EFFECT OF SUPPLEMENTATION OF BETA-CAROTENE AND ASCORBIC ACID ON LIPID PROFILES IN CARDIOVASCULAR PATIENTS

By

CHADALAVADA SRILAKSHMI

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN (DEEMED UNIVERSITY) COIMBATORE-43

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN FAMILY AND COMMUNITY SCIENCE

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Certified as bonafide research work

Signature of the Head of the Department

Signature of the Dean of Faculty of Home Science

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Signature of the Guide

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Contents

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Introduction

INTRODUCTION

Proper diet is the key to good health and vigour. Diet and nutrition are synonymous with health. It provides the necessary nutrients which give energy, promote growth and sustain the metabolic functions and repair processes that are essential for life. The preventive role of corrective nutrition is an ever evolving process. Inadequate and improper diets are not only responsible for undernutrition but also contribute to several chronic degenerative diseases such as cardiovascular diseases, diabetes and cancer (Ghafoorunissa and Krishnaswamy, 1995).

Diseases of the circulatory system account for an appreciable proprotion of total morbidity and mortality in adults through out the world. The different conditions assume varying degrees of importance in developing and affluent countries. For example, in developing countries rheumatic heart disease is common, but new cases of this disease are relatively infrequent in most affluent societies, where coronary heart disease (CHD) has assumed epidemic proportions. In which nutritional factors play an important role in aetiology and management (Mann, 1993).

Cardiovascular disease is responsible for about 40 per cent of deaths in developing countries. Simple accessible and cost effective preventive therapies that decrease the incidence of CVD could greatly effect public health, substantial interest has recently focused on the hypothesis that the naturally occuring antioxidant vitamins

such as vitamin E, C and Beta-carotene may prevent myocradial infraction, progression of coronary heart disease or strokes (Prabhat, 1995).

Cardiovascular disease can no longer be considered a problem of only affluent countries. With rapid industrialization, socio-economic development and increase in life expectancy, the state is now set for chronic diseases in India. In recent years, there has been a significant change in life style and dietary patterns with growing affluence. There is enough evidence linking dietary habits to cardiovascular diseases. New research findings in this area indicate the potential value of diets in prevention of such disorders. It is propably the right time to take of fresh look at the diets consumed by our population and formulate appropriate dietary guidelines to prevent and control these diseases (Ghafoorunissa and Krishnaswamy, 1995)

Everyone wishes to remain youthful even in oldage. Sensible and healthy food habits right from childhood coupled with good life styles care, not only delay ageing and increase the life space, but also add to the quality of life, "Health for all by 2000 A.D" and "All for Health" should encompass diseases due to inadequate as well as faulty diets. As we move up the ladder of economic properity, diet associated chronic diseases begin to assume significance. A changing demographic profile and technological progress can lead to many health problems. Coupled with sedentary life

styles, imbalanced diets can contribute to chronic degerative diseases.

Today coronary artery disease and hypertension have emerged as the most important cause of premature disability and death all over the world. Genetic susceptibility (heredity) and being a male, makes one more prone to develop these diseases in middle age. It is also known that coronary artery disease is an inavoidable effect of socio-economic development. It is therefore important to concentrate on factors which can reduce the risk of developing heart disease (modifiable risk factor). Results of several large scale studies show that certain strategies based on dietary modifications, is implimented, can decrease the morbidity associated with diseases of heart and blood vessels and bring about a regression (decrease) of the pathalogical (abnormal) process.

The limited information on heart diseases in our country indicates a higher prevalence in the urban population. further, Indians seems to develop the disease at an earlier age than people in the West. Even Asian immigrants in the West have higher incidence of heart disease and diabetes than the local population. It is therefore necessary to reduce the prevalence of these disorders by modifying and related risk factors.

The framingham (1994) data showed an unquestionable link between high blood cholesterol and heart disease.

Both high cholesterol and high triglycerides in the blood appear to be important risk factors for atherosclerosis. In adults less than 55 years of age, a cholesterol concentration greater than 200 mg/100 ml or a triglyceride concentrations greater than 200 mg/100ml, clearly indicates high fat content of the blood (Jaggi, 1990).

Hyperlipidemias of genetic origin are different from diet induced elevations of blood lipids. The enormous increase of blood lipids in hyperlipidemias is due to defects in certain metabolic processes. Therefore, in addition to regulating diets drugs may be required to control the blood lipids.

Thus, while it is urgent to develop new and better means of treating patients who have heart disease, it is more urgent to discover and implement methods of preventing the disease, some rules of personal health behaviour have been identified as important factor in a reduction of the risk of cardiovascular disease.

Given the link between diet and coronary heart disease and especially the importance of eating unsaturated fats, dietary advice and government action to reduce fat consumption in general and saturated fats both of animal and plant origin in particular are important weapons in the fight against coronary disease (Jose, 1996).

Increasing attention is being given to the role of certain micronutrients and other elements in the diet, some

have antioxidant properties, which may provide protection against atherosclerotic ischaemic heart disease (Jose, 1996).

Oxygen free radical reactions have been implicated in many chronic disease processes, including atherosclerotic cardiovascular disease. Recent studies on lipid metabolism have suggested that oxidative modification of low-density lioproteins accelarates atherogenesis. Micronutrient antioxidants, including alpha- tocopherol and Beta-carotene, how ever, can neutralize oxygens free radical and inhibit low density lipoproteins oxidation (Richard, 1995). This

Epidemiologic research on the role of carotenoids in the development of cardiovascular disease supports the hypothesis that carotenoids have preventive potential. This research includes, ecologic examinations of dietary intakes in relation to trends in cardiovascular disease mortality, case control studies of the differences in serum or adipose tissue concentrations of Beta-carotene between those developing myocardial infractions and those remaining free of this or other expressions of cardiovascular disease and cohort studies relating the development of cerebrovascular disease or even greater intima wall thickness to lower consumption of carotenoid rich foods. This research has foccused largely on Beta-carotene, one of several carotenoids in the U.S diet (Lenore and Susan, 1995).

Gerster; (1991) says that short term intake of Beta-carotene in doses of 300 microgram/day was shown tc lead to a modest but significant increase in serum HDL of healthy adults. In a 2 year study intakes of 20 mg a high significant increase in HDL lipoprotein was found suggesting a decreased cardiovascular risk with prolonged intake of moderate Beta-carotene concentration.

Vitamin C may help improve blood flow and possibly prevent heart attacks in people with coronary artery disease according to a preliminary study. It is an antioxidant, mopping of free radicals or unstable compounds, that damage the living of blood vessels leading to heart and presenting the normal flow of oxygen rich blood. The recommended daily allowance (RDA) of vitamin C is 60 milligrams but Dr. Hopkins recommends that patients should generally double their recommendation (Denise mann, 1997).

Hence the present study was undertaken with the following objectives.

1. To study the socio-economic and dietary pattern of the selected CVD patients
2. To analyse the lipid profile of the selected cardiovascular patients.
3. To observe the effect of supplementation of Beta-carotene, Beta-carotene and ascorbic acid on lipid profile.

Review of Literature

II REVIEW OF LITERATURE

Review pertaining to the study "Effect of supplmentation of Beta-carotene and ascorbic acid on lipidprofiles in cardiovascular patients" is as follows :

A. Prevalence of cardiovascular diseases

B. Web of risk factors for cardiovascular diseases

C. Dietary factors and cardiovascular disorders

D. Prevention of cardiovascular disease

E. Antioxidant vitamin and cardiovascular disorders

F. Role of Beta-carotene and ascorbic acid on lipidprofile

A. Prevalence of cardiovascular diseases

In 1988, coronary heart disease (CHD) accounted for about 36 per cent and cancer for 22 per cent of deaths in the U.S if stroke is included the figure for cardiovascular fatalities rises to about 43 per cent. In Europe great difference has been observed in CHD mortality between but also with in countries with a pronounced north - south gradient. The age standardized CHD death rate in 35 to 64 year old males was about 400 to 500 per 1,00,000 males in Finland, 150 in Belgium and only about 100 in Italy. Interestingly, life expectancy of 65 years of age is comparable in all industrialized countries and seems to have reached a plateau at around 72 years for males and 81 years for females. This suggest that, except in cases of premature death, life style determines the type of disease that will be the major cause of death rather than extending the actual life expectancy (Helgagerster, 1991).

A recent world health report states that as per data available for 1993. Heart attacks kill 9.7 millions per year is the second biggest killer. The mortality rate in India is raising rather steeply, it is estimated that 2.5 million persons die here yearly. In India victims are comparitively young (Chopra, 1995).

According to Johnston et al., (1994) coronary artery disease is common among the people who have total serum cholesterol more than 200 mg/dl. Total cholesterol values was positively related to mortality from heart disease in younger men and middle aged and older men and women.

Cardiovascular disease is more prevalent among 75 per cent of subjects who have HDL cholesterol less than 0.9 m.mol and also among the person those who have high total cholesterol to HDL cholesterol ratio (Meilahn et al., 1992).

Data from cardiovascular health study indicates that 36.1 per cent women and 38.7 per cent of the men has sub clinical atherosclerosis and cardiovascular disease and that the prevalence increased with age (Kuller et al., 1994). According to Chada et al., (1994) over all incidence rate of coronary heart disease was 19.7/1000 in urban area where compared to 9.2/1000 in rural areas in India.

The adult treatment programme conducted in 1989, CVD was listed as the underlying cause of death in nearly half of the persons with diabetes. Over the age of 55 years, persons who have diabetes are at high risk of developing coronary heart disease (Tinker, 1994).

Women with increased age combined with elevated systolic blood pressure, increased LDL, decreased HDL cholesterol and increased fasting blood sugar and cigarette smoking was associated with 3.5 fold increase in the prevalence of subclinical atherosclerosis (Kuller et al., 1994).

According to Hiroshi (1990) cardiovascular diseases claims 12 million lives each year and cancer 4.9 million. Much suffering and many deaths from these diseases could be prevented by adopting a healthy lifestyle, particularly in regard to Food and nutrition, moderating alcohol consumption and significantly reducing the use of tobacco.

In India, coronary heart diseases accounts for 10 to 15 per cent of all cardiovascular disease (Sucheta et al., 1992). According to ICMR (1992) it is estimated that there are nearly 20 million hypertensive and 15 million cases of CHD in India and a few epidemiological studies have indicated a higher prevalence in urban than in rural areas. Presently 10 per cent of mortality in India is due to heart diseases.

In the Western countries the incidence of heart attacks is said to be more in males belonging to higher income group. But in India, they occur equally among the rich as well as poor. From Gujarat in 1968, the occurrence of heart attacks occupation wise was reported as follows: Executives, professionals 28 per cent, clerks, teachers 23.5 per cent, manual workers 25.5 per cent, sedentary workers,

shopkeepers 15.5 per cent, the retired people 7.5 per cent (Jaggi, 1990).

B.Web of risk factors for cardiovascular diseases

According to Durairaj (1997) the common risk factors of coronary artery disease are :-

1. Age : males over 45 years and females over 55 years
2. Family history of ischamic heart disease or sudden cardiac death
3. Current cigarette smoking
4. High blood pressure
5. Low high density lipoproteins
6. High blood cholesterol
7. Diabetes mellitus and other such as obesity, physical inactivity and contraceptive pills

Atherosclerosis, in particular coronary heart disease is related to a number of risk factors such as obesity and lack of exercise. The importance of all the risk factors is related to their effects on increasing the levels of blood cholesterol. In 1-2 per cent of the population high blood cholesterol levels are caused by genetic abnormalities. But in developed countries, high blood cholesterol is caused mainly by an inadequate diet. Eipidemiological studies have shown that in populations with diets low in cholesterol and fats, coronary heart disease and thrombosis are less frequent than in other populations with high fat and cholesterol consumption (Jose, 1996).

John (1994) points out that the major reason for the greater heart attack risk among non-vegetarians is

because of cholesterol and saturated fat. All cholesterol and 70 per cent of saturated fat in the average diet come from animal products our bodies make cholesterol in sufficient amounts, so we don't need it in our diet.

1. Age and sex:

According to Devadas (1990), coronary heart disease usually manifests itself after the age of 50. Indians tend to develop coronary heart disease at an earlier age than people in Western countries. The highest incidence was found between 41 and 60 years. Serum cholesterol levels were significantly raised in CHD patients in the age group of 21-50 years.

Ayanian (1991) opines that since 1950, the incidence of coronary heart disease has risen among women while it has declined among men. Major advances in diagnosis and treatment have contributed to a decline in over all mortality from coronary heart disease in both sexes, over the past 20 years. However, some evidence suggests that there are systamatic differences between women and men in the application of these major advances (Antia, 1989).

There is an overwhelming evidence that men aged 25 to 55 are at high risk for CHD when their cholesterol level is 240 mg/dl or above while those with lower cholesterol readings have less risk. Premeno pausal women with cholesterol levels above 240 mg/dl develop CHD unless they have additional risk factors (Pekkanen, 1994).

2. Family history:

It is common for coronary disease to occur in several members of the same family. When coronary heart disease occurs in an individual under the age of 50-55 years, consideration should be given to investigating other members of the family for risk factors (Durairaj, 1997).

The occurrence of heart attacks in several members of a family is a common observation. It has been found that the incidence of heart attacks is nearly 4 times as frequent among siblings of persons with CHD as among siblings of persons without it. Genetic factors secure to be involved in predisposing some families to these diseases (Jaggi, 1990).

3. Cigarette smoking:

It is an important risk factor. Smokers have two or three times greater risk of developing ischamic heart disease than non-smokers. The risk increases with the number of cigarettes smoked and the number of years one has smoked. After stopping smoking cigarettes, the risk of coronary artery disease is progressively reduced, but it may take upto 20 years for it to fall to that of a non-smoker (Durairaj, 1997).

Studies from India have shows that tobacco smoker carries a three-fold higher risk for CHD. Death due to CHD is nearly three times higher among smokers as compared to that in non-smokers. Smoking leads to premature CHD, peripheral vascular disase and stroke (ICMR Bulletin, 1993).

4. High blood pressure:

According to Hauta (1993) there is cross sectional association between hypertension and cardiovascular disease. The most common risk factor for the development of coronary artery disease is elevation of systolic blood pressure.

High blood pressure is called "The silent killer", because it strikes without warning usually a person doesn't feels ill with this disease. Suddenly a blood vessel in the brain bursts, causing a stroke or the pressure causes severe damage to heart, kidney or eyes (Wasir, 1992).

5. Diabetes mellitus:

One of the most common cause of death in diabetes is coronary artery disease. The risk is markedly increased in younger diabetes.

Diabetes causes a lot of complications an important one being the narrowing of the blood vessels called atherosclerosis. This change occurs at an earlier age in diabetes and is more extensive. Hyperglycemia is known to affect aortic wall metabolism. Coronary artery disease is common in diabetes. Women who have diabetes suffer heart attacks more aften. In a study abroad, it was found that 46 per cent of apparently non-diabetic patients with atherosclerotic coronary heart disease had an abnormal glucose tolerence. In Kanpur 49 per cent patients with CHD were found to have impaired glucose tolerence.

Diabetes mellitus is one of the major hormonal disturbance that has profound influences on atherosclerosis.

The diabetes has elevated levels of LDL and VLDL lipoprotein cholesterol together with reduced HDL cholesterol a picture which is recognised as a hall mark of lipidprofile of ischamic heart disease (Datta, 1990).

Raheja (1990) says "today there is considerable epidemiological evidence to indicate that Indians have increases sucepitability, to diabetes mainly atherosclerotic heart disease, developing them at a comparitively younger age and often without associated risk factors.

6. Obesity

Many studies have shown a link between obesity and coronary artery disease. After age 25, a person starts gaining weight, unless he does something about the food he eats and the amount of daily activity. The reason is that, beginning at this age, the body starts to need less food because the metabolism in slowing down.

