater quantity may not improve the absorption of lycopene any further. Although most diets provide at least 15% of energy from fat, we cannot tell whether less than this amount of fat in the diet could have any differential effect on the absorption and serum concentrations of lycopene. The monounsaturated fat diet appeared to have benefits on serum lipids and susceptibility of LDL to oxidation. Further research may be able to elucidate the precise amount of total and monounsaturated fat required in the diet for optimal benefit.

Similar studies need to be conducted over a longer period in 'at risk subjects' like hypercholesteromics, hypertensives and diabetics, to investigate if high monounsaturated fat rich diets have any differential effect compared to a low fat, high carbohydrate diet, on the absorption of lycopene and lipid parameters in people with altered lipid metabolism or high levels of oxidant stress. Further studies are necessary to elucidate if different types of oils make a difference in the bioavailability of these carotenoids; for example if olive oil will have a differential effect compared to the oleic acid rich sunflower oil used in the present investigation, due to the presence of phenols and other phytochemicals, rather than just the oleic acid content.

Data is needed from population groups on the relationship between serum levels of lycopene and incidence of chronic diseases like CHD. Data is also needed on the dietary intake of lycopene rich foods required to achieve the serum lycopene levels that may be related to a lower incidence of CHD and other chronic diseases. Studies are also required on the comparative bioavailability of lycopene from different tomato products providing the same amount of dietary lycopene such as paste, puree, juice and soup.

The present studies were performed in an artificial setting where the source and amount of lycopene was controlled and the intake was limited to two week periods. In everyday life similar changes to those observed in male participants, which increase the serum lycopene levels, may be easy to achieve, provided a number of sources are added to Australians' usual diet. A combination of intake of different products like tomato paste, tomato puree, tomato sauce, baked beans, pasta sauce and tomato soup taken regularly in small amounts (two to three serves a day) in the diet may lead to an increase in the serum lycopene levels, in a manner which could be associated with a reduced risk of CHD.

Appendix A Diet Record

NameI.D.Please read these important instructions carefully

- Please record ALL the food and drinks consumed
- Please record the food at the time of eating and Not from memory at the end of the day
- You should include all the meals & snacks, plus sweets, drinks (including water) etc.
- Remember to include any additions to foods already recorded such as sauces, dressings or extras e.g. gravy, salad dressings, stuffing, sugar, honey, syrups etc., butter or margarine (e.g. added to bread, crackers, vegetables).
- If you do not eat a particular meal or snack, simply draw a line across the page at this point. This will show that you definitely have not eaten anything.

Describing food and drink- guidelines

 Give as many details as possible about the type of food that you eat e.g. brand name of food where applicable (e.g. Miracle margarine);

Type of: Breakfast cereal e.g. weetbix

Milk e.g. whole or skim milk

Cake or biscuit e.g. fruit cake, wheatmeal biscuit

Fruit e.g. fresh, dried, stewed

Soft drink e.g. regular or low calorie

Type of cheese, fish or meat e.g. cheddar, cod fillet, loin of pork.

- Please give details of the method of cooking all foods (e.g. fried, grilled, boiled, roasted, steamed, poached, and stewed).
- Write whether the fat on the meat or skin on poultry was eaten or not.

Recording the amounts of foods you eat

It is very important that you record the quantity of each food and drink you consume.

Here are some suggestions on how to record amounts:

• In household measurements:

Butter and margarine can be measured in teaspoons or tablespoons.

E.g. state the number of teaspoons (tsp.), tablespoons (tbsp.), cups etc. and state whether the spoons were level, rounded or heaped.

• Weights marked on packages:

Most convenience foods have their weight marked on the packaging and this can be quoted e.g. half of 425g can of baked beans.

Bread - indicate the size of the slice e.g. sandwich, medium or toasted

- Measure cooked food and not raw food.
- Measure the food after it is served.
- If you do not eat all the food you first served out, measure and write down how much is left. There is no need to measure the left over core, peel or bones.
- For a mixed dish like casserole, record the total amount eaten. There is no need to record the amount of each separate ingredient.
- For sandwiches and rolls, measure each ingredient as it is being made or if the sandwich is already prepared, name each ingredient and the number of sandwiches eaten.
- If at all possible, it would be very helpful to weigh your portions of foods on the scales provided. If it is not possible to weigh the food portion, please use the pictures on the attached sheets to indicate what sort of portion size you ate e.g. you might have 1 portion of spaghetti size A, 1 portion of meat size B or 2 slices of cheese size C.

It is very important that you do not adjust what you eat and drink because you are keeping a record. This is very easy to do but remember, we are interested in your eating habits, not the perfect diet!!!

