Effect Of Supplementation Of Spirulina On The Lipid Levels Of Selected Hyperlipidemic Subjects

By

D. Vidhya

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN - DEEMED UNIVERSITY, COIMBATORE - 641 043

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN FOOD SERVICE MANAGEMENT AND DIETETICS

APRIL - 1999

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CERTIFIED AS BONAFIDE RESEARCH WORK

SIGNATURE OF THE DEAN	SIGNATURE OF THE HEAD OF THE DEPARTMENT	SIGNATURE OF THE GUIDE

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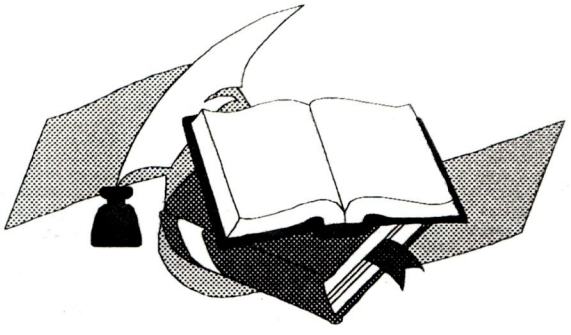

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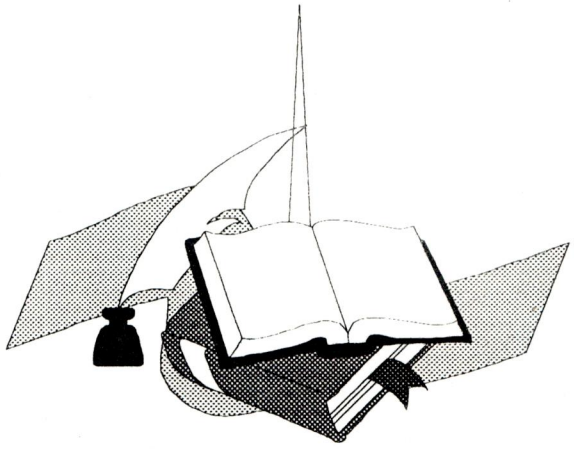


Introduction

I INTRODUCTION

Health means being sound in body, mind and spirit. The 30th World Health assembly decided that the main social target of governments and World Health Organisation(WHO) in the coming decades should be " health for all by 2000 A.D." Equity in health and health care must be placed higher on the public agenda (Mehta, 1998).

Health of the individual is affected by medical illness which may be caused both by infectious diseases and chronic diseases. Preventing and controlling chronic diseases is often a matter of choosing wiser life styles and creating better environments (World Health, 1997).

The World Health report (1997) made it clear that roughly two-third of deaths caused worldwide by chronic diseases in developing countries and also states that cardio - vascular disease account for nearly half of the total deaths.

The three major risk factors for cardio-vascular disease are hyperlipidemia, hypertension and cigarette smoking (Skinner **et al.**, 1996). The hyperlipidemias may occur from increased levels of cholesterol and triglycerides in a variety of lipoproteins.

The National Cholesterol Education Program, (1993) points out that approximately one half the population has cholesterol levels within the desirable range, one fourth in the borderline range and one fourth can be classified at high coronary heart disease risk.

Forty percent of the United States population had high blood cholesterol (Sempos **et al.**, 1993). In Uttar Pradesh, around 50 per cent population over age 20-29 years had high serum cholesterol level (Tiwari **et al.**, 1997).

The serum cholesterol is a potent indicator of risk of coronary heart disease. There is a strong association between the plasma cholesterol level measured early in adult life in men and cardiovascular disease in mid life (Klag **et al.**, 1993). In Framingham study it was found that men and women 35 to 44 years of age with serum cholesterol levels of 265 mg/100 ml or higher had a five times greater risk of developing heart disease than those with levels below 200 mg/dl.

Hypercholesterolemia, the most extensively measured lipid, is strongly associated with coronary heart disease. The degree of risk rises in proportion to the concentration of cholesterol (Kannel **et al.**, 1987).

The second report of the Expert Panel on Detection, Evaluation and Treatment of high blood cholesterol in Adults re - affirms that increased blood cholesterol level specifically low-density lipoprotein (LDL) cholesterol, increases risk for coronary heart disease (National Cholesterol Education Program, 1993).

Many population studies have shown that high density lipoprotein (HDL) cholesterol is a strong, negative, independent predictor of coronary heart disease incidence and mortality in men and women (Krause **et al.**, 1996). A high HDL-cholesterol level is now considered as a negative risk factor and a low HDL-cholesterol as a positive risk factor for coronary heart disease (National Cholesterol Education Program, 1993).

It was the combination of high triglycerides and low HDL-cholesterol predicted coronary heart disease (Castelli, 1992). There is positive relationship between coronary artery disease and plasma triglyceride (Patsch**et al.**, 1992).

A consensus panel at the National Institute of Health (NIH) concluded that the risk of coronary heart disease increases with high level of LDL cholesterol and decreases with high level of HDL cholesterol.

Elevated plasma triglyceride and very low density lipoprotein (VLDL) cholesterol and lower HDL- cholesterol are more common with non insulin dependent diabetes mellitus (American Diabetes Association, 1995). Lipid abnormalities have been identified as risk factors for macrovascular complications in diabetic patients (Garber **et al.**, 1992).

The serum cholesterol is higher in hypertensive males and females (Sangita Nayak, 1995). In Mumbai, about 57 percent of the hypertensive patients had total cholesterol greater than 200 mg/dl and 37 percent had LDL cholesterol more than 130 mg/dl (Joglekar **et al.**, 1996).