In general disease and death from heart disease are higher in direct relation to the degree of overweight. Data obtained in the FRAHIMGAM study, appears that obesity may accelarate atherosclerosis and its effect is more apparent before age 50. The relationship between obesity and atherosclerosis, is thus multifaceted, it is of considerable importance as a risk factor

Studies conducted by Croft et al, (1995) revealed that the cut off points for high waist to hip ratio (WHR) that define high risk for CVD has been suggested men (0.95) and women (0.80). A high WHR are associated with increased

risk for death over all and mortality due to CVD, diabetes, high levels of blood pressure and lipids and insulin.

Weight, height and waist and hip circumference were measured for women of urban and rural areas of South China, Mean BMI ranged from 20.1 per cent to 21.9 respectively. Mean waist hip ratio was 0.84 in men and 0.80 in women. After accounting for age and BMI, waist - hip ratio was associated negitively with fasting serum HDL cholesterol and Positively with serum triglycerols, total and LDL cholesterol, glucose and mean systolic blood pressure. BMI was associated in a similar direction with most of these risk factors (Folsom, 1994)

7. Physical inactivity:

The evidence is increasing that lack of physical exercise is a contributory factor to coronary artery disease. Studies have shown that life long exercise protects against coronary events independently of smoking, obesity, hypertension or parental death from heart disease. Although earlier studies have suggested that exercise has to be vigorous in order to be effective, more recent findings suggest that only moderate exercise is needed to achieve the benefit (Durairaj, 1997).

Francis (1989) suggests that physically active people have fewer heart attacks than the lazy. It is thought that the heart is protected by long term regular exercise including regular amounts of fairly intensive work such as sport participation and garden digging.

8. Stress:

Curan (1989) defines family stress as "a condition that arises when family life gets out of control". Sress dces play a role in aggravating several diseases. When stress becomes excessive it will bring rapid change in bcdy chemistry trigger the release of volumes that raise our blood pressure and heart rate (Hausel, 1991).

Constant worry and tension stimulates the actual grounds to produce more adrenaline and cortisone. This contributed to constructed arterier, high B.P and increased work for the heart (Sucheta et al, 1992).

9. Alcohol and Coffee:

There is now good evidence that moderate consumption of alcohol is associated with a lower risk of coronary artery disease than either totalism or heavy drinking. However, the evidence is not strong enough to encourage non-drinkers to take up drinking. Evidence against coffee is not strong, but some individuals develop rapid heart beat or even irregular heart rhythm with strong coffee. So too much strong coffee is to be avoided (Durairaj, 1997).

10. The contraceptive pill:-

A number of studies have indicated that taking the pill can increase blood pressure and blood cholesterol levels and make the blood to clot more. However, the modern pill tends to have lower doses of hormone that the ones involved in earlier studies (Durairaj, 1997).

C. Dietary factors and cardiovascular disorders

According to Jose (1996) the relationship between food intake and cardiovascular disease is discussed with particular reference to the consumption of saturated fats. Total dietary fats should not provide more than 30-35 per cent of calorific intake.

Increasing attention is being given to the role of certain micronutrients and other elements in the diet, some having antioxidant properties, which may provide protection against atherosclerotic ischaemic heart disease.

Proteins, preferably of plant origine, should provide about 15 per cent of calorie intake. There is evidence that plant proteins can reduce blood cholesterol levels (Jose, 1996).

According to Srilakshmi (1993) increasing fiber in the diet will serve to recuce cholesterol. Pectin (Apples, Guavas) lowers the level of serum cholesterol and enhance the excretion of faecal steriods. It has no effect on serum triglycerides and HDL cholesterol. Guargum had hypocholesteraemic effect. Legumes, vegetables and fruits can lower the level of serum cholesterol but the effect is usually small compared with the well known effect on HDL and serum triglycerides.

Studies carried out at NIN have shown that fenu greek seeds contain 50 per cent total fiber and 20 per cent of soluble fibre and they not only reduce serum lipids but

also reduce blood glucose. Therefore 20-25g of fenu greek seeds (4-5+SP) in two or three divided doses along with food are recommnended for diabetes and heart diseased patients.

The effects of various fatty acids and fatty acid classes, the effects of replacing hard fats by carbohydrate rich foods or oils on food lipids and lipoproteins and the implications of such dietary changes for risk of coronary heart disease are reviewed (Katan, et al, 1995).

Fish is an abundent source of long chain n-3 PUFA. These fatty acids decrease the fribrinogen levels, inhibit the activity of platelets and the clotting process. In addition these fatty acids reduce the blood levels of cholesterol and triglycerides (Ghafoorunissa and Krishnaswamy, 1995).

D.Prevention of cardiovascular disease

According to Durairaj (1997) primary prevention of coronary artery disease means prevention of death and non-fatal events in healthy population. Secondary prevention is the term used to desirable measures that are designed to prevent reinfraction and death due to coronary heart disease. The process of atherosclerosis begins in the teens and is often well advanced by the time one attains the age of 50 years. Atherosclerosis can be serious because it cuts down the blood flow. Hyperlipidaemia plays a major role in the genesis of atherosclerosis and coronary artery disease.

Therefore low intake of fats and cholesterol prevents the risk of cardiovascular diseases.

A review manifestation of coronary heart disease differ in women and men. Several risk factors in women such as elevated blood lipids and lipoproteins, body weight, hypertension and beneficially responsive to nutrition intervention. About 27 per cent of all women and 50 per cent of women aged 55 to 74 years are candidates for dietary intervention. It will be important to understand the effects of gender, menopausal status and age on dietary responsiveness in order to significantly reduce the incidence of CHD in women (Kris et al, 1993).

According to Jose (1996) the dietary recommendation for the prevention of arteriosclerosis are

1. Cholesterol intake should not exceed 300 mg/day
2. Total dietary fats should not provide more than 30-35 per cent of calorific intake. Saturated fats, mono-unsaturated fats and poly unsaturated fats should not provide more than 10 per cent, 10-15 per cent and 10 per cent respectively, of this intake, with a contribution of 0.4-0.6 per cent from n-3 polyunsaturated fats.
3. Carbohydrates, such as fibre starch and sugar should provide 45-50 per cent of caloric intake, fibre, which includes cellulose, semicellulose, legnin, pectin and Beta glucons, helps to reduce blood levels of cholesterol - LDL.

4. Proteins, preferably of plant origine, should provide about 15 per cent of caloric intake. There evidence that plant proteins can reduce blood cholesterol levels.
5. Fasting and weight loss reduce cholesterol - LDL levels, even in patients with heredity hypercholesterolaemia. Physical exercise is very important in this connection.
6. Excessive alcohol consumption should be avoided. Since it not only poisons the liver but can also damage the myocardium and increase the risk of hypertension and cerebrovascular accident ie., upto 30g per day increases the risk of arteriosclerosis.

E.Antioxidant vitamins and cardiovascular disorders:

Indian Council of Medical Research (1996) opines that among the dietary factors, apart from fat, fibre is yet another important factor which influences blood cholesterol. Fruits and vegetables are also rich in antioxidants like vitamin C,E and Beta-carotene. LDL-cholesterol, to be atherogenic has to undergo chemical reaction known as oxidation. The antioxidants prevent oxidation of LDL cholesterol and reduce the risk of coronary heart disease. Pulses like bengal gram are rich in fibre and are reported to reduce cholesterol levels. Cluster beans, psyllium and oats are also rich in soluble fibre. It is recommended that fibre intake should be around 40 grams per day.

Several epidemiological studies have shown that people who consume large amounts of vitamins, including Beta-carotene, have lower rates of coronary artery disease. The potential benefit of vitamins is supported not only by epidemiological investigators but also by laboratory research that suggests a possible mechanism for how antioxidant vitamins might lessen the adverse impact of cholesterol. The one piece of advice that is not controversial is to eat a diet rich in fruits and vegtables. These foods are rich in antioxidants and can also help people lose weight and lower their cholesterol levels. Studies consistently show that the people who eat more of these foods have a lower heart attack risk (Janet, 1995).

The use of large doses of vitamin E suppelements is associated with a significantly decreased risk of coronary heart disease. The data on vitamin C intake were essentially negative in both studies and were thus at variance with the findings of Enstrom et al, who showed that in a comparably large population both mortality from coronary heart disease and over all mortality were inversly correlated with vitamin C intake. In men who smoked, carotene intake was associated with a lower risk of coronary heart disease in the Health professional follow up study. But the fact of the matter is that we do have available a number of natural antioxidants, including vitamin E, vitamin C and Beta-carotene, that are generally considered not to be toxic (Daniel, 1993).

Antioxidant vitamins have been postulated to prevent coronary heart disease (CHD) by impeding the oxidation of lipoproteins. Benefits have been reported for vitamin C, Vitamin E and carotene when examined separately. A high total antioxidant vitamin score is associated with a reduced risk of CHD in women (JoAnn, 1992).

Plasma concentrations of vitamin C and E and carotene were significantly inversly related to the risk of angina. There was no significant relation with vitamin A smoking was a confounding factor. The increase relation between angina and low plasma carotene disappeared and that with plasma vitamin C was substantially reduced after adjustment for smoking. Vitamin E remained independently and inversly related to the risk of angina after adjustment for age, sex, smoking habit, blood pressure, lipids and relative weight. His finding suggest that high incidence of coronary heart disease may benefit from eating diets rich in natural antioxidants, particularly vitamin E (Riemersma et al, 1991).

Antioxidants such as ascorbate (AA) Alphatocopherol and Beta-carotene (BC), levels of which can be increased with diet without side effects could be a safe way in preventing LDL-oxidants. There was a significant inverse correlation between all 3 antioxidants and LDL oxidation rate (Ishwarlal et al, 1992).

Although nature provides a large number of mechanisms for defense against oxidation and free radical

induced damage, the predominant antioxidants protecting the LDL molecule are Alpha-tocopherol, retinyl stearate, V - tocopherol and Beta carotene. Alpha- tocopherol converts the peroxyl free radical to the much less reactive hydroperoxide, thus inhibiting the propagating step in lipid peroxidation. Beta-carotene, a plant derived hydrocarbon carotenoid is an effective scavenger of oxidizing radicals, particularly siglet oxygen. Ascorbic acid also derived from fruits and vegetables, is the first line of defense against oxygen radicles in the water soluble compartment and plays an important role in regenerating reduced alpha-tocopheral (Richard et al, 1995).

Substantial laboratory, animal and human data suggest that oxidation of low-density lipoprotein (LDL) cholesterol is an important step in the pathogenesis of atherosclerotic lesions. The major lipid-soluble anti-oxidants vitamins are vitamin E and Beta-carotene, a precursor of vitamin A. The major water soluble antioxidant is Vitamin C. Vitamin E is important in preventing oxidation of LDL cholesterol, Beta-carotene also prevents oxidation of LDL cholesterol whereas vitamin C prevents LDL cholesterol oxidation and perserves vitamin E and Beta-carotene levels during oxidative stress (Jha et al, 1995).

In the prospective observational studies, antioxidant supplemented group is compared with non-vitamin supplemented group. Persons who subsequently did and did not develop cardiovascular disease (Jha et al, 1995).

According to Frel (1995) the antioxidants may act as antiatherogens has stimulated a great number of basic research, studies and chemical intervention trials. Epidemiological data from two large prospective cohort studies suggest a strong protective effect of the nutrient antioxidants vitamin E and in smokers Beta-carotene against coronary heart disease. Increased intake of vitamin C, a known potent antioxidant nutrient also has been associated with substantially lowered cardiovascular disease mortality. Angina pectoris, a chemical indicator of cardiovascular atherosclerotic disease, is associated with decreased plasma concentrations of vitamin C and E and Beta-carotene.

Rapidly increasing evidence suggests that oxidation of low density lipoprotein is important in atherogenesis. As reviewed by Steinberg oxidized LDL is preferentially taken up by macrophages to create foam cells. Dietary antioxidants, particularly vitamin E can function as free radical scavengers to decrease the initiation and propagation of fatty acid oxidation. In addition of protecting LDL vitamin E can inhibit smooth muscle cell proliferation which is an important component of atherogenesis. Finally vitamin E may reduce the risk of CVD through its effect on platelet adhesion laboratory data. Recent animal and epidemiologic data also support the hypothesis that vitamin E may reduce risk of cardiovascular disease (Meir and Eric, 1995).

The study conducted showed that a supply of Beta-carotene (15 or 30 mg/day), a vitamin E (15 mg/day) and

vitamin C (30 mg /day) involved an increase of singlet oxygen protection of erythrocytes. This protection disappeared very quickly after 15 days of treatment (Postaire et al, 1996).

Gey (1995) says evidence on the relation between antioxidant micronutrients (Vitamin E, C, and Beta-carotene and others) intake and risk of cardiovascular diseases is reviewed.

According to Heseker (1994) High intakes of selenium, vitamin C and E and Beta-carotene are likely to be protective. Indeed studies suggest a co-relation between the incidence of coronary heart disease and levels of vitamin E or levels of seleinium suggesting that antioxidant status may be relevant to the human disease. Infact preliminary data from the physicians health study suggest that Beta-carotene reduces the risk of cardiovascular events in subjects with existing CHD.

F. Role of Beta-carotene and ascorbic acid on lipid profile

A recent analysis of this risk group demonstrated that those subjects receiving 50 ug Beta-carotene on alternate days experienced a statistically significant reduction in cardiovascular events.

Dietary Beta-carotene was associated with decreased cardiovascular mortality in a prospective cohort studies. Those assigned to 50 mg of Beta-carotene on alternate days had a 44 per cent reduction in all major coronary events and 49 per cent reduction in all major vascular events. The data

from these prospective studies provide support that Beta-carotene a therapy without clinically important side effects reduces the risk of cardiovascular disease. First, the universe association between antioxidant consumption and CVD second a temporal relationship between exposure and outcome and minimizes recall bias. Finally, the protective benefit of antioxidants is biologically possible, based on research linking oxidation of LDL and atherosclerosis (Richard et al, 1995).

Carotenoids are largely distributed in lipoprotein membranes and the lipid phases of intracellular structures usually together with vitamin E. Dietary intake of carotenoids has been associated with a reduced risk of several chronic diseases (Olson, 1993).

Dietary carotenoids have diverse biological functions and actions in areas of human health including chemoprevention, cardiovascular disease, cataract prevention and immune responses (Bendich, 1993).

According to (Witztum et al, 1993) studies on the ability of dietary supplementation of **Beta-carotene** to protect LDL from oxidative modification suggest that even a 20 fold enrichment of LDL with Beta-carotene provided enhanced protection against oxidation.

The function of Beta-carotene as an antioxidant are reviewed and evidence of its possible role in the prevention of atherosclerotic disease is presented. Possible mechanisms by which Beta-carotene may impede the progress on

of atherosclerosis are also considered (Gaziano et al, 1993).

Hughes et al (1995) says Beta-carotene in doses of upto 300 mg daily increases HDL-cholesterol with in two or four weeks in healthy subjects. Effect of high dose Beta-carotene upon serum lipids, lipoproteins and selected sex steroid hormones were investigated in 59 adult patients and 36 healthy controls. There is no alternation in sex steroid hormones but there is an increase of 10 per cent HDL-cholesterol.

Plasma vitamin 'C' concentration were found to be directly associated with plasma levels of high HDL-cholesterol. Therefore high levels of plasma vitamin C lowers the risk of cardiovascular disease (Halfrisch et al, 1994).

There is also evidence that vitamin C may protect against atherosclerosis by enhancing HDL-cholesterol levels. Recent epidemiological data confirms that vitamin C serum levels were significantly and positively associated with HDL-cholesterol predictive for lowering coronary risk. A study in Switzerland showed that men with low plasma vitamin C levels had a higher risk of ischemic heart diseasse or stroke, during 12 years follow up and vitamin C plasma levels below 26.1 mmol/lit. which is associated with an intake of 50 mg vit/day increased risk of mortality from cardiovascular diseases.