DAY 2 - Date

Record ALL food and drink consumed during the day including sweets, snacks, 'nibbles', sauces and dressings.
Please record: METHOD OF COOKING (e.g. *boiled* pasta)

METHOD OF COOKING (e.g. *boiled* pasta) TYPE OF FOOD (e.g. boiled *wholegrain* pasta) QUANTITY OF FOOD (e.g. 6 *heaped* T boiled wholegrain pasta)

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK	
EARLY MORNING			
BREAKFAST			
DURING MORNING			
MIDDAY			

DAY 2 - continued

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK	
DURING AFTER- NOON			
EVENING MEAL			
DURING EVENING/ BEDTIME SNACK		-	

Sample of photographic portion size



PHOTOGRAPH 5

Appendix B Food Frequency Questionnaire

Listed below are some of the foods we want you to think how often you consume and tick the right box. If there are any food that you eat with tomatoes in it, but is not listed please add them to the end of the list.

For serving size- give the best possible explanation of amount in either weight or house hold measure, i.e. tea spoon/ table spoon/ cup.

Food	Serving	Daily	5-6	3-4	1-2	1-2	Rarely
	Size		times a week	times a week	times a week	times a	or never
Tomato raw			WEEK			month	
Tomato							
dried							
Canned							
tomato							
Tomato							
sauce							
Tomato							
puree							
Tomato							
soup							
Tomato							
juice							
Baked							
beans							
Pasta/							
spaghetti in							
tomato							
paste/ sauce							
Stew/							
casserole or							
curry with							
tomatoes							
Pizza							
Quiche							
Cheese							
burger							
Salsa sauce							

Food	Serving	Daily	5-6	3-4	1-2	1-2	Rarely
	Size		times a	times a	times a	times a	or never
			week	week	week	month	
Vegetable							
soup with							
tomatoes							
Apricot dry							
Minestrone							
soup							
Water							
melon							
Guava/							
guava							
nectar							
Grapefruit,							
raw, pink or							
red							
Pink							
grapefruit							
Juice							
Bloody							
Mary							

Appendix C Low Carotenoid Diet

Following is the list of foods we need you to avoid for three days prior to commencing either diet period one or two. You can choose any of the foods from the **FREE** list. Do not take any of the foods from the **AVOID** list.

FREE

AVOID

Fruits (maximum 2 serves day)

Apple (limit 1 day) Orange (limit 1 day) Banana Berries Grapes Kiwi fruit Pineapple Pear Honey dew melon

Vegetables

Peas (limit ¹/₂ cup day) Corn (limit ¹/₂ cup day) Potato (unrestricted) Eggplant Cabbage Cauliflower Cucumber Radish Onion- white Beetroot-canned and drained Mushrooms Lettuce- iceberg only Pink grape fruit Watermelon Cantaloupe Mango Apricots (fresh and dried) Peaches Nectarines Papaya Plums

Tomatoes Pumpkin Beans Carrots Broccoli Dark green leafy vegetables, e.g., spinach, Chinese greens, dark lettuces Capsicum- red and green Celery

FREE

Spreads and flavourings

Jam- only berry	Tomato sauce or ketchup
Marmalade	Tomato paste and soup
Honey	Tomato puree
Peanut butter	Fruit chutneys
Vegemite	Cocktail sauce
No oil dressing	Apricot based sauces and marinades
Soy sauce	Parsley
Ovster sauce	Basil
Black bean sauce	Oregano
Mustard sauce	Barbecue sauce
Garlic	Mavonnaise
Ginger	5
Salt	
Black pepper	
Cereals	
Any breakfast cereal except those with	Cereals with added ß-carotene
added ß-carotene	
Bread- white, wholemeal, mixed grain, rye	
Boiled rice or pasta (brown or white)	Tomato based sauces on rice or pasta
Lentils	
Meat, fish, poultry, eggs	
All types	Dishes with added tomato based
	sauces

AVOID
FREE

AVOID

Dairy

Reduced fat and skim milk	Full cream milk, custard
Cottage and ricotta cheese	All other cheeses
Natural, vanilla or berry fruit yoghurt	All other fruit yoghurts
	Ice Cream and chocolate

Fats and oils

Margarine and butter (limit to 2 teaspoons per day) Oil

Beverages

Flavoured mineral water and soft drinks
(e.g., fanta, coke)
Orange juice
Tomato juice
Guava nectar or juice
Pink grape fruit juice
Apricot nectar
Vegetable juices
Red wine
Fruit based liqueurs

Others

Any foods containing β-carotene e.g. Uncle Toby All- Bran Foods containing colours (no 160, 161, 162, 163, 165) e.g. mayonnaise Foods containing annatto extracts e.g. icecream

Appendix D Basic Menu on the Two Diets (For women)

Following is the diet to be taken for the next 16 days.