The National Institute of Health Consensus Development Panel, convened in 1985 to assess the level of knowledge about obesity determined that a 20 percent

increase in body weight substantially increase the risk of hypertension, coronary artery disease and lipid disorders. In women higher body mass index (BMI) are associated with higher triglyceride and total cholesterol and LDL cholesterol levels and lower HDL cholesterol levels (Denke **et al.**, 1994).

A single risk factor is not responsible for any disease. There are many risk factors associated with hyperlipidemia. The controllable risk factors are obesity (Matter, 1986), mental stress (Mattiason **et al.**, 1990), high amount of dietary intake of cholesterol and saturated fatty acids (Dietschy, 1998), a daily intake of six or more cups of boiled black coffee (WHO, 1998), alcohol (Gaziono, 1993), smoking (Dallongeville **et al.**, 1996) and oral contraceptive use (Kakis **et al.**, 1993) and the uncontrollable risk factors are age (Krause **et al.**, 1996), sex-female sex hormones raise HDL- cholesterol (Deutsch, **et al.**, 1993) and genetic predisposition (Deutsch, **et al.**, 1993).

As already mentioned life style factors has to be changed to manage any disease. Diet is used to treat people who have elevated blood lipids. The total fat content should be reduced to about 30 to 35 percent of calories. Cholesterol and saturated fats are often moderately restricted. Some plant fibres especially the soluble carbohydrate (pectin and guar gum) found in citrus fruits, apples, legumes, oat and barley can have significant hypocholesterolemic effects (Asha Kavatra **et al.**, 1991). Weight loss is especially useful in bringing VLDL cholesterol to normal which is achieved by lowering caloric intake and vigorous exercise (Dengel **et al.**, 1998).

The report of Indian Medical Gazette (1990) indicates that one percent reduction in plasma cholesterol concentration in middle aged men should result in a two percent reduction in the incidence of coronary heart disease. So, hyperlipidemia should be managed properly in order to reduce the risk of heart disease. Now-a-days many foods are used as health food. Spirulina, a blue green algae is now becoming a health food worldwide.

Spirulina, is a multicellular, filamentous cyanobacterium belonging to an algae of class cyanophyta. The World Food conference of the United Nations declared spirulina as 'The Best Food For Tomorrow'. Spirulina has the approval ofFood and Drug

Administration Act (FDA) of United States of America. International Health bodies hail spirulina as 'The Greatest Superfood on Earth'.

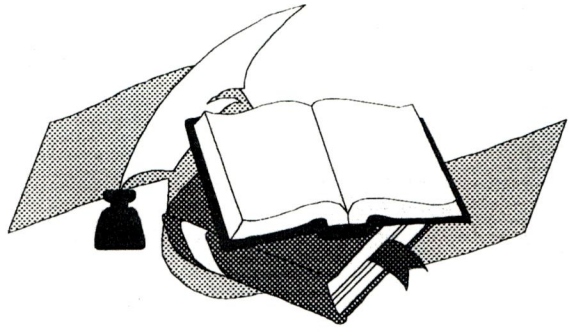
Spirulina has a long history of human usage in Mexico and central Africa, where it grows naturally in the alkaline lake. It is gaining popularity in recent years as a food supplement (Kapoor **et al.**, 1993). Spirulina has over 60 percent protein content, the highest among the foods. It is a fine blend of vitamins A,B,C,E and biotin and is considered the richest vegetarian sources of vitamin B 12 (Bamji **et al.**, 1995). Spirulina has a ß-carotene content 10-12 times that of carrot and an iron content 5-6 times that of leafy vegetables. Further in addition to being rich in trace elements and essential aminoacids, spirulina also provides poly unsaturated fatty acids which is in the form of gamma linolenic acid (Venkataraman, 1993).

Spirulina is attributed with many beneficial effects and has been promoted as a natural health and slimming food in United States and Europe. Treatment of acute anaemia, improvement of haemoglobin levels in human and also control of diabetes by reducing blood sugar have been reported following administration of spirulina. Spirulina is used as sports nutrient and space food (Benemann, 1998). It has got a hypocholesterolemic effect because of its high gamma-linolenic acid content (3-4 percent), which acts directly upon lipid metabolism by preventing accumulation of fats and cholesterol (Seema Arora, 1996).

Dearth of study regarding the hypolipidemic effect of spirulina insisted the investigator to take up the present study with the following objectives:

To

1. Identify the hyperlipidemic patients
2. Assess their
   (a) dietary pattern
   (b) life style pattern
   (c) clinical status
3. .Supplement spirulina to the selected hyperlipidemic subjects.
4. Evaluate the impact of spirulina on blood lipid levels.



Review of Literature

II REVIEW OF LITERATURE

The literature pertaining to the present study on the "Effect of supplementation of spirulina on the lipid levels of selected hyperlipidemic subjects" is discussed under the following headings

A. Incidence of hyperlipidemia

B. Controllable and uncontrollable risk factors of hyperlipidemia

C. Lipid as risk factor

D. Management of hyperlipidemia

E. Spirulina

1. The Best Food for Tomorrow
2. Management of hyperlipidemia with spirulina

A. Incidence of hyperlipidemia

A survey conducted among United States population (Third National Health and Examination Survey) showed that 40 per cent had high blood cholesterol level (Sempos, 1993).

In Brazil, in females, the prevalence of hypercholesterolemia was 30 per cent and hypertriglyceridemia was 30.4 per cent, for males prevalences were 24 per cent for hypercholesterolemia and 26 per cent for high LDL and 27.6 per cent for hypertriglyceridemia. (Lessa **et al**., 1997).

Sanchez-Muniz **et al**. (1996), pointed out that among 169 Spanish people, high total cholesterol was seen in 5.4 per cent, high triglyceride levels in 3.7 per cent, low levels of HDL cholesterol in 0.7 per cent and high LDL cholesterol in 1.4 per cent.