Recently a reduction of 10.3 per cent in systolic blood pressure was found in an elderly cohort after a 6 week supplementation of 250 mg vitamin c/day (Weber et al, 1996).

Catharine et al (1995) reported that mortality from stroke was highest in those with the lowest vitamin C status. A similar gradient in risk was present for plasma ascorbic acid concentrations. No association was found between vitamin C status and risk of death from coronary heart disease. Therefore antioxidant vitamins are potentially important in the prevention of cerebro vascular disease Vitamin C is the most important dietary antioxidant in terms of intake.

The free radical scavenging activity of ascorbic acid was investigated by assessing their ability to inhibit the peroxidation of linoleic acid in sodium doecyl sulphate (SDS) micells at 37 ^{o}C. Ascorbic acid was observed to inhibit the peroxidation in a concentration dependent fashion with an IC50 value of 17 mm (Kaufman, 1995).

Methodology

III METHODOLOGY

The methodology pertaining to the study on 'Effect of supplementation of Beta-carotene and Ascorbic acid on lipid profiles in cardiovascular patients" was done in the following sequence.

A. Selection of the venue

B. Selection of the sample

C. Framing the interview schedule and conducting the survey

D. Assessment of nutritional status

   a. Anthropometric measurements

   b. Recording the mean food and nutrient intake

   c. Analysis of serum for lipid levels

E. Supplementation with Beta-carotene and Ascorbic acid for CVD patients

A. Selection of the venue:

For the conduct of the study the venue selected was a private Cardiac Centre in Vijayawada where adequate cardiovascular diseased patients were available and the management was very co-operative and also the patients were willing to participate in the above study. This cardiac centre is specially catering the needs of the patients suffering from cardiovascular diseases and also attached with a well equipped bio-chemistry laboratory where the lipid profile was done for all the patients.

The selected subjects were eager to know their lipid profile and hence provided full support for the completion of the study. They are interested in lowering their total cholesterol and triglycerides levels and readily accepted the supplementation of Beta-carotene and ascorbic acid.

B. Selection of the Sample

Two hundered patients (124 men and 76 women) suffering from cardiovascular diseases, both in-patients and out-patients coming for regular check-up were selected at random for the study. Patients selected were from low, middle and high income groups and were in the age range of 45 to 70 years. Among the 200 patients 20 hyperlipidemic patients were selected for supplementation of Beta-carotene and ascorbic acid. Among the 20 sub samples 6 male patients and 4 female patients are supplemented with Beta-carotene and 2 male patients and 8 female patients were supplemented with both Beta-carotene and ascorbic acid. group 'B'.

Ten patients were supplemented with 500mg of spirulina containing 600 microgams of Beta-carotene (Natoxid tablet) and the other group was supplemented with 600 microrgrams of Beta-carotene and 500 mg of ascorbic acid (Celin tablet) per day.

C. Framing the interview schedule and conducting the survey:

According to Kothari (1991) the best mehtod of collection of data is the interview schedule method in which

the investigator fills up the proforma containing a set of questions.

Hence an interview schedule was formulated and the schedule includes questions regarding the age, sex, type and the size of family, levels of education, occupation, family income per month, dietary pattern and dietary practices, family history, habit of smoking and drinking alcohol, exercise schedule and intake of Beta-carotene and ascorbic acid.

Using the interview schedule thus prepared (Appendix I) the information relevant to the research work was collected from all the selected cardiovascular patients.

D. Assessment of nutritional status

Nutritional assessment is the process where by the state of nutritional health of an individual is determined. It includes anthropometric, chemical, bio-chemical and dietary data. The conclusions reached through nutritional assessment become the basis for the development of intervention programme in the community and for the planning and implementation of nutritional care for individuals.

According to Robinson (1986) nutritional status refers to the health of an individual as it is affectec by the intake and ultilization of nutrients. Normal nutrition implies a sufficiency of nutrients and energy intake neither deficiency nor excess that affords the highest level of wellness.

a. Anthropometric measurements :

Anthropometry deals with comparative measurements of the body. These measurements permit estimation of body fat, muscle tissue and bone. They may inlcude height, weight, head, arm and chest circumference, waist and hip ratio, skin fold to body fat.

Body weight is a composit of body water, lean tissue adipose tissue and bone. Weight is the basic indicator in clinical practices. Beam balance scales are accurate, if carefully looked after. For older people, light weight under pants or shorts or other minimum clothing of known weight will usually be retained. Theoretically weighing should not be done after a full meal, nor with a full bladder (Jelliffe, 1989).

Standard height in preferably measured against a fixed scale attached to a wall. The client is told to stand errect with feet together and heels back against the wall and eyes looking straight a head. In this study, for two hundred patients height and weight measurements were recorded. The most commonly used ratio is the quetlet's index or Body Mass Index (BMI). BMI is body weight in kilograms divided by the height in meters square. BMI of 25-30 is an indiction of being overweight and above 30 indicates obesity (Gopalan et al,1989). Abdominal obesity is often measured as the waist to hip ratio (WHR), which is the ratio of obesity in the upper trunk to that is the lower trunk. A high WHR is

associated with increased risk for death. Circumferences were measured to the nearest inch at the waist (umbilicus) and at the hip (maximum posterior extension between the illic crest and buttocks). The WHR was calculated by dividing waist girth by hip girth (Janet et al, 1995).

b. Recording the mean food and nutrient intake

Dietary assessment and dietary status are important parts of the larger picture of characteristics that, taken to-gether, describe the relationships between diet, health and disease. The characteristics may include nutrients of other food constituents, foods, food groups dietary patterns (Maurice et al, 1994).

For the sub-sample food weighment survey was conducted. In which the actual food intake of the subjects was weighed for three days and the mean food intake/individual was calculated. Then the nutrient intake of the patients were calculated with the help of the "Nutritive value of Indian Foods" (ICMR, 1995). Thus the mean food and nutrient intakes was recorded for the patients and compared with recommended dietary allowances suggested by ICMR.

C. Analysis of serum for lipid levels

Blood lipid levels have been shown to be related to the incidence of myocardial infarction, cerebrovascular accident and hypertension. The blood lipid profile is used to determine the accounts of different lipids in the blood in

order to assess the risk of presence of cardiovascular disease. Hyperlipidemia is characterized by an elevated level of serum cholesterol and triglycerides which are carried in the blood bound with specific proteins called lipoproteins (Aronson,1990).

Lipoproteins are packages of cholesterol and protien that carry fat and cholesterol in the blood stream. Low density lipoprotein (LDL) carry cholesterol from the liver to the blood vessels and other tissues and High density lipoprotein (HDL) scavenge cholesterol from the blood vessels and other tissues to the liver. Elevated LDL levels are below 130 mg/dl, HDL should be above 35 mg/dl. Elevated blood levels of triglycerides is also an independent risk factor for heart disease.

Lipid profile was done for the sub-sample of twenty cardiovascular patients before the supplementation and after 30 days of supplementation and finally after 60 days of supplementation.

5 ml of blood was drawn, serum was separated and used for the analysis of lipid profile namely serum total cholesterol, serum triglycerides and high density lipoproteins. The lipid profile was estimated with the help of the kits available using the following methods. The procedure for the estimation is given in Appendix II.

1.	Total cholesterol	Enzymatic - colorimetricmethod suggested by Allain et al(1974)
2.	Triglycerides	Enzymatic method suggested by Bucolo et al (1981)

3.	High density lipoproteins	Enzymatic method suggested by Lopex et al (1977) and Allain et al (1974)

4. Very low density lipoproteins - The very low density lipoproteins values are calculated from the triglyceride values, using the following formula

$$\text{VLDL cholesterol} = \frac{\text{Triglycerides}}{5}$$

5 is a constant factor.

5. Low density lipoprotein - The low density lipoprotein cholesterol values are calculated from the triglycerides, HDL and total cholesterol values using the following formula

LDL = Total cholesterol - HDL cholesterol - VLDL-C

The above calculations are applicable only if triglyceride concentration is not more than 400 mg/dl

E. Supplementation with Beta-carotene and Beta-carotene with ascorbic acid

After analysing the lipid profile of the selected subjects and recording their total cholesterol and other lipid levels, one group of 10 heart patients (Group A) were supplementated with Beta-carotene tablet/day (Plate I) for a period of 60 days.

The Beta-carotene tablet selected for supplementation was "NATOXID" containing 500 mg spirulina in each tablet providing 600 micro gram of Beta-carotene. This

was selected because it contains spirulina and is not toxic to the human body in excess and also, it stores in the liver like the food vitamin. It is cheap and easily available in the market.

The second group of 10 patients were supplemented with Beta-carotene and ascorbic acid tablet (Plate II). The name of the tablet is Celin, the amount of ascorbic acid given was 500 milligram/day along with Beta-carotene tablet (Natoxid) regularly for a period of 60 days.

To study the effect of supplementation of antioxidants, blood profile was analysed for the patients after 30 days and also after 60 days of supplementation period.

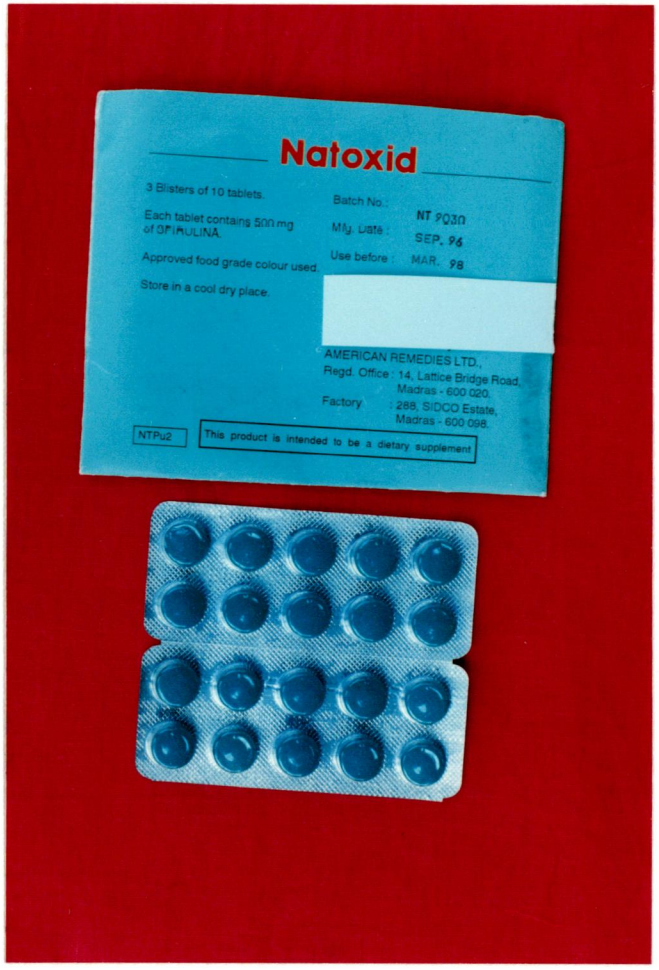

PLATE - I

BETA-CAROTENE TABLETS

PLATE - II

ASCORBIC ACID TABLETS

Results and Discussion

IV. RESULTS AND DISCUSSION

The results and discussion pertaining to this study is depicted under the following headings.

A. Background information of the selected patients
   1. Age and sex of the patients
   2. Occupation of the selected patients
   3. Educational status
   4. Income pattern
   5. Family history of the diseases
   6. Symptoms associated with cardiovascular disease
   7. Dietary modification of the selected patients

B. Possible factors responsible for altering the lipid profile.
   1. Type of oils used
   2. Consumption of prepared foods
   3. Smoking pattern of the male patients
   4. Alcoholic consumption pattern

C. Weight and height pattern of the selected patients
   1. Mean weight and height of the patients
   2. BMI values of the selected patients
   3. Waist/Hip ratio of the patients

D. Food and nutrient intake of the patients
   1. Food intake of the male patients
   2. Food intake of the female patients
   3. Nutrient intake of the male patients
   4. Nutrient intake of the female patients

E. Lipid profile of the selected patients

1. Supplementation with Beta-carotene

2. Supplementation with Beta-carotene and ascorbic acid

A. Background information of the selected patients

1. Age and sex of the patients

The age and sex of the patients are presented in Table I.

TABLE I

AGE AND SEX OF THE PATIENTS

S.No.	Age (in years)	Sex: Males N	Males %	Female N	Female %	Total N	Total %
1.	30-40	-	-	2	2.6	2	1.3
2.	40-50	10	8.2	12	15.4	22	11.8
3.	50-60	82	67.2	42	53.8	124	60.5
4.	> 60	30	24.6	22	28.2	52	26.4
	Total	122	100	78	100	200	100

Among the 200 cardiovascular patients selected for the study 60.5 per cent of the patients were in the age group of 50-60 years among whom 67.2 per cent are males and 53.8 per cent are females. About 26.4 per cent of the patients were in the age group of above 60 years in which 24.6 per cent are males and 28.2 per cent are females. Among the 11.8 per cent of the patients in the age group of 40-50 years, eight point two per cent are males and 15.4 per cent are females. And 1.3 per cent of the females are in the age group of 30-40 and none of the male member was in the above age group.

2. Occupational of the selected patients

The occupation of the patients are presented in Table II.

TABLE II

OCCUPATION OF THE SELECTED PATIENTS

S.No.	Occupation	Sex					
		Male		Female		Total	
		N	%	N	%	N	%
1.	Sedentary	48	39.3	78	100	126	63.0
2.	Moderate	56	45.9	-	-	56	28.0
3.	Heavy	18	14.8	-	-	18	9.0
	Total	122	100	78	100	200	100

Among the 122 male patients 39.3 per cent are doing sedentary activity and 45.9 per cent of males are doing moderate activity and 14.8 per cent are doing heavy work. Among the 78 female patients selected all of them are doing only sedenatry activity and none of them are belong to the other activity groups.

Regular aerobic exercise is very important in keeping people control blood pressure and blood lipid profiles. The level for disease control say that the risk for CVD is doubled for inactive people as compared with active people (Nieman, 1993).

According to Joseph (1992) who are physically active have a lesser chance of developing coronary problems than those who are sedentary, so there is a good justification for taking up some form of regular exercise.

3. Educational status

The following Table III gives the educational sttus of the selected patients.

TABLE III

EDUCATIONAL STATUS OF THE SELECTED PATIENTS

S.No.	Educational status	Sex: Male N	Male %	Female N	Female %	Total N	Total %
1.	Illiterate	30	24.5	34	43.5	64	32
2.	Upto elementary school	36	29.5	20	25.6	56	28
3.	Upto high school	30	24.5	18	23.4	48	24
4.	Upto higher secondary	12	9.8	3	3.8	15	7.5
5.	Upto graduation level	10	8.2	2	2.5	12	6
6.	Upto post-graduate level	4	3.2	1	1.2	5	2.5
	Total	122	100	78	100	200	100

Out of 122 male subjects 24.5 per cent are illiterate. Twenty nine point five per cent had elementary school education, whereas 24.5 per cent went upto high school, 9.8 per cent had higher secondary education and 8.2 per cent are graduates and 3.2 per cent are post graduates. Among the 78 females 32 per cent are illitarates, 28 per cent were studied upto elementary school, 24 per cent had high school education, whereas 7.5 per cent had higher secondary education 6 per cent are graduates and 2.5 per cent are post graduates.

4. Income pattern

Table IV shows the income pattern of the patients

TABLE IV

INCOME PATTERN OF PATIENTS

S.No.	HUDCO (1994) classification	Income/month in Rs.	Number	Percentage
1.	Low	1250-2650	118	59
2.	Middle	2651-4450	56	28
3.	High	4451 and above	26	13
	Total		200	100

Among the 200 patients 59 per cent had an income in the range of Rs. 1250-2650, 28 per cent had an income in the range of Rs. 2651-4450 and 13 per cent had an income in the range of Rs.4,451 and above.

According to HUDCO (1994) the low income group belongs to an income range of Rs.1250-2650, the middle income group ranges between Rs.2651-4450 and the high income ranges from Rs.4,451 and above.