It is important that you consume each food in the amounts prescribed. We have tried to make the diet as varied as possible, but there are limitations as to what you can and cannot eat.

Meal Plan

Breakfast	
• Muesli (as provided)	1 serve
	(1 oasted with oil on)
• Milk (reduced fat fortified)	200 gm
	8
Mid-Day	
• Tomato soup (as provided)	1 serve
• Biscuit (provided)	2 (only on MUFA
diet)	
Lunch	
• Sandwich	
-Bread (mixed grain or rye or whole mea	al) 2 slices(average
	slice 30gm)
-Protein source (see protein options for h	unch) 1 serve
-Salad vegetables (see list for options)	10
-Margarine (as provided)	10 gm
• Fruit (see list for options)	1 piece
Afternoon	
• Bread white	1slice
• Honey/ berry jam	10gm
Dinner	
• Tomato dish (provided) 1 s	erve (cooked with oil)
• Protein source (see meat options)	1serve
Carbohydrate source (see carbohydrate optio	ons) 1serve
• Vegetables(frozen peas and corn mix)	100 gm
• Oil for cooking (provided)	5 gm
Beverages and yoghurt	
• Limit tea/coffee to 2 cups a day	
Yogurt low fat (natural or strawberry)	200gm
Additional milk	100 gm

Food options list

Lunch Protein options

Choose one of the following

	e	
٠	Tuna in brine, drained	100 gm
٠	Australian Salmon in brine, drained	50 gm
٠	Cottage cheese, low fat	100 gm
٠	Ham, lean	50 gm
٠	Corned beef, lean	75 gm
٠	Chicken, baked lean no skin	50 gm
٠	Egg, whole hard-boiled	1 average (50gm)

Lunch salad vegetables

You may include any of the following salad vegetables

- Ice-berg lettuce
- Cucumber
- Beet-root, canned and drained
- Salad onion (white)
- Mushrooms •
- Radish

Fruit options

Choose one of the following

٠	Apple	1 average
•	Banana	1 average

Pear 1 average •

Dinner Protein options

Choose one of the following (uncooked weight)

- Beef, lean trimmed of all fat 150 gm • Chicken, lean no skin 150 gm • • Lamb, boneless lean trimmed of all fat 150 gm
- Lamb chump chop raw, lean 125 gm
- Fish, white flesh variety 150 gm •
- Pork, butterfly steak grill lean 150 gm

Rice and other carbohydrate options

Choose one of the following

- Rice, boiled 150 gm
- Pasta, boiled 150 gm
- Potato, boiled or baked 300 gm

Special Instructions

- **Tomato Soup-** we ask you to have 300 ml of soup **every alternate day** starting from the 1st day of the study period. Heat your soup for two minutes on high in a microwave. If possible, try to use the same appliance and same bowl for soup for the duration of the study.
- **Tomato dishes** Remove one portion of the tomato dish from the freezer night before consumption and leave it to thaw in the refrigerator. Heat for two minutes on high in the microwave before consumption.

Biscuit (MUFA diet)

We ask you to have biscuit in the same meal as the soup.

Oil

• We have provided you with a daily cooking allowance of oil. You may use this oil to cook your meat and/or vegetables. Please make sure that you consume all the oil in the amounts prescribed.

Frozen vegetables

• We ask you to have the **pea and corn mix** for the majority of the study. You can choose any brand as long as you keep it consistent between diet period one and two.

Tomato products

- Please do not take any tomato products including
 - -Tomato sauce or other tomato based sauces such as Barbecue sauce and Cocktail sauce
 - -Tomato juice
 - -Tomato puree
 - -Tomato salsa
 - -Raw tomatoes in salad or sandwich

General Guidelines

• It is important that you eat the quantities of food in the amounts specified. If you have any difficulties, please do not hesitate to call us.

It is also important that you neither gain nor lose weight on this diet.

If you feel that the prescribed diet is too little or too much for you, please let us know straight away, so that we can make changes for you.

- Fruit, bread or milk can be taken at times other than those suggested in the meal plan.
- You must not eat any other foods except those specified for the diet period.
- We ask that you maintain your usual physical activity pattern. If you become ill during the diet period, please inform us as soon as possible.
- You may add two teaspoons of sugar daily to your tea or coffee.

Flavourings

• To enhance the taste of your meal(s), you may add any of the following flavourings

-Soy sauce, oyster sauce, black bean sauce

- -Salt
- -Pepper
- -Garlic
- -Ginger
- -Mustard
- -Lemon
- -Spices

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