Knight **et al**. (1993) found out hyper-cholesterolemia was present in nearly one quarter of the non-Asians but prevalied in less than one eighth of Asian men out of 288 samples.

Sircar (1992) poined out the incidence of hyperlipidemia as 1.04 per cent (ie) 2.78 million people in India.

Among 300 individuals in Rajasthan, 8 per cent had high cholesterol levels, 6.7 per cent had high LDL cholesterol levels, 29.7 per cent had low HDL cholesterol, 6 per cent had mild hypertriglyceridemia (Gupta, 1994). In Varanasi, a

study conducted by Tiwari **et al**. (1997) pointed out that 15 per cent males and 18 per cent females had high total cholesterol and LDL-cholesterol, 25 per cent males and 10 per cent females had low levels of HDL cholesterol.

A study of coronary risk factors was carried out by Dwivedi (1997), in 80 healthy subjects in Delhi belonging to lower, middle and upper socio-economic segments, dyslipidemia was observed in 36.1 per cent, 21.6 per cent and 42.2 per cent respectively.

B. Controllable and uncontrollable risk factors of hyperlipidemia

1. Controllable risk factors

They are

a. Obesity

b. Dietary intake of fat

c. Coffee

d. Alcohol

e. Smoking

f. Oral contraceptive use

a. Obesity

A study conducted by Rabkin **et al**. (1997), showed that the prevalence of dyslipidemia was related to BMI, as LDL cholesterol and triglyceride concentrations were higher and HDL cholesterol concetrations lower in those with higher BMI.

Arai **et al**.(1994), in their study about obese subjects pointed out the reason for decreased levels of serum HDL-cholesterol in obese subjects and they said that the high levels of plasma cholesteryl ester transfer protein may partly explain the reduced levels of serum HDL cholesterol in obese subjects.

b. Dietary intake of fat

A study conducted by Judd **et al**. (1994) showed that the dietary trans mono unsaturated fatty acids raise LDL-cholesterol levels compared with oleic acid, but to a lesser degree than saturated fatty acids. For every one per cent increase in total energy intake from saturated fatty acids, a 2.7 mg/dl increase in plasma cholesterol level is predicted. (Katan, 1994).

According to Denke **et al**. (1994), a 25 mg increase in dietary cholesterol would raise serum cholesterol by 1 mg/dl.

C. Coffee

Several epidemiologic studies suggest an association between coffee intake and increased levels of serum cholesterol (Haffner **et al**., 1991).

The most recent study on coffee's role in heart disease, done at Stanford University found that sedentary man who drink three cups of coffee or more a day may be at higher risk of developing heart disease than those who drink less coffee (WHO, 1998).

A study conducted by Fried **et al**. (1992) showed that a heavy consumption of filtered caffeinated coffee (>720 ml/day) leads to a statistically significant increase in plasma total cholesterol, LDL cholesterl and HDL cholesterol.

The presence of high amounts of palmitic acid in coffee may be partly responsible in raising cholesterol levels (Thelle, 1997).

d. Alcohol

Low levels of alcohol may be protective against coronary heart disease. Moderate alcohol consumption is associated with an increase in HDL cholesterol, on the other hand, high alcohol intake raises triglyceride levels (Willet **et al**., 1986).

Krause **et al**.(1996) states that alcohol affects both triglyceride and HDL-cholesterol levels. The effects of alcohol on triglyceride levels are dose-dependent and are greater in persons with triglycerides over 150 mg/dl.

Wine drinkers had higher HDL-cholesterol levels than drinkers of beer and spirits (Kimball **et al**. 1992).

e. Smoking

High serum cholesterol and high blood pressure resulting from cigarette smoking increase mortality from heart disease (Neaton **et al**., 1992).

Krause (1996) states that smoking decreases HDL-cholesterol (6-8 mg/dl) and increases VLDL-cholesterol. A study conducted by Senti **et al**. (1998) showed that there is link between smoking and triglyceride metabolism.

Dallongeville **et al**. (1996) conducted a study among 90 smokers and non-smokers and the results of this study showed that smokers had significantly higher levels of mean serum triglyceride, VLDL – cholesterol and lower values of HDL-cholesterol than non-smokers.

f. Oral contraceptive use

Women using oral contraceptives have higher plasma triglyceride concentrations than non-users (Burkman, **et al**. 1998).

Walsh **et al**. (1993) conducted a study about the effect of low dose oral contraceptives on VLDL and LDL metabolism and in this study five premenopausal women consumed oral contraceptives containing 0.035 mg ethinyl estradiol and it was found that this oral contraceptive substantially raises levels of VLDL.

Mental stress, anxiety and lack of sleep also raises serum cholesterol. (Mattiason **et al**., 1990).

2. Uncontrollable risk factor

They are

a. Age

b. Sex

c. Heredity

a. Age

As age increases, the cholesterol level will increase. Plasma levels of HDL-cholesterol are negatively associated with age. A decline in plasma levels of total cholesterol and HDL-cholesterol is particularly common in the elderly. BMI and plasma total cholesterol rose among younger age groups whereas levels declined in older people aged 65 to 79 years (Wilson **et al**., 1994).

Krause (1996) states that the total cholesterol and LDL cholesterol increase with aging. Over the 45 year period from age 20 to 65, total cholesterol in men increases by 13 per cent and in women over the same period the increase is 21 per cent.

b. Sex

A study conducted by Deutsch **et al**. (1993), showed that women have higher level of HDL-cholesterol than men. Female sex hormones raise HDL-cholesterol whereas male sex hormones lower HDL-cholesterol.