Since, the subjects were selected from private cardiac centre, patients were available from all the three income groups.

.5. Family history of diseases

Table V denotes the family history of the selected patients.

TABLE V

FAMILY HISTORY OF THE DISEASES

S.No.	Type of diseases	Father N	Father %	Mother N	Mother %	Others N	Others %	Total N	Total %
1.	Diabetes mellitus	35	17.5	22	11	Nil	-	57	28.5
2.	Hypertension	35	17.5	25	12.5	Nil	-	60	30.0
3.	Cardiovascular disease	19	9.5	14	7	Nil	-	33	16.5
4.	Cancer	-	-	2	1	Nil	-	2	1
5.	No information	-	-	-	-	48	24	48	24
	Total	89	44.5	63	31.5	48	24	200	100

Among the 200 patients 28.5 per cent had a family history of diabetes mellitus, 30 per cent had a family history of hypertension, 16.5 per cent had a family history of cardiovascular disease and only 1 per cent had a family history of cancer whereas 24 per cent of the patients doesn't seems to know the details about the family history of the diseases.

6. Symtoms associated with cardiovascular diseases

Table VI highlights the symptoms associated with heart disease as experienced by the patients.

TABLE VI

SYMPTOMS ASSOCIATED WITH CARDIOVASCULAR DISEASE

S.No.	Symptoms	Male N	Male %	Female N	Female %	Total N	Total %
1.	Hypertension	40	32.7	10	12.8	50	25
2.	Anginapectoris	76	62.5	45	57.6	121	60.5
3.	Asthma	4	3.2	18	23.0	22	11
4.	Backpain	2	1.6	5	6.4	7	3.5
	Total	122	100	78	100	200	100

With regard to the symptoms of the patients 25 per cent had hypertension among whom 32.7 per cent are males and 12.8 per cent are females, 60.5 per cent had anginapectoris among whom 62.5 per cent are males and 57.6 per cent are females, 11 per cent of the patients had asthma in which 3.2 per cent of them are males and 23 per cent are females and only 3.5 per cent had back pain among whom 1.6 per cent males and 6.4 per cent are females.

7. Dietary modification of the selected patients

Table VII depicts the details about the foods restricted by the patients after the onset of the disease.

TABLE VII

FOODS RESTRICTED

S.No.	Foods Restricted	Male		Female	
		No	%	No	%
1.	Roots and tubers	120	98.4	78	100
2.	Oily foods	122	100	78	100
3.	Mutton	84	68.8	78	100
4.	Egg	19	15.6	78	100

Out of 200 patients 98.4 per cent of the males and 100 per cent females restricted roots and tubers in the diet. All the patients restricted oily foods and 68.8 per cent of the males and 100 per cent of the females restricted mutton. Only 15.6 per cent of the males and 100 per cent of the females were restricted egg in their daily diet.

B. Possible factors responsible for altering the lipid profiles

1.Types of oils used

Table VIII shows the type of oils used by the

selected patients

TABLE VIII

TYPE OF OILS USED BY THE SELECTED PATIENTS

S.No.	Type of oils used	Before N	Before %	After N	After %
1.	Groundnut oil	172	86	50	25
2.	Gingelly oil	4	2	3	1.5
3.	Palm oil	11	5.5	13	6.5
4.	Refined oil	14	7	35	17.5
5.	Sunflower oil	10	5	113	56.5

Among the 200 patients a major proportion of 86 per cent used used groundnut oil before the onset of the disease, 2 per cent gingelly oil before the occurrance of the disease and 1.5 per cent used after the onset. Palm oil was used by 5.5 per cent before and 6.5 per cent after the occurrance of the disease. Refined oil was used by 7 per cent and it was increased to 17.6 per cent after the onset of the disease. Use of sunflower oil was increased upto 56.5 per cent after the onset of the disease.

High intakes of saturated fatty acids increase blood levels of total and LDL cholesterol and accelarates the processes of atherosclerosis and thrombosis along with blood pressure and arrhythmias. On the other hand polyunsaturated fatty acids have opposite effects and is important for prevention of blood clotting (Ghafoorunissa and Krishnaswamy, 1995).

9. Consumption of prepared foods

Table IX depicts the frequency of consumptcn of prepared foods by the selected patients before and after the onset of the disease.

TABLE IX

CONSUMPTION OF PREPARED FOODS BY THE SELECTED PATIENTS

S.No.	Prepared foods consumed	Daily		Weekly		Monthly	
		B	A	B	A	B	A
1.	Baked	-	-	22.5	18	57	54.5
2.	Fried	-	-	-	-	77	77
3.	Pickles	59	38.5	21.0	18.5	16.5	16.5
4.	Fatty foods	15.5	7.5	26.5	14.5	31.0	30.0

B- Befpre : A - After

With regard to the consumption of prepared foods, baked foods were consumed weekly by 22.5 per cent before and 18 per cent after the incidence of the heart disease, monthly by 57 per cent before and 54.5 per cent after the incidence of heart disease. Fried foods were consumed by 77 per cent monthly before and after the incidence of heart disease. Pickles were consumed daily by 59 per cent of the patients before and it was rediced to 38.5 per cent after the incidence of the disease. Fat rich foods were consumed daily by 15.5 per cent before and 7.5 per cent after the incicence of heart disease. It was consumed weekly by 26.5 per cent before and 14.5 per cent after the incidence of heart disease whereas 31 per cent consumed monthly before and 30 per cent after the incidence of the heart disease.

3. Smoking pattern of the male patients

Table X indicates the smoking pattern of the selected patients.

TABLE X

SMOKING PATTERN OF THE MALE PATIENTS

S.No.	Smoking pattern	Before		After	
		N	%	N	%
a.	Yes	68	55.7	7	5.7
	No	54	44.3	-	-
b.	Type				
	Beedi	9	13.2	1	1.5
	Cigarette	53	77.9	4	5.9
	Tobacco	6	8.8	2	2.9
c.	No smoked				
	10-20	27	39.7	7	5.7
	20-30	27	39.7	nil	nil
	30-40	14	20.6	nil	nil

With regard to the smoking pattern of the selected male patients 55.7 per cent found to be smoking and 43.5 per cent were not having the habit of smoking before the onset of the disease. After the diagnosis of the disease only 57 per cent were found to be continueing the habit. With regard to the type smoked 13.2 per cent were smoked beedi, 77.9 per cent smoked cigarettes and 8.8 per cent smoked tobacco before the onset of the disease. It was interesting to note that after the onset of the disease that 1.5 per cent, 5.9 per cent and 2.9 per cent were smoked beedi, cigarette and tobacco respectively.

Among the patients smoked 39.7 per cent smoked 10-20 cigarette/beedi per day, 39.7 per cent smoked 20-30 cigarette/beedi per day and 20.6 per cent used 30-40 cigarette/beedi per day before the onset of the disease. Whereas only 5.7 per cent still continue to smoke 10-20 cigarette/beedi per day.

The risk for heart attack increases with the number of cigarettes you smoke as well as the duration. It decreases if one stops smoking (Ghafoorunissa and Krishnaswamy 1995).

The possibility that cigarette smokers have an increased need for vitamin C have been confirmed by an analysis of Hanes II data. Those who used supplements regularly had more protection and vitamin C serum levels than those who used them irregularly. It appears that smokers may need upto 30mg more/day than non smokers. Intakes above that, how ever, provide no additional protection (Helen, 1989).

4.Alcohol consumption pattern

Alcohol is a social evil. It adds to problems at home and work, to crimes and accidents. In addition to the heart, alcohol also damages the liver, brain and nervous system and is casually related to cancers. It is sensible to avoid alcohol altogether for your sake and for the sake of others (Ghafoorunissa and Krishnaswamy, 1995).

Table XI presents the alcohol consumption pattern of the selected patients.

TABLE XI

ALCOHOL CONSUMPTION PATTERN OF THE SELECTED PATIENTS

S.No.	Consumption pattern	Before N	Before %	After N	After %
a.	Yes	16	13.10	nil	nil
	No	106	86.90	nil	nil
b.	Type				
	Arrack	4	25.00	nil	nil
	Whisky	5	31.25	nil	nil
	Brandy	5	31.25	nil	nil
	Wine	2	12.50	nil	nil

Among the patients 13.1 per cent have the habit of consuming alcohol, and 86.9 per cent didn't have the habit of using alcohol. Among the patients used alcohol 25 per cent used arrack, 31.25 per cent used whisky and the same percentage used brandy and 12.5 per cent used wine before the onset of the disease. It was appealing to note that after the onset of the disease none of the patients are consuming alcohol this may be because of the advice of the doctors.

C. Weight and height pattern of the selected patients

1. Mean weight and height of the selected patients

Table XII depicts the weight and height patterns of the patients.

TABLE XII

MEAN WEIGHT AND HEIGHT OF THE SELECTED PATIENTS

S.No.	Sex	Mean weight (in Kg)	Reference weight (ICMR) in kgs	Mean Height (in cms)	Reference Height (ICMR) in cms
1.	Males	62.7	60	163.9	177
2.	Females	59.4	50	158	164

The above table depicts that among the selected male patients 49.1 per cent were having normal BMI values and 18.8 per cent were found to be having first grade obesity and 5.7 per cent were recorded under second grade obesity and 4 per cent were in the third grade obesity. The remaining patients were having their BMI value less than the normal values.

With regard to the female patients 51.5 per cent were in the normal category, 32 per cent and 3.8 per cent were having grade I and grade II obesity respectively. None of the female patients having the grade III obesity.

From this table it is clear that even though obesity was found to be one of the major risk factor for the precipitation of heart disease, people having less than the normal BMI values were also suffering from cardiovascular diseases.

3. Waist/hip ratio of the selected patients

Table XIV shows the waist/hip ratio of the selected patients.

TABLE XIV

WAIST/HIP RATIO OF THE SELECTED PATIENTS

S.No.	Waist/hip ratio	Males		Females	
		N	%	N	%
1.	0.7 -0.75	10	8.2	17	21.9
2.	0.76-0.85	26	21.3	33	42.3
3.	0.86-0.90	71	58.2	18	23.0
4.	0.91-0.95	nil	nil	nil	nil
5.	0.96-1.00	15	12.3	10	12.8
	Total	122	100	78	100

Cut off points for hight waist to hip rati (WHR) that may define risk for CVD has been suggested for men (0.95) and women (0.80). The evidence reviewed that high WHR is associated with CVD mortality. For identifying persons at high risk for death, cut off points for WHR have been specified at 0.95 for men and 0.80 for women. These values are based primarily on mortality data from Europear population (Croft, et al, 1995).

In the survey conducted, out of 122 male patients 8.2 per cent were at 0.7-0.75 and 21.3 per cent were at 0.76 to 0.85 cut off points whereas 58.2 per cent were nearer to the risk that is 0.86 to 0.90. None were at 0.91-0.95 but 12.3 per cent were at the high risk that is 0.96 to 1.00. Among 78 female patients 21.9 per cent were having WHR between 0.7-0.75, and 42.3 per cent were at the risk that is between 0.76 to 0.85. Twenty three per cent were also at the risk between 0.86 to 0.90. None were in between 0.91 to 0.95. At the least 12.8 per cent were at high risk between 0.96 to 1.00 cut off points.

Waist/hip ratio in one of the parameter to found out the risk for the cardiovascular diseases. With regard to this parameter all the male patients asre having the ratio as 0.9 whichis the cut off point given by Croft et al, (1995).

D. Food and nutrient intake of the patients

1. Food intake of selected male patients

Table XV shows the intake of food by the selected patients.

TABLE XV

MEAN FOOD INTAKE OF SELECTED MALE PTIENTS

S.No.	Foods (g)	RDA (ICMR) (1994)	Actual intake	Percentage of deficit or surplus
1.	Cereals	460	218	- 52
2.	Pulses	40	53	+ 34
3.	Green leafy vegetables	40	81	+104
4.	Roots and tubers	50	49	- 1
5.	Other vegetables	60	68	+ 14
6.	Fruits	30	62	+108
7.	Milk and milk products	150	325	+116
8.	Sugar and jaggery	20	5	- 71
9.	Fleshy foods	30	nil	nil
10.	Oil	40	8	- 79

From the Table XV it was found that the cereal intake was deficit by 52 per cent from the ICMR recommended dietary allowance. The pulse intake was excess by 34 pe cent. Leafy vegetables was deficit by 104 per cent with regard to the consumption of roots and tubers, sugar and jaggery and fats and oils it was deficit by 1 per cent, 71 per cent and 79 per cent respectively other vegetables. Fruits and milk and milk products were found to be surplus by 14 per cent, 108 per cent and 116 per cent respectively.

2. Food intake of selected female patients

Table XVI gives the information about the food intake of the selected female patients.

TABLE XVI

MEAN FOOD INTAKE OF FEMALE SELECTED PATIENTS

S.No.	Foods (g)	RDA ICMR	Actual intake	Percentage of deficit or surplus
1.	Cereals	410	217	- 47
2.	Pulses	40	54	+ 35
3.	Green leafy vegetables	100	74	- 26
4.	Other vegetables	40	62	+ 55
5.	Roots and tubers	50	43	- 14
6.	Fruits	30	60	100
7.	Milk and milk products	100	312	+212
8.	Sugar and jaggery	20	10	- 50
9.	Fleshy foods	30	nil	nil
10.	Oil	20	11	- 45

Regarding the food intake of the selected patients it was found that cereal intake green leafy vegetable intake, roots and tubers, sugar and jaggery and oil was deficit by 47 per cent, 26 per cent, 14 per cent, 50 per cent and 45 per cent respectively. Intake of pulses, other vegetables, fruits and milk and milk products were found to be excess by 35 per cent, 55 per cent, 100 per cent and 212 per cent respectively.

3. Nutrient intake of the selected male patients

Table XVII shows the intake of nutrients of the selected male patients.

TABLE XVII

MEAN NUTRIENT INTAKE OF THE SELECTED MALE PATIENTS

S.No.	Nutrient	RDA ICMR 1991	Actual intake	Percentage of deficit or surplus
1.	Energy (k.cal)	2425	1532	- 36
2.	Protein (g)	60	50	- 16
3.	Fat (g)	20	21	+ 5
4.	Calcium (mg)	400	1027	+156
5.	Iron (mg)	28	9	- 66
6.	Beta carotene (µg)	2400	2488	+ 3
7.	Thiamine (mg)	1.2	2.5	+ 115
8.	Riboflavin (mg)	1.4	1.6	+ 14
9.	Vitamin C (mg)	40	133	+ 234
10.	Niacin (mg)	16	7	- 51

From the table it was found that energy intake was deficit by 36 per cent when campared with recommended dietary allowance. Protein intake was deficit by 16 per cent,iron by 66 per cent and niacin was deficit by 51 per cent with regard to the consumption of fat, calcium, Beta-carotene, thiamine and riboflavin were surplus by 5 per dent, 156 per cent,3 per cent, 115 per cent, 14 per cent respectively. Vitamin C intake was also surplus by 234 per cent.

4. Nutrient intake of the selected female patients

Table XVIII gives the information about the mean nutrient intake of the selected female patients.

TABLE XVIII

MEAN NUTRIENT INTAKE OF THE SELECTED FEMALE PATIENTS

S.No.	Nutrients	RDA ICMR 1991	Actual intake	Percentage of deficit or surplus
1.	Energy (k.cal)	1875	1434	- 23
2.	Protein (g)	50	43.8	- 12
3.	Fat (g)	20	19	- 3
4.	Calcium (mg)	400	990	+148
5.	Iron (mg)	30	9.8	- 67
6.	Beta-carotene (µg)	2400	2480	+ 3
7.	Thamine (mg)	0.9	3.4	+213
8.	Riboflavin (mg)	1.1	1.3	+ 18
9.	Vitamin C (mg)	40	114	+186
10.	Niacin (mg)	12	8	-30

From the above table it was found that energy intake was deficit by 23 per cent when compared with recommended dietary allowance. Protein intake was deficit by 12 per cent, fat by 3 per cent. Iron by 67 per cent and niacin by 30 per cent. With regard to the consumption of calcium, Beta-carotene, thiamine and riboflavin were surplus by 148 per cent, 3 per cent, 213 per cent and 18 per cent respectively. Vitamin C intake was also surplus by 186 per cent. Individual intake is given in Appendix IV.

E.Lipid profile of the selected patients

1. Supplementation with Beta-carotene tablets

Table XIX shows the mean values of blood lipid prfiles of the selected patients before and after the supplementation for 30 days and 60 days period.

TABLE XIX

LIPID PROFILE OF THE SELECTED PATIENTS SUPPLEMENTED WITH BETA-CAROTENE TABLETS

S.No.	Lipid levels mg/dl	Desirable levels (NCEP, 1989)	Lipid profile values Initial Mean+S.D I	After 30 days Mean+SD II	Final Mean+SD III	Group	't' value
1.	Total cholesterol	< 200	206.3+23.18	201.9+23+99	196.1+20.78	IVs II IVs III	6.41** 2.71*
2.	Triglycerides	< 150	173.6+17.68	169.9+17.48	157.4+14.37	IVs II IVs III	4.60** 2.56*
3.	HDL-cholesterol	> 50	53.9+4.65	56.6+3.53	59.2+3.93	IVs II IVs III	2.56* 5.68**
4.	LDL-cholesterol	< 130	119.3+22.72	111.24+24.8	108.44+20.90	IVsII IVsIII	3.68** 2.78*
5.	VLDL-cholestrol	< 30	34.1+4.04	34.06+3.51	31.46+2.88	IVsII IVsIII	0 2.14NS

** Significant at P < 0.01

* Significant at P < 0.05

NS Not significant

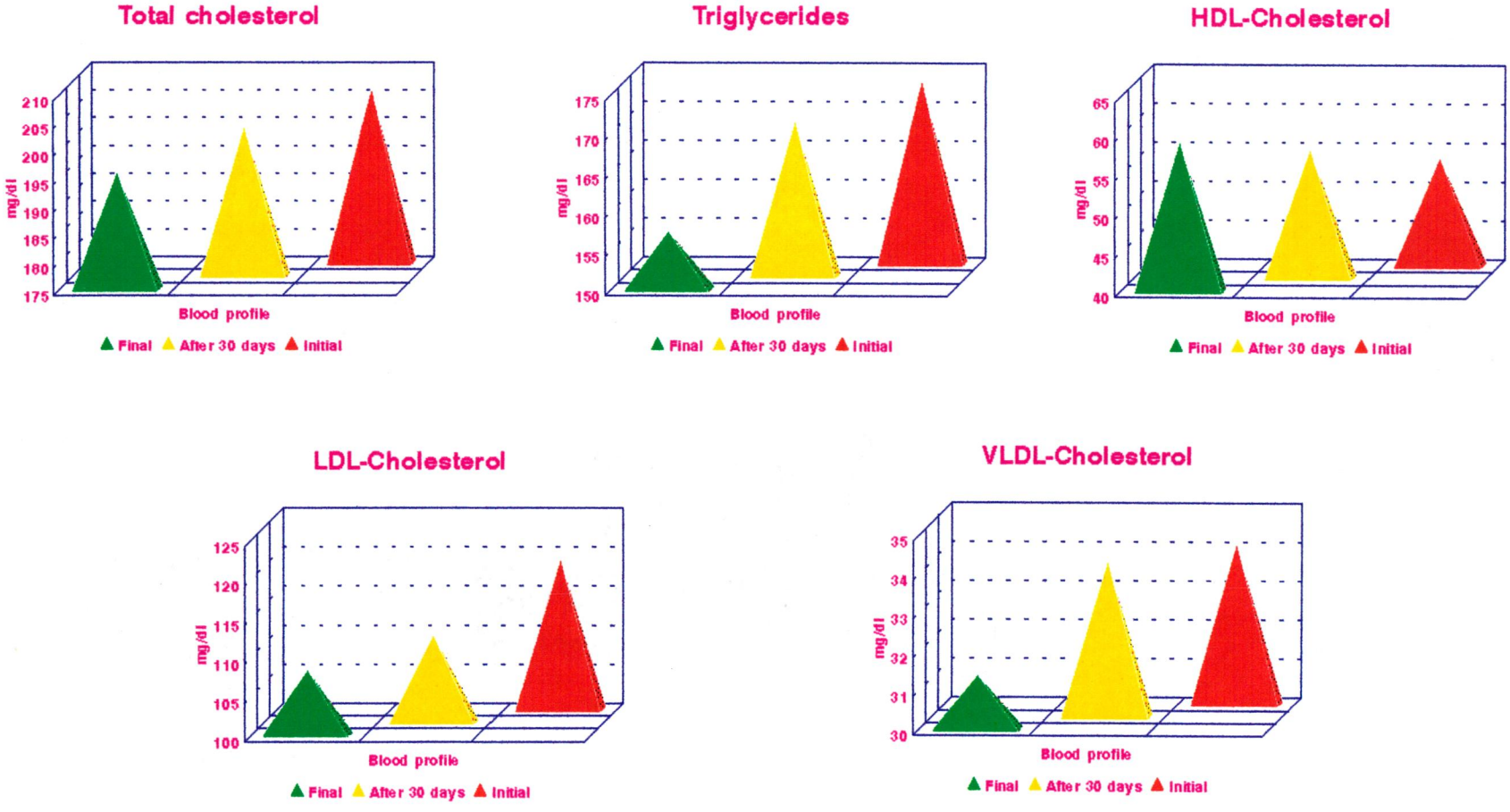

Figure 1
Lipid profile of the selected patients
supplemented with Beta-carotene tablets

SEA

The mean total cholesterol level of the selected cardiovascular patients was recorded as 206.3 mg/dl and it was reduced to 201.9 mg/dl after 30 days supplementation of Beta-carotene tablets. After 60 days of supplementation (final) it was the mean value was reduced to 196.1 mg/dl. When these values were statistically analysed and it showed significant difference at $P<0.01$ level between initial value and the values after 30 days and 60 days of supplmentation.

The mean triglyceride level of the selected cardiovascular patients was found to be 173.6 mg/dl initially and it was reduced to 169.9 mg/dl and 157.4 mg/dl after 30 days and 60 days of supplementation, when statistically analysed it showed a significant difference at ($P<0.01$) between the first and second group and the difference between the first and third group.was only at $P<0.05$.

The mean high density lipoprotein level was recorded as 53.9 mg/dl initially and it was increased to 56.6 mg/dl after 30 days and 59.2 mg/dl after 60 days of supplementation. From this it was revealed that Beta-carotene in effective in increasing the good cholesterol namely HDL-cholesterol n the blood. When these values were statistically analysed the difference was and found to be significant at $P<0.05$ between first and second group and between the first and third group, it was significant at $P<0.01$.

Low density lipoprotein cholesterol level was found to be 119.3 mg/dl before the supplementation period and the

level was reduced to 111.24 mg/dl and finally 108.44 mg/dl after 30 days and 60 days of supplementation respectively. When the values were analysed statistically it showed significant difference at $P<0.01$ between first and second group and at $P<0.05$ betwen first and third group.

The mean values of very low density lipoprotein cholesterol was 34.1 mg/dl before supplementation and it was reduced to 31.46 mg/dl after the supplementation period. These two values when statistically analysed were found to be not significant. Hence it was found that the supplementation of Beta-carotene had no significant effect on lowering the verylow density lipoprotein cholesterol. Figure I shows the comparision between before and after supplmenntation of Beta-carotene.

The lipid research clinics, coronary primary prevention trial and follow up study offered a unique opportunity to prospectively study the relationship between total serum carotenoid levels and coronary heart disease in a population of hyperlipidemic men. This study concluded the participants with higher serum carotenoid levels had a decreased risk of incident of CHD. This finding was stronger among men who were smoked (Morris et al, 1994).

2. Supplementation with Beta-carotene and ascorbic acid tablets

Table XX represents the mean values of blood lipid profile of the selected patients before and after the supplementation for 30 days and 60 days period.

TABLE XX

LIPID PROFILE OF THE SELECTED PATIENTS SUPPLEMENTED WITH BETA-CAROTENE AND ASCORBIC ACID TABLETS

S.No.	Lipid levels mg/dl	Desirable levels (NCEP, 1989)	Lipid profile values Initial Mean+S.D I	After 30 days Mean+SD II	Final Mean+SD III	Group	't' value
1.	Total cholesterol	< 200	232.9±39.63	228.8±41.48	216.1±40.37	IVsII IVsIII	4.75** 4.50**
2.	Triglycerides	< 950	189.8±35.84	185.5±36.15	164.8±34.4	IVsII IVsIII	12.35** 11.19**
3.	HDL cholesterol	> 50	52.8±7.32	56.5±6.69	58.8±5.85	IVs II IVs III	7.74** 3.32**
4.	LDL cholesterol	< 130	142.3±38.56	135.2±40.38	124.2±38.45	IVsII IvsIII	6.39** 12.27**
5.	VLDL cholesterol	< 30	37.2±7.77	37.1±7.37	33±6.91	IVsII IV III	0.17NS 2.16NS

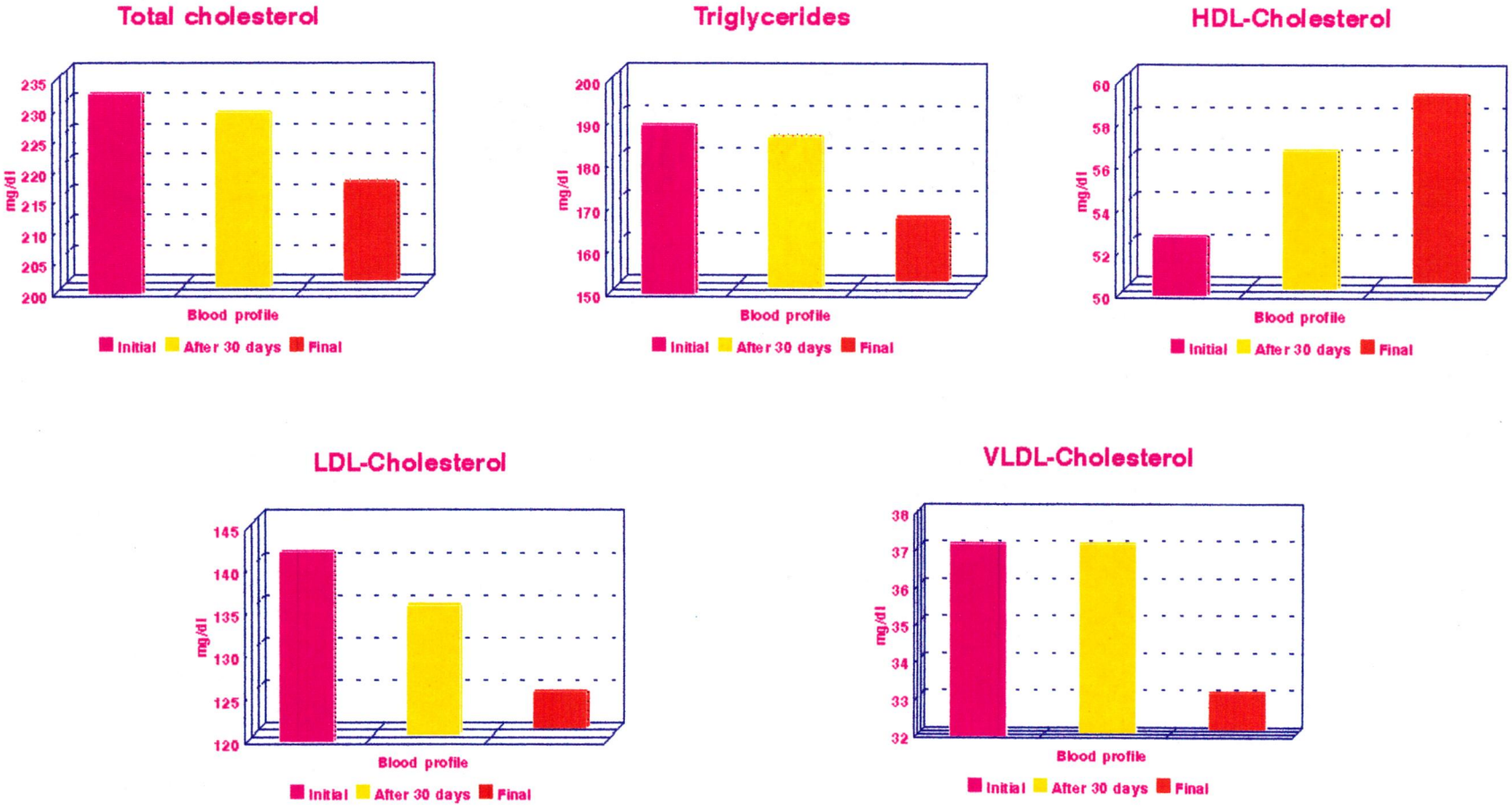

Figure 2
Lipid profile of the selected patients supplemented with Beta-carotene and ascorbic acid tablets

S69

The mean total cholesterol level of the selected cardiovascular patients was recorded as 232.9 mg/dl initially and it was lowered to 228.8 mg/dl after 30 days and it was reduced finally to 216.1 mg/dl after 60 days of supplementation. These three values were statistically analysed and the difference between the first and second group and first and third group was significant at $P<0.01$.

The mean triglyceride value of the selected cardiovascular patients was found as 189.9 mg/dl initially and it was reduced to 185.5 mg/dl after 30 days of supplementation and it was further reduced to 164.8 mg/dl after 60 days of supplementation with Beta-carotene and Ascorboic acid tablets. The difference between the initial value and supplementation after 30 days and 60 days were satistically analysed were found to be significant at $P<0.01$.

The high density lipoprotein was 52.8 mg/dl before supplementation and 56.5 mg/dl after 30 days and it was increased to 58.8 mg/dl after 30 days and it was increased to 58.8 mg/dl after 60 days of supplementation. The supplementation of Beta-carotene and ascorbic acid was effective in increasing the high density lipoprotein level with the statistical difference at $P<0.01$ between the groups.

The low density lipoprotein was recorded as 142.3 mg/dl initially and it was reduced to 135.5 mg;dl and 124.2 mg/dl after 30 days and 60 days of supplementation respectively. These values were when statistically analysed

it showed significant differenct at $P<0.01$ between initial value and after 30 days of supplementation and 60 days of supplementation.

With regard to the VLDL cholesterol the mean value was 37.2 mg/dl and was reduced to 33 mg/dl after 2 months of supplementation period. However the difference were found to be not significant between the groups. Individual values are given in Appendix V. Figure 2 shows the lipid level before and after supplementation

Hence it was found that the supplementation of Beta-carotene and ascorbic acid tablets were significantly effective in lowering total cholesterol, triglycerides, low density lipoprotein cholesterol and also effective in increasing High density lipoprotein cholesterol. But this supplementation had no significant effect on lowering the very low density lipoprotein cholesterol.

As suggested by the results of epidemiologic studies, there seems to be a protective effect of fruit and vegetable consumption on the concentration of cardiovascular disease risk factors such as cholesterol. The DAMET study shows that 4 week of administration of 400g fruit and vegetables/day resulted in lower concentrations of total cholesterol and LDL cholesterol. The diet, however, also resulted in lower intakes of energy, cholesterol and fat (Lenore and Susan, 1995).

Summary and Conclusion

SUMMARY AND CONCLUSION

The present study entitled "Effect of supplementation of Beta-carotene and ascorbic acid on lipid profile in cardiovascular patients" has the objective to observe the effect of supplmentation of Beta-carotene, Beta-carotene and ascorbic acid together on lipid profile in cardiovascular patients.