In a 10-year longitudinal study of women going through menopause, average serum cholesterol increased by 19 per cent in the perimenopausal period (Van Beresteijn, 1993).

Rifici **et al**. (1992) pointed out that estrogen has been shown to inhibit LDL oxidation which may help to explain the lower rates of coronary heart disease seen in premenopausal women.

Postmenopausal women who are at increased risk of coronary heart disease have greater prevalence of smaller LDL than premeenopausal women of the same age (Campos, 1988).

C. Heridity

According to a study conducted by Deutsch **et al**. (1993), it was clear that the genetic predisposition is reflected in high levels of LDL-cholesterol in the blood.

A genetic epidemiological study of serum lipid and lipoprotein levels was conducted among families of Marevaris residents in Calcutta by Majumder **et al**. (1996) showed the estimated genetic heritability for HDL-cholesterol was 80 per cent while that for triglyceride was 55 per cent. Since genetic effects are strong, individuals whose parents have low HDL-cholesterol and high serum triglyceride are obviously at a high risk for developing coronary heart disease.

C. Lipid as risk factor

Serum lipid is a risk factor for the following diseases:

1. Coronary heart disease

Blood cholesterol concentration was directly related to mortality from coronary heart disease (CHD) (Chen **et al**., 1991). According to Shipley **et al**. (1991), reducing plasma cholesterol concentrations in middle age may influence the risk of death from CHD in old age.

Patsch **et al**. (1992) opined that there is positive relationship between CAD and plasma triglyceride. According to Castelli (1992) it was the combination of high triglyceride and low HDL-cholesterol predicted CHD.

Buring **et al**. (1992), pointed out that high levels of HDL-cholesterol have an inverse relation with myocardial infaction. The ratio of total cholesterol to HDL-cholesterol is a better measure of risk for CHD (Kinosian, **et al**. 1994)

2. Diabetes Mellitus

According to Garber **et al**. (1992), lipid abnormalities have been identified as risk factors, macrovascular complications in diabetic patients. Patients with hypertriglyceridemia are insulin resistant, glucose intolerant and hyperinsulinemic (Shen **et al**., 1993). Patients with diabetic mellitus have an increased risk for coronary heart disease due to hyperglycemia, hypertension and dyslipidemia (Garg, 1998).

3. Hypertension

A study conducted by Sangita Nayak (1995) showed that serum cholesterol level is higher in hypertensive males and females. Park **et al**. (1996), has pointed out that the prevalence of diabetes and hypertension increased as either cholesterol or triglyceride level increased.

4. Other diseases

The study conducted by Kawasaki **et al**. (1997) has shown that hyperlipidemia is a novel etiologic facgtor in deep vein thrombosis. Gisbert **et al**. (1997) has pointed out that hyperlipidemia is a frequent finding in liver transplant recipients and after liver transplantation 12 per cent had both elevated cholesterol and triglyceride levels.

The study conducted by Lindenstrom **et al**. (1994) showed that the risk of stroke is increased with plasma cholesterol concentrations greater than 8 ml/L.

Saraya **et al**. (1995) in a study has shown that the mean plasma cholesterol and triglyceride values were higher in male gallstones patients as compared to controls and in female patients only plasma triglycerides were elevated.

D.Management of hyperlipidemia

According to Stein (1994), the initial approach to the treatment of hyperlipidemia should involve diet, increased physical activity and weight reduction, if target are not achieved drug therapy should be followed. Diet therapy is an important tool in the management of hyperlipidemia. Restriction in the diet helps the hyperlipidemic individual to maintain his blood cholesterol in the normal levels and thus can reduce the risk of heart disease (Antia, 1997).

1. Diet

a. Energy

Reduction in body weight and blood lipids is accomplished by lowering calorie intake (Anitta, 1997). Geil **et al**. (1995) in their study, gave American Heart Association's step 1 diet (30 per cent of energy from fat, 50 to 60 per cent from carbohydrate, 10 to 20 per cent from protein and less than 300 mg cholesterol per day) to hypercholesterolemic subjects for eight weeks and found reduction in serum total cholesterol by 9.2 per cent for women and 7.2 per cent for men and serum LDL-cholesterol by 9.2 per cent for women and 9.8 per cent for men.

b. Carbohydrate

Patients gradually introduced a high carbohydrate (65 per cent of energy) and low fat diet (20 per cent) may achieve a significant reduction of total cholesterol and LDL-cholesterol (Ullmann **et al**., 1997). Barnard (1991) has showed that a diet high in complex carbohydrate and fibre and low in fat and cholesterol combined with exercise reduced the total cholesterol and LDL-cholesterol by 23 per cent.

c.Fat

For the prevention of hyperlipidemia the total fatcontent should be reduced to about 30 to 35 per cent of calories. The content of saturated fat should be limited to 10 per cent of calories and polyunsaturated fatty acids (PUFA) should not exceed 10 per cent of calories and the remaining calories should be monounsaturated fatty acid (MUFA) (Jackson, **et al**. 1989).

Berry **et al**. (1992) suggested that a diet enriched with MUFA can reduce plasma concentrations of total and LDL-cholesterol as long as the amount of dietary saturated fatty acids is not increased.

Sabate **et al**. (1993), suggested that incorporating moderate quantities of walnuts into the recommended cholesterol lowering diet decreases the serum levels of total cholesterol. O'Desa **et al**. (1990), pointed out that lean beef can be included in cholesterol lowering diets.

Howelingen **et al**. (1990), showed that fish intake increases the HDL-cholesterol and decreases triglyceride content. Eicosa pentaenoic acid and omega - 3 fatty acid in the fish lowered total cholesterol and increased the proportion of HDL - cholesterol (Ackman, 1986).

d.Fibre

Glore (1994), suggested that soluble fibre - pectins, gums, mucillages, algae polysaccharides and some hemicelluloses-in legumes, oats, fruits and psyllium lower serum cholesterol and LDL-cholesterol.