The patients selected were from all the three income groups that is low, middle and high class. The subjects selected for the survey were mostly in the age group of 50-60 years (60.5 per cent) and 26.4 per cent were in the age group of above 60 years. Others are in between 40-50 and 30-40 years of age group. The survey was conducted with the help of an interview schedule.

Most of the selected patients are sendentary workers (63 per cent). Among them all the females are sedentary workers and among 122 males 39.3 per cent are sedentary, 45.9 per cent are moderate and rest of them are heavy workers.

Regarding their educational status 32 per cent are illiterates, 28 per cent had education upto elementary school and 24 per cent had education upto high school whereas 7.5 per cent, 6 per cent and 2.5 per cent had their education upto higher secondary, graduation level and post graduate level respectively.

With regard to the family history of diseases 28.5 per cent had a family history of diabetes mellitus, 30 per cent had hypertension, 16.5 per cent had cardiovascular

diseases and 1 per cent had cancer expressed by the patients. Among those 60.5 per cent had the symptom of angina pectoris, 25 per cent expressed hypertension whereas 11 per cent and 3.5 per cent expressed asthma and back pain respectively.

In relation to dietary modification 100 per cent of the patients restricted oily foods, 98.4 per cent restricted roots and tubers, 68.8 per cent restricted mutton and 15.6 per cent restricted egg.

From the data it was revealed that 56.5 per cent were using sunflower oil after the onset of the disease and 17.5 per cent were using refined oil whereas 25 per cent were using groundnut oil even One point five per cent and 6.5 per cent were using gingelly oil and palm oil respectively. Before the onset of the disease 86 per cent used groundnut oil and only 7 per cent and 5 per cent used refined oil and sunflower oil respectively.

About the consumption of prepared foods, it was found that most of them were using pickles and fatty foods before the onset of the disease and it was reduced after the on set of the disease.

Regarding the smoking pattern 55.7 per cent had the habit of smoking before and 5.7 per cent had the habit after the onset of the disease. Among them 13.2 per cent smoked beedi, 77.9 per cent smoked ciagarette and 8.8 per cent smoked tobacco before the onset of the disease and after the onset of the disease 1.5 per cent, 5.9 per cent and 2.9 per cent were smoking beedi, cigarette and tobacco respectively.

About the number smoked 39.7 per cent smoked 10-20 and 20-30 per day, whereas 20.6 per cent smoked 30-40 per day before the onset of the disease. After the onset of the disease only 5.7 per cent smoking between 10-20 both beedi/cigarette.

About the alcohol consumption pattern only 16 per cent had the habit of consuming alcohol before the onset of the disease. Among them maximum were consumed whisky and brandy and least were consumed arrack and wine. None of them had the habit of consuming alcohol after the incidence of cardiovascular disease.

With regard to the mean weight and height of the selected patients among males the weight was 62.7 kg and themean height was 163.9 cms. And among females the mean weight was 59.4 kg and the mean height was 158 cms.

Among the male patients selected 23 per cent were found to be in grade I obesity 7 per cent and 5 per cent were in grade II and grade III obesity respectively. Whereas 25 per cent of the female patients were in grade I obesity and 3 per cent were in grade II obesity. Among the male patients selected 12.3 per cent were having risk for cardiovasculasr disease According to their waist/hip ratio calculation. among the women patients 42 per cent were falling under the risk category.

Food intake of the male patients were surplus with regard to the consumption of pulses, green leafy vegetables, fruits, milk and milk products. At present their diet was deficit for cereals, sugar and jaggery and fats and oils.

Food intake of the female patients was surplus for pulses, Other vegtables, fruits and milk and milk products. Their diet is deficit for cereals, leafy vegetables, roots and tubers, sugar and jaggery and fats and oils.

With regard to the nutrient consumption both male and female patients consume surplus amount of calcium, thiamine, riboflavin and vitamin-C.

Supplementation of Beta-carotene tablet fcr cardiovascular patients was found to be very effective. There was significant reduction in total cholesterol triglycerides and LDL cholesterol level and also this is effective in increasing the HDL-cholesterol level. However there is no significant difference in VLDL-cholesterol level. Thus it is concluded that supplementation of Beta carotene is effective in altering the lipid profile for the hyperlipidemic patients.

Supplementation of Beta-carotene along with ascorbic acid was highly effective in reducing the total cholesterol, triglycerides and LDL cholesterol and is also effective in increasing the HDL-cholesterol. However it is not helping for the reduction in VLDL-cholesterol.

Thus the result of the supplementation study concluded that both ascorbic acid and Beta-carotene supplementation for the patients suffering from cardiovascular diseases can prevent and reduce the risk of mortaility among patients suffering from CVD. Since these

two supplements were available as tablet form it is so convinient for the patients to use this regularly.

RECOMMENDATIONS

1. To reduce the risk of cardiovascular disease diet councelling is highly effective
2. Physical exercise must be compulsory for all the patients to reduce body weight,BMI and waist/hip ratio.
3. Supplementation of hypolipidemic foods and nutrients can be recommended for all the patients for reducing the unwanted cholesterol and to increase the good cholesterol in blood.

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APPENDIX - I

PROFORMA TO STUDY THE EFFECT OF SUPPLEMENTATION OF BETA-CAROTENE AND ASCORBIC ACID ON LIPID PROFILES IN CARDIOVASCULAR PATIENTS.

1. NAME OF THE INTERVIEWEE :

   ADDRESS :

2. NAME OF THE INTERVIEWER :
3. DATE :
4. AGE: HEIGHT: WEIGHT: SEX:

   HIP: WAIST:
5. EDUCATIONAL QUALIFICATIONS :
6. MODE OF OCCUPATION :

   SEDENTARY :

   MODERATE :

   HEAVY :
7. NON-OCCUPATIONAL ACTIVITY :

   TYPE OF ACTIVITY :
8. TYPE OF FAMILY :
9. COMPOSITION OF THE FAMILY :

ADULTS	CHILDREN	TOTAL

10. MONTHLY INCOME :
11. INCOME FROM OTHER SOURCES :
12. TOTAL FAMILY INCOME PER MONTH:
13. EXPENDITURE ON FOOD PER MONTH:
14. WHETHER VEGETARIAN OR NON-VEGETARIAN :
15. TYPES AND AMOUNT OF FATS AND OILS CONSUMED PER DAY :

TYPE	AMOUNT	
	BEFORE	AFTER
Ghee		
Vanaspathy		
Gingelly Oil		
Groundnut Oil		
Cocunut Oil		
Palm Oil		
Refined Oil		
Sunflower Oil		
Bran Oil		
Butter		

16. CONSUMPTION OF PREPARED FOODS:

FOODS	QUALITY		FREQUENCY					
	BEFORE	AFTER	DAILY		WEEKLY		OCCASIONALLY	
			BEFORE	AFTER	B	A	BEFORE	AFTER
Cakes								
Biscuits								
Pickles								
Sweets								
Ice cream								
Puddings								
Puffs								
Vadai								
Bonda								
Chips								
Vattal								
Jams								
Pappad								
Squash								

17. NUMBER OF MEALS CONSUMED PER DAY:

18. FAMILY HISTORY:

S.No.	Diseases	Relation to subject	Age in years	Education	Occupation	Marital Status
1.	Diabetes					
2.	Blood pressure					
3.	Heart disease					
4.	Kidney disorder					
5.	Obesity					
6.	Others specify					

19. SUBJECTS HISTORY:

Diabetes

Blood pressure

Kidney disorder

Heart disease

Obesity

Hyperlipidemia

Liver disease

Any other

20. How long do you suffer from this disease:

21. Do you have any following symptoms at present ?

High blood pressure

Angihaectoris

22. Food consumption pattern: B: Before A: After

FOOD	AMOUNT CONSUMED					
	DAILY		WEEKLY		OCCASIONALLY	
	B	A	B	A	B	A
Cereals						
Pulses						
G.L.V.						
Roots & tubers						
Other vegetables						
Fruits						
Milk						
Curds						
Meat						
Mutton						
Beef						
Pork						
Chicken						
Fish						
Egg						
Butter milk						
Butter						
Ghee						
Sugar						
Jaggery						

23. Have you made any dietary modification after diagnosing of the disease : YES / NO

A) IF YES

FOODS INCLUDED	REASONS

FOODS EXCLUDED	REASONS

24. Do you have any knowledge about Beta-Carotene rich foods?
25. If yes, Mention amount and frequency of consumption?
26. Do you know lipid lowering effect of Beta-Carotene: YES/NO
27. Do you take coffee often : YES/NO
28. If yes, specify the quantity:
    a) 2-4 cups
    b) 4-5 cups
    c) 6-8 cups
29. a) Do you smoke: YES/NO
    b) If yes how long since you have been smoking
    c) Type of smoking material used

TYPE	QUANTITY	FREQUENCY	
		BEFORE	AFTER
Beedi			
Cigarette			
Tobacco			
Others			

30. Do you have the habit of using alcoholic drinks ? YES/NO

If yes how long since you have been using

Type of drinks	Quantity	Frequency		
		Daily	Weekly	Occasionally
Arrack				
Toddy				
Whisky				
Brandy				
Wine				
Beer				
Rum				

31. Do you do any exercise? YES/NO

If yes

TYPE	DURATION	
	BEFORE	AFTER
Jogging		
Walking		
Swimming		
Yoga		
Others		

HEALTH DETAILS

Blood profile:

Blood cholesterol level :

Blood tryglyceride level :

HDL cholesterol :

LDH cholesterol :

VLDL cholesterol :

33. What are the causes that you think would have caused this disease?

a) Faulty dietary habits
b) Irregular eating habits
c) Heridity
d) Smoking
e) Alcohol
f) Lack of exercise
g) Not aware

HDL – CHOLESTEROL

Quantitative estimation of serum HDL – Cholesterol

PEG – PAP Method

Read absorbance at 500 nm (490 to 550 nm) or with Green filter.

- **Enzymatic colorimetric method**
- **Easy automation**
- **Not affected by time or temperature variation during precipitation and sedimentation**
- **Better differentiation at low values**
- **Rapid results**
- **Simple & convenient method**
- **Excellent correlation with ultra centrifugation method**

ENZOKIT®

SUMMARY

Cholesterol, Cholesterol esters, triglycerides and phospholipids are linked with varying proportions of apoproteins to form lipoproteins. They are grouped into four classes as (i) high density lipoprotein (HDL), (ii) low density lipoprotein (LDL), (iii) very low density lipoprotein (VLDL) and (iv) chylomicrons. High density lipoprotein (HDL) is a small particle of plasma lipoproteins with density 1.063 – 1.21 g/ml. It consists of 50% protein, 20% cholesterol, 30% phospholipid and traces of triglycerides. Lipoproteins, specially cholesterol containing lipoproteins are associated with atherosclerosis and coronary heart diseases (CHD). The greater part of cholesterol in circulating plasma is in the form of LDL. In normal subjects, LDL is produced from VLDL. LDL is taken up by body cells and utilised and surplus cholesterol from LDL is freed. The free cholesterol from tissues is picked up by HDL, metabolised, transformed, recirculated and excreted in various ways.

PRINCIPLE

Chylomicrons, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) of serum are precipitated using buffered polyethylene glycol (PEG – 6000).

After centrifugation, high density lipoproteins (HDL) are in the supernatant. The Cholesterol in the HDL fraction is estimated by an enzymatic method using cholesterol esterase, cholesterol oxidase, peroxidase, 4 – aminoantipyrine and phenol.

CLINICAL SIGNIFICANCE

A distinct inverse relationship exists between HDL – Cholesterol (HDL – C) levels and CHD, that is, lower the HDL – C concentration, greater the risk of CHD and vice-versa. Usually the values of LDL – C and HDL – C are examined simultaneously and compared to study CHD risk assessment. However, Framingham's study reflects that HDL – C is a very important independent marker for CHD risk factor at any given level of LDL – C. For instance although CHD is most prevalent when LDL – C levels are high and HDL – C levels low, yet CHD is fairly uncommon even at very high levels (220 mg/dl) of LDL – C provided HDL – C levels are also high (65-85 mg/dl). On the other hand CHD is common when both LDL – C and HDL – C levels are very low (e.g. 100 mg/dl and 25 mg/dl respectively).

LDL – C can be calculated by using Friedwald and Fredrickson's formula:

$$\text{LDL – Cholesterol} = \text{Total Cholesterol} - \left[\frac{\text{Triglycerides}}{5} + \text{HDL – Cholesterol}\right]$$

ACCURACY AND PRECISION

Validation studies establish that ENZOKIT HDL – CHOLESTEROL has excellent accuracy and reproducibility.

For **accuracy**, comparative studies were conducted on random samples using ENZOKIT HDL – CHOLESTEROL and a reference method. The resultant coefficient of correlation was 0.970 and the corresponding regression equation was $y = 0.95x - 1.42$.

For **precision**, within run and between run studies were carried out using samples having low and high normal values (35 to 82 mg/dl). The maximum coefficient of variation obtained was 3.2%.

FOR BEST RESULTS

- While pipetting the STANDARD (REAGENT 3) rinse the tip atleast once, with the STANDARD SOLUTION.
- Wipe the tip properly from outside and dispense the STANDARD SOLUTION in the CHROMOGEN REAGENT completely.
- To minimise 'outside the tip carryover' it is advisable not to dip the tip more than 1 or 2mm in the STANDARD SOLUTION/SAMPLE.

REFERENCES

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RANBAXY DIAGNOSTICS
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LT – HDL – 1

ENZOKIT®

HDL– CHOLESTEROL

(PEG – PAP Method)

Code HDL 40 : 2 x 20 ml Kit

CONTENTS

Reagent 1 (2 bottles) : BUFFER
Store at 2°– 8° C. Active Ingredients
- Phenol

Reagent 2 (2 bottles) : ENZYMES
Store at 2°– 8° C. Active Ingredients
- Cholesterol esterase
- Cholesterol oxidase
- Peroxidase
- 4-Amino antipyrine
- Sodium cholate

Reagent 3 (1 bottle) : STANDARD
Store at 2°– 8° C. (Cholesterol 50 mg/dl)
Ready to use

Reagent 4 (1 bottle) : PRECIPITATING
Store at 2°– 8° C. REAGENT
Active Ingredients
- Polyethylene Glycol 6000

PREPARATION OF WORKING SOLUTION

Dissolve contents of one bottle of Reagent 2 with bottle of Reagent 1. Mix well and store at 2°– 8° C. This is the chromogen reagent.

STORAGE AND STABILITY

ENZOKIT HDL– CHOLESTEROL reagents are stable till the expiry date indicated on the labels when stored at 2°– 8° C. The chromogen reagent is stable for four weeks at 2°– 8° C and for one week at 20°– 25° C. when stored in an amber coloured vial.

SPECIMEN COLLECTION AND STORAGE

- Serum (fasting) is preferred to plasma.
- Plasma collected with use of heparin as anticoagulant may also be used.
- Serum or plasma should be separated immediately.
- No deproteinization of serum is required.

PRECAUTIONS

ENZOKIT HDL– CHOLESTEROL is for *in vitro* use only. Avoid contact with skin, eyes and clothes.

INTERFERING SUBSTANCES

ENZOKIT HDL– CHOLESTEROL is free of interference from moderately high levels of bilirubin and haemoglobin.

PROCEDURE

I. HDL– CHOLESTEROL SEPARATION

Pipette into centrifuge tube	Quantity
Sample	0.3 ml
Precipitating Reagent	0.3 ml

Mix well, keep at R.T. for 10 minutes and then centrifuge at 4000 rpm for 10 minutes or at 2000 rpm for 20 minutes, to obtain a clear supernatant. Proceed to step II.

II. HDL– CHOLESTEROL ESTIMATION

A. For instruments requiring 1.0 ml volume :

Pipette into test tubes :	Blank (B)	Standard (S)	Test (T)
Chromogen Reagent	1.0 ml	1.0 ml	1.0 ml
Standard (50 mg/dl)	–	0.1 ml	–
Sample (Supernatant from Step I)	–	–	0.1 ml

Mix and incubate at 37° C for 10 minutes or for 20 minutes at R.T. (25° to 30° C).

B. For colorimeters requiring 3.0 ml volume :

Pipette into test tubes:	Blank (B)	Standard (S)	Test (T)
Chromogen Reagent	1.0 ml	1.0 ml	1.0 ml
Standard (50 mg/dl)	–	0.2 ml	–
Sample (Supernatant from Step I)	–	–	0.2 ml
Mix and incubate at 37° C for 10 mins. or at R.T. (25° to 30° C) for 20 mins.			
Distilled Water	2.0 ml	2.0 ml	2.0 ml

NOTE : For laboratories using instruments of cuvette capacity 2.5 ml (eg. ERMA) chromogen reagent, standard and sample volumes remain the same; distilled water volume may be reduced to 1.5 ml.

C. For colorimeters requiring 5.0 ml volume :

Pipette into test tubes:	Blank (B)	Standard (S)	Te (T
Chromogen Reagent	1.0 ml	1.0 ml	1.0
Standard (50 mg/dl)	–	0.4 ml	
Sample (Supernatant from step I)	–	–	0.
Mix and incubate at 37° C for 10 mins. or at R.T. (25° to 30° C) for 20 mins.			
Distilled Water	4.0 ml	4.0 ml	4. ml

NOTE : The read out requirement for most colorimeters is 4. ml, even though 5.0 ml is specified. In such cases the distilled water volume may be reduced to 3.0 ml after the incubation step. This will increase the sensitivity proportionately.

Mix and read absorbance of test (A_T) and standard (A_S) and the reagent blank (A_B) at 500 nm wavelength (490 to 550 nm) or with Green filter.

The colour developed is stable for 1 hour at room temperature, if protected from direct light.

CALCULATIONS

HDL – Cholesterol concentration (mg/dl)

$$= \frac{A_T - A_B}{A_S - A_B} \times 100$$

To convert mg/dl to mmol/l, use factor :
mmol/l = mg/dl x 0.026

REFERENCE VALUES

	Expected Values	Standard risk	High risk
Men	35 – 55 mg/dl	55 mg/dl	<35 mg/dl
Women	45 – 65 mg/dl	65 mg/dl	<45 mg/dl
Ratio : $\frac{\text{Total Cholesterol}}{\text{HDL – Cholesterol}}$		3 to 6	more than

LINEARITY

The method is linear upto 200 mg/dl.

TRIGLYCERIDES

Quantitative estimation of serum triglycerides

GPO-PAP Method

Read absorbance at 546 nm (530 to 570 nm) or with Green Filter

- **Enzymatic colorimetric method**
- **More specific and sensitive than Acetylacetone method**
- **Single step**
- **Low sample volume**
- **10 minutes test**
- **Single working reagent**
- **No cumbersome extraction and saponification**
- **Easy automation**

ENZOKIT

RANBAXY DIAGNOSTICS

ENZOKIT Triglycerides uses the GPO-PAP method for estimating serum triglycerides. Use of lipase enzyme to liberate glycerol from triglycerides and sequential enzyme system for estimating glycerol makes the method specific. The method is sensitive due to the high molar extinction coefficient of the final colour complex and consequently the sample volume is decreased. The final colour developed is within the visual range.

PRINCIPLE

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipase. In the presence of ATP and glycerokinase. the glycerol is converted to glycerol-3-phosphate. The glycerol-3-phosphate is then oxidised by glycerol-3-phosphate oxidase to yield hydrogen peroxide. Hydrogen peroxide reacts in the presence of peroxidase with ESPAS (N-ethyl-N-sulfopropyl-m-anisidine) and 4-aminoantipyrine to form a coloured complex. The intensity of the colour developed is proportional to trigycerides concentration and is measured photometrically at 546 nm (530 to 570 nm) or with Green filter.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{Glycerokinase}} \text{Glycerol-3-Phosphate} + ADP$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{Glycerol-3-phosph. Oxidase}} H_2O_2 + \text{Dihydroxyacetone Phosphate}$$

$$H_2O_2 + \text{Aminoantipyrine} + \text{ESPAS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H_2O$$

Serum triglycerides estimation is of significant importance in the investigation of hyperlipoproteinaemia. The levels may be found elevated in atherosclerosis, diabetes mellitus, glycogen storage diseases like Von Gierke's disease, alcoholism, secondary hyperlipoproteinemia and nephrotic syndrome. It also forms a part of patients lipid profile.

ACCURACY AND PRECISION

Validation studies establish that ENZOKIT Triglycerides has exellent accuracy and reproducibility.

For **accuracy,** compartative studies were conducted on random samples using ENZOKIT Triglycerides and a reference method. The resultant coefficient of correlation was 0.986 and the corresponding regression equation was $y = 1.084 x + 0.332$

For **precision** within run and between run studies were carried out using samples having normal and abnormal values (59-282 mg/dl). The maximum coefficent of variation obtained was 0.86%.

AUTOMATED APPLICATION

- Application sheets for use on specific semiautomatic, batch and auto analyzers are available on request.

REFERENCES

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RANBAXY
LABORATORIES LTD.
NEHRU PLACE, NEW DELHI-110019

LT TGS 31-1202

ENZOKIT
TRIGLYCERIDES

(GPO-PAP)

Cat. No. D12603 3x10ml Kit

Cat. No. D12601 5x20ml Kit

CONTENTS

		Cat. No. D12603	Cat. No. D12601
Reagent 1	: BUFFER	(3 bottles)	(5 bottles)
Store at 2° to 8°C.	: Active ingredients		
	• Pipes buffer		
	• ESPAS		
Reagent 2	: ENZYMES	(3 bottles)	(5 bottles)
Store at 2° to 8° C.	: Active ingredients		
	• Lipoprotein lipase		
	• Glycerokinase		
	• Glycerol-3-Phosphate oxidase		
	• Peroxidase		
	• 4-Aminoantipyrine		
	• ATP		
Reagent 3	: STANDARD	(1 bottle)	(1 bottle)
Store at 2° to 8° C.	: (Triglycerides 200 mg/dl) Ready to use		

PREPARATION OF WOKING SOLUTION

Dissolve contents of one bottle of Reagent 2 with contents of one bottle of Reagent 1. Mix well and store at 2° to 8° C. This is the chromogen reagent.

STORAGE AND STABILITY

ENZOKIT Triglycerides reagents are stable till the expiry date indicated on the lables when stored at 2° to 8° C. The enzyme chromogen reagent is stable for three weeks at 2° to 8°C when stored in amber coloured vial.

SPECIMEN COLLECTION AND STORAGE

- Serum (fasting) is preferred to plasma.
- Plasma collected with use of heparin as anti-coagulant may be used.
- Plasma collected with use of anticoagulant containing fluoride or oxalate should be avoided.

Samples should be used on the same day. If necessary they may be preserved in a refrigerator at 2° to 8° C for four days. Samples should be brought to room temperature before use.

PRECAUTIONS

ENZOKIT Triglycerides is for *in vitro* use only. Avoid contact with skin, eyes and clothes.

INTERFERING SUBSTANCES

Haemolysis and high bilirubin contents interfere marginally with the test, only in high concentrations.

PROCEDURE

A. For automated instruments requiring 1 ml volume:

Pipette into test tubes:	Blank (B)	Standard (S)	Test (T)
Chromogen Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	—	0.01 ml	—
Sample	—	—	0.01 ml

Mix and incubate at 37° C for 5 minutes or at R.T. (25° to 30 C°) for 20 minutes. Read absorbance of test (A_T), standard (A_S) and reagent blank (A_B) against distilled water at 546 nm wavelength (530 to 570 nm) or with Green filter.

B. For colorimeters/spectrophotometers requiring 3.0 ml volume:

Pipette into test tubes:	Blank (B)	Standard (S)	Test (T)
Chromogen Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	—	0.02 ml	—
Sample	—	—	0.02 ml
	Mix and incubate at 37° C for 10 mins or at R.T. (25° to 30° C) for 20 mins.		
Distilled Water	2.0 ml	2.0 ml	2.0 ml

Note: For laboratories using instruments of cuvette capacity 2.5 ml (eg ERMA) chromogen reagent, standard and sample volumes remain the same, distilled water volume may be reduced to 1.5 ml.

Mix and read absorbance of the test (A_T), standard (A_S) and the reagent blank (A_B) against distilled water at 546 nm wavelength (530 to 570 nm) or with Green filter.

C. For colorimeters requiring 5.0 ml volume:

For use in this procedure, dilute 0.1 ml of sample and standard by adding 0.4 ml of distilled water or normal saline, mix.

Pipette into test tubes:	Blank (B)	Standard (S)	Test (T)
Chromogen Reagent	1.0 ml	1.0 ml	1.0 ml
Diluted Standard	—	0.2 ml	—
Diluted Sample	—	—	0.2 ml
	Mix and incubate at 37° C for 10 mins or at R.T. (25° to 30° C) for 20 mins.		
Distilled Water	4.0 ml	4.0 ml	4.0 ml

Note: The read out requirement for most colorimeters is 4.0 ml, even though 5.0 ml is specified. In such cases the distilled water volume may be reduced to 3.0 ml after the incubation step. This will increase the sensitivity proportionately.

Mix and read absorbance of the test (A_T), standard (A_S) and the reagent blank (A_B) against distilled water at 546 nm wavelength (530 to 570 nm) or with Green filter.

The colour developed is stable for 1 hour at room temperature, if protected from direct light.

CALCULATIONS

Triglycerides concentration (mg/dl)

$$= \frac{A_T - A_B}{A_S - A_B} \times 200$$

To convert mg/dl to mmol/l, use equation:

mmol/l = mg/dl x 0.0114

NORMAL VALUES

Serum Triglycerides

Men: 60-165 mg/dl Women: 40-140 mg/dl

0.68-1.88 mmol/l 0.46-1.60 mmol/l

LINEARITY

The method is linear upto 1000 mg/dl for procedure A and 600 mg/dl for procedure B&C. For sample values higher than the linearity limits given above dilute the samples suitably with 0.9% saline and repeat the assay. Apply proper dilution factor to calculate the final result.

RANBAXY DIAGNOSTICS

LT/TG3

CHOLESTEROL

Quantitative estimation of serum cholesterol

CHOD-PAP Method

Read absorbance at 500 nm (490 to 550 nm) or with Green Filter

- **Enzymatic colorimetric method**
- **More specific and sensitive than chemical methods**
- **Free from non – cholesterol steroids**
- **High linearity**
- **Uniformity with the recommended and preferred method for HDL Cholesterol**
- **Low sample volume**
- **10 minutes test**
- **Single working reagent**
- **No corrosive reagents**
- **No deproteinisation**
- **Easy automation**

ENZOKIT®

RANBAXY DIAGNOSTICS

for estimating serum cholesterol. The cholesterol esterase and cholesterol oxidase enzymes make the method very specific. Non-cholesterol substances are not estimated. There is minimal interference from substances like bilirubin. The method is very sensitive due to the high molar extinction coefficient of the final colour complex and consequently the sample volume is decreased to 10 µl. The intensity of the final colour complex is within the visual range. The enzymatic method for cholesterol estimation can be extended to estimate HDL-cholesterol.

PRINCIPLE

Cholesterol esters are hydrolysed by cholesterol esterase to free cholesterol and fatty acids. Free and liberated cholesterols are oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide is liberated. The hydrogen peroxide produced, couples with 4-aminoantypyrine and phenol in the presence of peroxidase to form a coloured compound. The intensity of the colour developed is proportional to cholesterol concentration and is measured photometrically at 500 nm wavelength (490 to 550 nm) or with Green filter.

$$\text{Cholesterol esters} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red Quinone} + H_2O$$

CLINICAL SIGNIFICANCE

Increased levels of cholesterol may be found in coronary artery diseases, diabetes mellitus, hypothyroidism, nephrotic syndrome and hepatic malfunctions like obstructive jaundice and cirrhosis of the liver. Cholesterol levels may be low in acute hepatitis, malnutrition, anaemias, hyperthyroidism and Gaucher's disease. Normal cholesterol levels can be altered by age, stress, pregnancy and hormonal imbalance.

erol has excellent accuracy and reproducibility.

For **accuracy**, comparative studies were conducted on random samples using ENZOKIT Cholesterol and a reference method. The resultant coefficient of correlation was 0.997 and the corresponding regression equation was $y = 1.089x - 14.30$

For **precision**, within run and between run studies were carried out using samples having low abnormal and high normal values (100 to 260 mg/dl). The maximum coefficient of variation obtained was 4.20%.

AUTOMATED APPLICATION

- Application sheets for use on specific semi-automatic, batch and auto analyzers are available on request.

FOR BEST RESULTS

- While pipetting the STANDARD (REAGENT 3) rinse the tip atleast once, with the STANDARD SOLUTION.
- Wipe the tip properly from outside and dispense the STANDARD SOLUTION in the CHROMOGEN REAGENT completely.
- To minimise 'outside the tip carryover' it is advisable not to dip the tip more than 1 or 2 mm in the STANDARD SOLUTION/ SAMPLE.

REFERENCES

Allain C.C., et al., Clin. Chem., 20: 470-475 (1974).

RANBAXY
LABORATORIES LIMITED
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LT CCS 41 7202

(CHOD-PAP)

Code CCS 40 — 2 x 20 ml Kit
Code CHC 100 — 2 x 50 ml Kit

CONTENTS

Reagent 1
Store at 2° to 8°C.
(2 bottles)
: BUFFER
: Active ingredients
- Phenol

Reagent 2
Store at 2° to 8°C
(2 bottles)
: ENZYMES
: Active ingredients
- Cholesterol esterase
- Cholesterol oxidase
- Peroxidase
- 4-Amino antipyrine
- Sodium cholate

Reagent 3
Store at 2° to 8°C
(1 bottle)
: STANDARD
(Cholesterol 200 mg/dl)
Ready to use.

PREPARATION OF WORKING SOLUTION

Dissolve contents of one bottle of Reagent 2 with contents of one bottle of Reagent 1. Mix well and store at 2° to 8°C. This is the chromogen reagent.

STORAGE AND STABILITY

ENZOKIT CHOLESTEROL reagents are stable till the expiry date indicated on the labels when stored at 2° to 8°C. The chromogen reagent is stable for four weeks at 2° to 8°C when stored in amber coloured vial.

SPECIMEN COLLECTION AND STORAGE

- Serum (fasting) is preferred to plasma.
- Plasma collected with use of heparin as anticoagulant may be used.
- No deproteinization of serum is required.

Samples should be used on the same day. If necessary, they may be preserved in a refrigerator (2° to 8° C) for one week.

INTERFERING SUBSTANCES

Haemolysis and high bilirubin content interfere marginally with the test, only in high concentrations.

PROCEDURE

A. For automated instruments requiring 1.0 ml volume:

Pipette into test tubes:	Blank (B)	Standard (S)	Test (T)
Chromogen Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	—	0.01 ml	—
Sample	—	—	0.01 ml

Mix and incubate at 37° C for 10 minutes or at R.T. (25° to 30°C) for 20 minutes. Read absorbance of test (AT) and standard (As) against the reagent (B) at 500 nm wavelength (490 to 550 nm) or with Green filter.

B. For colorimeters requiring 3.0 ml volume:

Pipette into test tubes:	Blank (B)	Standard (S)	Test (T)
Chromogen Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	—	0.02 ml	—
Sample	—	—	0.02 ml
	Mix and incubate at 37° C for 10 mins or at R.T. (25° to 30° C) for 20 mins.		
Distilled Water	2.0 ml	2.0 ml	2.0 ml

Note: For laboratories using instruments of cuvette capacity 2.5 ml (eg ERMA) chromogen reagent, standard and sample volumes remain the same, distilled water volume may be reduced to 1.5 ml.

Mix and read absorbance of test (AT) and standard (As) against the reagent (B) at 500 nm wavelength (490 to 550 nm) or with Green filter.