Kestin **et al.**(1990) pointed out that rice bran reduced plasma lipids. Barley(McIntosh**et al.**.,1991), wheat germ (Cara **et al.**., 1991) and corn fibre (Shane **et al.** ., 1995), had hypocholesterolomic effect. Anderson **et al**. (1990) showed that 25 grams of ß-glucan rich oat bran per day lowered serum total cholesterol and LDL-cholesterol by 5.4 per cent and 8.5 per cent.

According to Story **et al**. (1986), beans lowered serum cholesterol levels. Sandstron **et al**. (1994), showed that pea fibre lowered the fasting and postprondial serum triglyceride concentrations.

Results of the study conducted by Mishra **et al**. (1993) indicate that 100 grams of fenugreek seed powder produced significant reduction in the serum total cholesterol, LDL cholesterol, VLDL cholesterol and triglyceride levels. Usha (1998) suggested that five stalks of curry leaves per day for three months, reduces serum cholesterol level.

Spiller **et al**. (1991), showed that 15 grams of guar gum reduced total plasma cholesterol and LDL-cholesterol and hypercholosterolemic adults. A study

conducted by Wolever **et al**. (1994), showed that psyllium containing cereal reduced serum total cholesterol, LDL-cholesterol and HDL-cholesterol.

e. Protein

The soy-protein associated with a statistically significant decrease in the plasma concentration of LDL-cholesterol as well as in the ratio of plasma LDL to HDL-cholesterol (Wong **et al**., 1998). Bakhit **et al**. (1994), showed that consumption of 25 grams of soybean protein per day with or without soybean fibre reduced the total cholesterol concentration in hyperlipidemic individuals.

f. Minerals

Bell **et al**. (1992) showed that calcium supplementation produced small decreases in LDL-cholesterol level in hypercholosterolemic men. In a double blind placebo controlled trial, 1200 mg of calcium carbonate lowered LDL-cholesterol by 4.4 per cent and increased HDL-cholesterol by 4.1 per cent.

g. Vitamins

Slattery **et al**. (1995) reported that dietary intake of vitamin A, C, E and ß-carotene are associated with HDL-cholesterol levels. Nuner **et al**. (1995) indicated that combination of vitamin C and E was better in altering the lipid levels than vitamin E or vitamin C alone is consistent with the ability of vitamin c to improve the antioxidant effect of vitamin E.

2. Exercise

Williams **et al**. (1994) has pointed out that in excercises plasma HDL-cholesterol levels increased most in men with high baseline HDL-cholesterol levels and lease in men with low baseline levels.

A study conducted by Dengel **et al**. (1998) have shown that a six month program of aerobic exercise and weight loss lowered total cholesterol by 14 per cent and triglyceride by 34 per cent and raised HDL-cholesterol two fold.

Wood **et al**. (1988), compared diet alone to diet and exercise during weight loss. Persons who exercised during weight loss had more healthful lipid profiles and in particular higher HDL levels, than persons who lost weight simply through energy restriction.

3. Drug

A study conducted by Furmaga (1993) showed that four antilipemic drugs - colestipol, gemfibrozil, lovastatin and niacin were used in the clinic. When supplemented for three months, gemfibrozil increased HDL-cholesterol concentration by 9.2 per cent reduced triglyceride levels by 38 per cent and significantly lowered the ratio of total cholesterol to HDL-cholesterol (Miller, **et al.**, 1993).

Arca **et al.** (1994), showed that lovastatin therapy reduced both total cholesterol and LDL-cholesterol values in hypercholosterolemic post menopausal women.

Niacin is valuable tool in the management of hyperlipidemia because it rises HDL-cholesterol and reduced LDL-cholesterol and triglyceride levels (Luria, 1988).

E. Spirulina

1. The Best Food for Tomorrow

Christopher Hills - the father of spirulina is the pioneer in the field of using spirulina as a human food. Spirulina has the highest protein content among the natural foods ranging from 46-70 per cent (Cliferri, 1983). The Net Protein Utilisation (NPU) value of spirulina is 62 per cent (Switzer, 1982). Spirulina also contains a rapid-acting and efficient carbohydrate called rhamnose (Ray, 1985).

Sharma (1992) opines that spirulina is a rich source of natural bio-chelated vitamins, containing all the vitamins in highly bio-available form. Annapurna **et al.** (1991) has pointed out that spirulina is one of the richest sources of natural beta-carotene, the provitamin A.

Spirulina is also a good source of vitamin B-12 (2 μg/g) and it is the only food of plant origin which contains B-12 vitamin (Bamji **et al.**, 1995). Spirulina also contains Vitamin B-1, Vitamin B-2, Vitamin B-3, Vitamin B-6, vitamin E, folic acid, biotin, inocitol and pantothenic acid (Weisburger, 1991).

Spirulina algae is very rich in iron, the total iron content is around 89 mg/100g (NIN, 1989). Robert Henrikson (1989) pointed out that spirulina contains

good amount of bio-chelated calcium magnesium, manganese, potassium copper, zinc, chromium and selenium.

According to Dillon **et al**. (1995), spirulina has about 5-6 per cent essential fatty acids, of which gamma linolenic acid is 30 per cent, one of the highest natural sources available (Roughhan, **et al**., 1989).

Studies at the National Institute of Nutrition, Hyderabad revealed that feeding 2 gms of spirulina to youngchildren remove the problem of conjunctial xerosis and bitot spots because of its beta carotene content.

A study conducted in Japan by Takeuchi (1998), with eight women who were treated with four grams of spirulina for 30 days showed increased in their average blood haemoglobin content by 21 per cent.

Babu (1989), in his study has proved that spirulina has a hypoglycemic effect in non insulin dependent diabetic patients.

A double cross-over study conducted in Germany by Becker **et al**. (1986) concluded that, faster weight reduction might be possible with five grams of spirulina supplemented for a period of four weeks, with virtually no side effects.

Mathew **et al**. (1995) evaluated the chemopreventive activity of spirulina in oral cancer in pan tobacco chewers, complete regression of lesions was observed in 45 per cent subjects.

Spirulina has rich chlorophyll which has the ability to heal wounds (Seema Arora, 1996). In Vietnam, spirulina is sold as lactogil, to increase lactation in nursing mothers (Venkatraman, 1993).

Spirulina is now available in powder and tablet form. In Japan, snack chips are also available. Fruit and snack bars enriched with spirulina cookies enriched with spirulina are also available now-a-days. Studies have shown spirulina as a possible space food (McKelvey, 1990).The NIN has conducted toxicological studies and has proved spirulina to be safe (NIN, 1988).

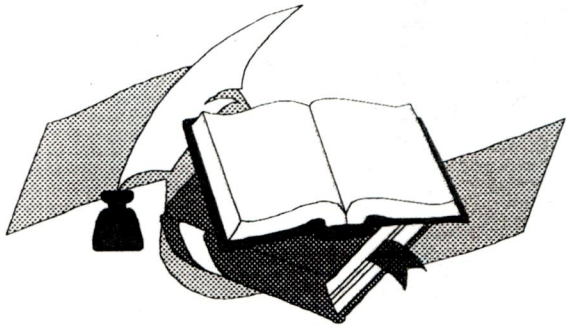
2. Management of hyperlipidemia with spirulina

In Japan, a clinical study on the anti cholesterolemic property of spirulina was conducted by Nayaka (1988). Thirty male volunteers with high cholesterol, mild

hypertension and hyperlipidemia, showed effective lowering of serum cholesterol, triglyceride and LDL levels after consumption of spirulina for eight weeks. They consumed 4.2 grams of spirulina (8 tablets of 500 mg) daily for eight weeks. The total serum cholesterol level dropped significantly by 4.5 per cent within four weeks from 224 mg/dl to 233 mg/dl.

The effect of spirulina on hypercholesterolemic patients was carried out by Ramamoorthy **et al**. (1996). Thirty ischaemic heart disease patients without any complications of the disease and with hypercholesterolemia, two grams and four grams of spirulina was supplemented for three months. Results indicated that spirulina plays a key role in weight reduction, loweringblood cholesterol levels and improving the lipid profile of patients.

Gamma linolenic acid (GLA) an essential fatty acid is known to reduce cholesterol and prevent fat accumulation (Nichols, 1986). GLA is not widely available in our diet but linoleic acid (LA) is available. LA is converted to GLA in the presence of an enzyme (delta-6 desaturase). This enzyme is inhibited by saturated fats and alcohol which results in high blood pressure and high cholesterol. Spirulina is very rich in GLA which overcomes the enzyme block and helps in alleviating cholesterol and high blood pressure (Carter, 1989).



Methodology

III METHODOLOGY

The methodology pertaining to the study on "Effect of supplementation of spirulina on the lipid levels of selected hyperlipidemic subjects" is discussed under the following headings

A. Selection of area

B. Selection of hyperlipidemic subjects

C. Collection of background information of the hyperlipidemic subjects

D. Assessment of the life style pattern of the subjects

E. Assessment of clinical status of the subjects

F. Survey of dietary pattern of the subjects

G. Quantification of food intake of the subjects and calculation of nutrients

H. Supplementation of home diets of the subjects with spirulina capsules

I. Evaluation of the impact of supplementation

A. Selection of area

The study was carried out in Coimbatore city. The hyperlipidemic subjects were selected from two hospitals in the city.

B. Selection of hyperlipidemic subjects

Individuals who come for the master health check up in the two hospitals were surveyed and male hyperlipidemic subjects were selected in the age group of 40-60 years by convenient sampling. Total cholesterol and low density lipiprotein (LDL) cholesterol increase with aging over the 45 year period from age 20 to 65, total cholesterol in men increases by 13 percent and in women over the same period the increase is 21 percent (Krause, 1996).

Eighteen subjects were selected and they were divided into three groups (I, II and III) of six each based on their cholesterol levels. Group I subjects were taken as control and group II and group III subjects served as experimental subjects.

C. Collection of background information of the hyperlipidemic subjects

In order to elicit information on the socio economic background of the subjects, an interview schedule was formulated (Appendix I A). Details regarding

educational status, occupation, income of the subject and total income of the family of the subjects were collected through the interivew schedule.

D. Assessment of the life style pattern of the subjects

Life style factors are associated with 50 percent or more of the leading causes of death (Dever, 1986). To assess the life style pattern of the subjects, informations regarding intake of coffee, tobacco, smoking pattern, alcoholism and exercise pattern were collected through interview schedule (Appendix I B).

E. Assessment of clinical status of the subjects

The body dimensions, blood pressure and blood lipid levels of the subjects were recorded and informations regarding the age of onset of hyperlipidemia, symptoms and familial tendency of hyperlipidemia were collected through an interview schedule (Appendix I C).

1. Assessment of body dimensions of the subjects

Anthropometry has become the most widely used tool in nutritional assessment (Foster, 1992). Among the various anthropometric measurements weight, height and waist and hip circumferences were used in this study.