C. For colorimeters requiring 5.0 ml volume:

For use in this procedure, dilute 0.1 ml of sample and standard by adding 0.4 ml of distilled water or normal saline, mix.

	Blank (B)	Standard (S)	Test (T)
Chromogen Reagent	1.0 ml	1.0 ml	1.0 ml
Diluted Standard	—	0.2 ml	—
Diluted Sample	—	—	0.2 ml
	Mix and incubate at 37° C for 10 mins or at R.T. (25° to 30° C) for 20 mins.		
Distilled Water	4.0 ml	4.0 ml	4.0 ml

Note: The read out requirement for most colorimeters is 4.0 ml, even though 5.0 ml is specified. In such cases the distilled water volume may be reduced to 3.0 ml after the incubation step. This will increase the sensitivity proportionately.

Mix and read absorbance of test (AT) and standard (As) against the reagent (B) at 500 nm wavelength (490 to 550 nm) or with Green filter.

The colour developed is stable for 1 hour at room temperature, if protected from direct light.

CALCULATIONS

Cholesterol concentration (mg/dl) $= \frac{A_T}{A_S} \times 200$

To convert mg/dl to mmol/l, use factor:
mmol/l = mg/dl x 0.026

NORMAL VALUES

Serum cholesterol : 150–250 mg/dl

LINEARITY

The method is linear upto 700 mg/dl for procedure A and 500 mg/dl for procedures B&C. For sample values higher than the linearity limits given above dilute the samples suitably with 0.9% saline and repeat the assay. Apply proper dilution factor to calculate the final result.

RANBAXY DIAGNOSTICS

LT CCS 41-1202

APPENDIX - III

WEIGHT, HEIGHT AND BMI OF THE SUBJECTS

S.No.	Weight in kg.	Height in m.	BMI	S.No.	Weight in kg.	Height in m.	BMI
1.	68	1.61	25.95	26.	55	1.56	22.42
2.	70	1.61	26.71	27.	50	1.64	18.38
3.	55	1.66	19.91	28.	43	1.76	13.73
4.	62	1.66	22.30	29.	92	1.81	27.77
5.	120	1.70	41.52	30.	45	1.58	17.87
6.	50	1.56	20.32	31.	55	1.61	20.90
7.	54	1.56	21.95	32.	65	1.76	20.76
8.	55	1.56	22.35	33.	58	1.61	22.13
9.	80	1.66	28.77	34.	73	1.64	26.83
10.	48	1.51	20.74	35.	40	1.61	15.26
11.	50	1.76	15.94	36.	44	1.61	16.79
12.	61	1.80	18.82	37.	70	1.56	28.45
13.	50	1.61	19.00	38.	46	1.58	18.25
14.	50	1.58	19.84	39.	108	1.76	34.60
15.	55	1.58	28.82	40.	57	1.56	23.17
16.	56	1.76	17.87	41.	73	1.70	25.32
17.	57	1.51	24.89	42.	83	1.71	28.14
18.	80	1.56	32.52	43.	50	1.66	17.99
19.	68	1.71	23.00	44.	43	1.51	18.67
20.	59	1.74	19.21	45.	58	1.61	21.32
21.	62	1.51	26.87	46.	75	1.64	27.57
22.	58	1.66	20.86	47.	83	1.64	26.30
23.	70	1.61	26.71	48.	62	1.70	21.45
24.	50	1.53	21.00	49.	50	1.56	20.32
25.	58	1.61	22.13	50.	50	1.66	17.98

S.No.	Weight in kg.	Height in m.	BMI	S.No.	Weight in kg.	Height in m.	BMI
51.	61	1.56	24.79	86.	70	1.58	24.89
52.	55	1.64	20.22	87.	57	1.51	12.47
53.	65	1.66	23.38	88.	41	1.81	18.86
54	57	1.58	22.61	89.	61	1.80	26.61
55.	62	1.61	23.66	90.	74	1.66	34.55
56.	65	1.70	22.49	91.	85	1.56	29.41
57.	50	1.56	20.32	92.	80	1.64	16.59
58.	57	1.53	24.05	93.	38	1.51	15.82
59.	62	1.58	24.60	94.	44	1.66	16.61
60.	70	1.61	26.71	95.	49	1.71	21.05
61.	62	1.53	26.16	96.	50	1.53	20.32
62.	65	1.56	26.42	97.	50	1.56	16.18
63	52	1.53	21.94	98.	45	1.66	22.79
64.	67	1.64	24.63	99.	62	1.64	18.65
65.	70	1.70	24.22	100.	50	1.64	18.65
66.	53	1.81	16.01	101.	61	1.71	20.67
67.	59	1.66	21.22	102.	91	1.71	30.84
68.	40	1.86	11.46	103.	80	1.70	27.68
69.	59	1.51	25.54	104.	60	1.68	23.80
70.	72	1.66	25.89	105.	50	1.53	21.05
71.	65	1.61	25.89	106.	70	1.61	20.71
72.	65	1.61	25.89	107.	45	1.70	15.57
73.	54	1.58	21.42	108.	70	1.66	25.17
74.	56	1.64	20.58	109.	68	1.64	25.00
75.	55	1.61	20.99	110.	48	1.51	20.74
76.	53	1.51	22.94	111.	60	1.66	21.58
77.	95	1.70	32.87	112.	57	1.76	18.21
78.	68	1.66	24.46	113.	70	1.61	26.71
79.	54	1.58	21.42	114.	40	1.58	15.87
80.	62	1.64	22.79	115.	64	1.64	23.52
81.	55	1.64	20.22	116.	64	1.61	24.42
82.	56	1.56	22.76	117.	64	1.56	26.00
83.	50	1.76	16.18	118	48	1.81	14.52
84.	64	1.58	24.39	119.	65	1.64	23.89
85.	55	1.56	22.35	120.	57	1.58	22.61

S.No.	Weight	Height	BMI	S.No.	Weight	Height	BMI
121.	65	1.56	26.42	161.	60	1.81	18.14
122.	67	1.51	29.00	162.	60	1.56	24.39
123.	78	1.81	23.58	163.	64	1.56	26.00
124.	53	1.76	16.90	164.	75	1.61	28.62
125.	72	1.66	25.89	165.	61	1.51	26.55
126.	67	1.64	24.63	166.	56	1.58	22.22
127.	68	1.76	21.73	167.	66	1.56	26.82
128.	53	1.51	22.82	168.	53	1.80	16.50
129.	62	1.81	18.73	169.	70	1.66	25.17
130.	58	1.76	18.50	170.	48	1.51	20.74
131.	58	1.68	23.00	171.	55	1.51	24.00
132.	59	1.64	21.69	172.	42	1.56	17.00
133.	67	1.61	25.57	173.	75	1.71	25.42
134.	66	1.71	22.54	174.	58	1.71	19.60
135.	64	1.58	25.39	175.	52	1.56	21.13
136.	41	1.61	15.64	176.	90	1.51	38.96
137.	52	1.56	21.13	177.	103	1.58	41.00
138.	51	1.76	16.29	178.	64	1.56	26.00
139.	41	1.56	16.66	179.	79	1.56	32.11
140.	71	1.76	22.70	180.	55	1.56	22.35
141.	74	1.64	27.20	181.	45	1.58	17.85
142.	55	1.61	20.99	182.	57	1.58	22.61
143.	92	1.66	33.00	183.	45	1.58	42.20
144.	60	1.64	22.00	185.	52	1.80	16.20
145.	54	1.56	21.95	186.	92	1.51	40.10
146.	65	1.66	23.38	187.	60	1.61	22.90
147.	48	1.51	20.74	188.	58	1.61	22.18
148.	65	1.51	28.21	189.	54	1.56	22.95
149.	63	1.74	20.60	190.	68	1.71	23.00
150.	43	1.51	18.67	191.	74	1.61	22.24
151.	70	1.61	26.71	192.	103	1.71	35.00
152.	58	1.58	23.00	193.	42	1.56	17.20
153.	68	1.56	27.64	194.	96	1.57	41.50
154.	50	1.58	19.84	195.	51	1.66	18.30
155.	58	1.61	22.13	196.	53	1.66	19.10
156.	75	1.56	30.48	197.	47	1.51	20.4
157.	70	1.64	25.73	198.	55	1.51	23.80
158.	57	1.51	24.89	199.	65	1.71	22.20
159.	48	1.61	18.32	200.	59	1.71	20.00
160.	70	1.64	25.73				

APPENDIX - IV

MEAN FOOD INTAKE OF THE MALE PATIENTS

S.No.	Cereals g	Pulses g	G.L.V g	Roots & tubers g	Other veg's g	Fruits g	Milk & m. pdts g	Sugar & Jaggery g
1.	250	70	75	70	60	100	500	10
2.	225	60	65	40	70	-	350	5
3.	220	70	85	20	75	35	250	5
4.	200	60	60	25	50	50	350	-
5.	250	50	100	40	100	40	499	10
6.	190	50	120	70	65	100	300	5
7.	230	40	30	30	60	60	200	5
8.	180	80	120	100	70	115	250	5

MEAN FOOD INTAKE OF THE FEMALE PATIENTS

S.NO.	Cereals g	Pulses g	G.L.V g	Roots & tubers g	Other veg's g	Fruits g	Milk & m. pdts g	Sugar & Jaggery g
1.	260	50	90	20	40	-	350	10
2.	235	40	40	20	65	40	300	15
3.	195	40	120	50	60	100	300	10
4.	170	45	55	35	50	70	300	10
5.	220	55	30	55	90	45	250	15
6.	200	5	85	45	60	35	400	20
7.	150	60	60	65	45	50	450	-
8.	210	70	40	80	50	25	250	5
9.	250	50	75	30	70	125	300	10
10.	220	75	85	30	85	35	300	10
11.	215	60	100	50	55	80	150	10
12.	280	50	110	40	85	120	400	10

MEAN NUTRIENT INTAKE OF THE MALE PATIENTS

S.No.	Energy k.cal	Protein (g)	Fat (g)	Ca (mg)	Iron (mg)	Beta-carotene (mg)	Thiamine (mg)	Riboflamin (mg)	Vit C (mg)	Niamine (mg)
1.	1650	50	15	1115	8.0	2520	2.9	1.2	170	8.20
2.	1725	45	25	920	10.0	2000	1.4	1.6	95	8.00
3.	1462	40	36	1000	8.5	2390	3.4	1.3	110	8.50
4.	1500	70	20	1123	7.0	2400	3.7	1.3	140	10.00
5.	1325	30	17	1121	10.0	2800	1.5	1.3	100	9.00
6.	1500	45	17	950	8.5	3060	1.9	1.3	185	5.00
7.	1781	60	20	1000	8.0	2320	1.4	1.8	95	7.00
8.	1320	63	19	990	15.0	2415	4.5	1.5	175	7.00

MEAN NUTRIENT INTAKE OF THE FEMALE SELECTED PATIENTS

S.No.	Energy k.cal	Protein (g)	Fat (g)	Ca (mg)	Iron (mg)	Beta-carotene (mg)	Thiamine (mg)	Riboflamin (mg)	Vit C (mg)	Niamine (mg)
1.	1100	73	15	1000	9	2200	3.8	1.4	130	10
2.	1700	50	20	1100	10	2360	3.4	1.1	100	9
3.	1480	40	20	800	8	2800	2.2	1.3	180	6
4.	1200	43	25	950	8	2460	4.5	1.3	80	9
5.	1367	30	20	1000	16	2440	3.4	1.1	70	7
6.	1752	50	25	1120	10	2480	4.2	1.5	65	8
7.	1348	40	20	953	11	2420	1.5	1.3	100	9
8.	1560	50	18	1050	10	2340	2.8	1.6	110	8
9.	1300	45	15	1010	8	2400	3.1	1.1	190	7
10.	1920	35	20	1026	9	2320	4.3	1.7	76	8
11.	1160	30	15	755	7	2350	3.9	1.2	95	9
12.	1330	40	20	1120	12	3200	4.2	1.2	180	10

APPENDIX - V

COMPARISON OF LIPID PROFILES OF THE SELECTED PATIENTS WITH BETE-CATOTENE SUPPLEMENTATION

TOTAL CHOLESTEROL

S.No.	Initial Values mg/dl	Value after 30 days mg/dl	Value after 60 days mg/dl
1.	184	180	175
2.	201	193	211
3.	168	160	173
4.	223	221	192
5.	224	219	203
6.	217	215	210
7.	200	196	192
8.	250	245	240
9.	203	200	190
10	193	190	175

TOTAL TRIGLYCERIDE

S.No.	Initial Values mg/dl	Value after 30 days mg/dl	Value after 60 days mg/dl
1.	160	154	150
2.	170	162	155
3.	211	204	161
4.	168	164	143
5.	170	168	142
6.	160	158	160
7.	163	162	193
8.	201	200	155
9.	163	159	155
10.	170	168	163

HDL CHOLESTEROL

S.No.	Initial Values mg/dl	Value after 30 days mg/dl	Value after 60 days mg/dl
1.	50	58	60
2.	50	57	59
3.	50	53	65
4.	53	53	58
5.	60	57	54
6.	60	61	62
7.	55	55	55
8.	48	52	55
9.	53	57	59
10.	60	63	65

LDL CHOLESTEROL

S.No.	Initial Values mg/dl	Values after 30 days mg/dl	Values after 60 days mg/dl
1.	115	91	85
2.	120	104	131
3.	96	66	76
4.	137	135	105
5.	130	128	121
6.	125	122	118
7.	112	109	105
8.	162	153	146
9.	117	111	100
10.	99	93	77

VLDL CHOLESTEROL

S.No.	Initial Values mg/dl	Values after 30 days mg/dl	Values after 60 days mg/dl
1.	32	31	30
2.	28	32	31
3.	42	41	32
4.	33	33	29
5.	34	34	29
6.	32	32	30
7.	33	32	32
8.	40	40	38
9.	33	32	31
10.	34	33	32

COMPARISON OF LIPID PROFILES OF THE SELECTED SUBJECTS WITH BETA-CAROTENE AND ASCORBIC ACID SUPPLEMENTAION

TOTAL CHOLESTEROL

S.No.	Initial Values mg/dl	Values after 30 days mg/dl	Values after 60 days mg/dl
1.	201	196	203
2.	220	217	206
3.	247	244	204
4.	230	227	202
5.	238	237	224
6.	225	217	209
7.	240	238	228
8.	330	329	320
9.	220	213	200
10.	178	170	164

TOTAL TRIGLYCERIDE

S.No.	Initial Values mg/dl	Values after 30 days mg/dl	Values after 60 days mg/dl
1.	170	165	152
2.	167	161	147
3.	165	161	149
4.	147	145	128
5.	235	233	220
6.	242	239	134
7.	186	180	175
8.	240	235	228
9.	186	180	228
10.	160	156	144

HDL CHOLESTEROL

S.No.	Initial Values mg/dl	Values after 30 days mg/dl	Values after 60 days mg/dl
1.	47	53	62
2.	52	54	61
3.	63	65	55
4.	54	58	61
5.	50	51	55
6.	51	56	57
7.	40	45	48
8.	50	55	57
9.	56	60	62
10.	65	68	70

LDL CHOLESTEROL

S.No.	Initial Values mg/dl	Values after 30 days mg/dl	Values after 60 days mg/dl
1.	120	110	111
2.	135	131	116
3.	151	147	119
4.	147	140	115
5.	141	139	125
6.	126	113	125
7.	163	157	145
8.	232	227	217
9.	127	117	104
10.	81	71	65

VLDL CHOLESTEROL

S.No.	Initial Values mg/dl	Values after 30 days mg/dl	Values after 60 days mg/dl
1.	28	33	30
2.	33	32	29
3.	33	32	30
4.	29	29	26
5.	47	47	44
6.	48	48	27
7.	37	36	35
8.	48	47	46
9.	37	36	34
10.	32	31	29