The height and weight of the subjects were recorded following the standard procedure given by Jelliffe (1989). From the recorded height and weight of the subjects, body mass index (BMI) values were calculated using the formula.

$$BMI = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}}$$

One of the most accepted method to measure obesity is measurement of BMI (Mahapatra, **et al.**, 1998). Body Mass Index is strongly related to lipid abnormalities (Rabkin **et al.** 1997).

Waist and hip circumferences of the subjects were noted. Waist to hip ratio was calculated to estimate body fat distribution and central fat accumulation (Garrow, 1987).

2. Determination of blood pressure of the subjects

The blood pressure of the selected subject was measured by using a sphygmomanometer. The blood pressure was noted in a relaxed state among the subjects.

3. Determination of lipid levels of the subjects

Serum total cholesterol, triglycerides high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol of the individuals were estimated to know their lipid levels. Five millilitres of venous blood was drawn from all the individuals for the estimation.

The serum total cholesterol of the subjects were estimated by using ZAK's method (Appendix II). High triglyceride levels has been associated with increased prevalence of coronary artery disease. The serum triglyceride levels of the subjects were estimated by using the enzymatic method (Appendix III) devised by span kit.

High HDL cholesterol level protects against heart disease. Hence in this study serum HDL cholesterol levels were estimated by using the method given in span kit (Appendix IV).

The serum LDL cholesterol was calculated using the following formula given by Friedewald (1972):

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL cholesterol}$$

VLDL cholesterol values were computed using Friedewald formula (1972):

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

F. Survey of dietary pattern of the subjects

Details regarding the type of diet, meal pattern of the subjects, their frequency of consumption of common foods with special reference to fleshy foods, milk

and oil were collected through interview schedule (Appendix ID). Informations regarding their awareness about the importance of diet in hyperlipidemia were also collected.

G. Quantification of food intake of the subjects and calculation of nutrients.

The days food intake of the subjects were collected by 24 hour recall method with simple household measures. Raw equivalent of the foods consumed was noted and from this the nutrient intake was calculated by using food composition table of Gopalan **et al.** (1996).

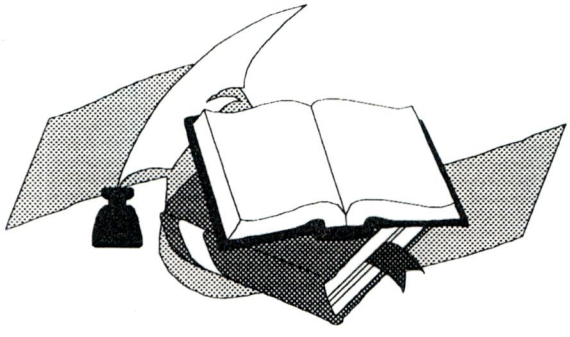
H. Supplementation of home diets of the subjects with spirulina capsules.

Spirulina capsules (each capsule consists of 500 miligram of spirulina) were given to the subjects at the beginning of the study period. Group II subjects consumed one gram of spirulina (two capsules) twice a day after the food in the morning and night. On a total, they took two grams of spirulina (four capsules) per day for a period of 30 days.

Group II subjects took one gram of spirulina (two capsules) thrice a day after the food in the morning, afternoon and night. They consumed three grams of spirulina (six capsules) per day for a period of one month. The dosage was given after consultation with the physician.

I. Evaluation of the impact of supplementation

Biochemical tests formed the main tool for evaluation of impact of supplementation. Serum total cholesterol, triglycecides and HDL cholesterol levels were again estimated at the end of one month and serum LDL cholesterol and VLDL cholesterol were calculated. Body weight (to calculate BMI) and blood pressure of the subjects were noted to know the impact of spirulina.



Results and Discussion

IV RESULTS AND DISCUSSION

The results of the present study on "Effect of supplementation of spirulina on the lipid levels of selected hyperlipidemic subjects" are discussed under the following headings

A. Background information of the hyperlipidemic subjects
B. Life style pattern of the subjects
C. Clinical status of the subjects
D. Dietary pattern of the subjects
E. Impact of spirulina on
    1. Body weight
    2. Blood pressure
    3. Lipid levels
F. Correlation between intake of selected nutrients and blood lipid levels

A. Background information of the hyperlipidemic subjects

The background information of the selected hyperlipidemic subjects was studied by administering an interview schedule. The results are presented below:

1. Age of the subject

The age of the subjects selected was surveyed and it was found that out of the total of 18 subjects, 14 subjects were above 51 years, seven were between 51-55 years and seven were between 56-60 years. Plasma levels of HDL-cholesterol are negatively associated with age (Wilson **et. al.,** 1994).

2. Educational qualification of the subjects

Table I shows the educational qualiification of the subjects.

TABLE I
EDUCATIONAL QUALIFICATION OF THE SUBJECTS

Educational status	Group I (n=6)	Group II (n=6)	Group III (n=6)	Total (n=18)
Middle school	-	1	1	2
High School	1	2	1	4
Higher secondary	2	1	1	4
Under graduate	3	2	2	7
Post graduate	-	-	1	1

All the subjects in this study were literate. The table shows that seven out of 18 subjects were undergraduates and only one was a post graduate.

3. Occupational status of the subjects

The occupational status of the subjects is presented in Table II.

TABLE II
OCCUPATIONAL STATUS OF THE SUBJECTS

Occupational status	Group I (n=6)	Group II (n=6)	Group III (n=6)	Total (n=18)
Typist	1	3	1	5
Lecturer	-	-	1	1
Engineer	1	1	-	2
Business	3	2	3	8
Agriculturist	1	-	1	2

The results revealed that out of 18 subjects, eight were doing business. Only two were engaged in heavy agricultural work.

4. Total monthly income of the families of the subjects

The total monthly income of the families of the subjects ranged from Rs.6000 to Rs.11000.

B. Life style pattern of the subjects

1. Coffee drinking

Out of the 18 subjects, majority that is 16 drank coffee (all in group I and five out of six in each of group II and group III).

The quantum of coffee taken by the subjects is given in Table III.

TABLE III
QUANTUM OF COFFEE TAKEN BY THE SUBJECTS

Quantum of coffee taken		GroupI (n=6)	Group II (n=6)	Group III (n=6)	Total (n=18)
ml	cups				
0-200	0-1	-	-	1	1
200-400	1-2	5	2	2	9
400-600	2-3	1	1	1	3
600-800	3-4	-	2	1	3

The table indicates that only one subject took below 200 ml or 1 cup per day whereas three subjects took 600-800 ml or 3-4 cups per day. Sedentary man between age of 30-55, who drinks three cups of coffee or more a day may be at higher risk of developing heart disease (WHO, 1998).

The type of coffee taken by the subjects is given in Table IV.

TABLE IV
TYPE OF COFFEE TAKEN BY THE SUBJECTS

Type of coffee	Group I (n=6)	Group II (n=6)	Group III (n=6)	Total (n=18)
Filter coffee	1	2	2	5
Instant Coffee	5	3	3	11

The consumption of instant coffee was more than that of filter coffee. None took boiled coffee. According to Bak **et. al.** (1989), boiled coffee produces even greater elevations in plasma lipids than filtered coffee.

2. Smoking pattern

The smoking pattern of the male subjects was studied because smoking is a risk factor for heart disease (Neaton **et. al.,** 1992).

The number of subjects smoking and the number of cigarettes smoked per day is tabulated in Table V.

TABLE V
NUMBER OF CIGARETTES SMOKED PER DAY

Number of cigarettes smoked	Group I (n=6)	Group II (n=6)	Group III (n=6)	Total (n=18)
0-10	4	2	1	7
10-20	-	3	1	4
20-30	1	-	1	2
Total	5	5	3	13

The table indicates that only 13 out of 18 subjects smoked. In group I and group II, five out of six smoked. Six out of 13 smoked more than one pack (ten cigarettes). Smoking more than a pack of cigarette is a risk factor for heart disease.

Smokers had significantly higher mean levels of serum triglyeride, VLDL cholesterol and lower levels of HDL - cholesterol than non-smokers (Dallongeville et. al., 1996).

3. Intake of pan with tobacco

Out of the 18 subjects four subjects chewed pan (one out of six in group II and three out of six in group III). One subject in group II and two subjects in group III chewed pan with tobacco three times a day and one subject in group III chewed six times a day.

4. Alcohol consumption

Nine out of 18 subjects took alcohol (three from group I or control group, two from group II and four from group III).

The type and frequency of alcohol intake by the subjects is presented in Table VI.

TABLE VI
TYPE AND FREQUENCY OF ALCOHOL INTAKE BY THE SUBJECTS

Type of alcohol	Group I (n=6)		Group II (n=6)			Group III (n=6)		
	Weekly	Occasi onally	Thrice a week	Weekly	Occasi onally	Thrice a week	Weekly	Occasi onally
Beer	-	-	-	1	-	-	-	2
Whisky	1	1	1	-	1	1	1	-
Brandy	-	1	1	-	1	1	-	-
Wine	-	-	-	1	-	-	-	-
Toddy	-	1	-	-	-	-	1	-

Out of nine subjects one subject (from group I) took wine (weekly), two subjects took toddy - one from group I (occasionally) and one from group III (weekly) and six subjects took whisky (two in each of group I, II and III).

The amount of alcohol intake by the subjects is given in Table VII.

TABLE VII
AMOUNT OF INTAKE OF ALCOHOL BY THE SUBJECTS

Amount (ml)	Group I (n=6)	Group II (n=6)	Group III (n=6)	Total (n=18)
30	1	-	1	2
60	-	-	2	2
120	2	2	1	5

Five out of nine subject took 120 ml of alcohol (two in each of group I and group II and one in group III). Moderate alcohol (10-50 ml) consumption is associated with an increase in HDL - cholesterol. On the other hand, high alcohol intake raise triglyceride level (Willett **et al.**, 1986).

5. Type of exercise

Out of the 18 subjects, five subjects exercised, of this three subjects were from control or group I (one walked daily and the other two weekly) and two subjects are from experimental group or group II (one walked daily and the other did yoga daily). Exercise training has major effects on lowering triglyceride levels in hyperlipidemic subjects (Lampman **et al.**, 1991).

C. Clinical status of the subjects

1. Height, weight and BMI of the subjects

The height, weight and BMI of the subjects is presented in Table VIII.

TABLE VIII
HEIGHT, WEIGHT AND BMI OF THE SUBJECTS

Group	Subject No.	Height (cm)	Weight (kg)	BMI
I (n=6)	1	168	72.0	25.5
	2	175	78.0	25.4
	3	170	75.0	25.9
	4	165	70.0	25.7
	5	178	75.0	25.3
	6	165	72.0	25.2
II (n=6)	1	168	71.5	25.3
	2	164	71.0	25.4
	3	168	72.0	25.5
	4	168	72.0	25.5
	5	167	70.0	25.1
	6	165	72.5	25.6
III (n=6)	1	156	62.0	25.8
	2	170	75.0	25.9
	3	168	71.5	25.3
	4	168	72.0	25.5
	5	175	78.5	25.6
	6	170	72.0	25.0

It is evident from the table that all the subjects had BMI in the range of 25-26. The classification scheme endorsed by WHO defines overweight as a BMI from 25 to 29.9. According to Denke et al., (1993), a higher BMI ($\geq$ 21 to < 30) was associated with higher plasma triglyceride levels, lower HDL- cholesterol levels and higher total cholesterol levels.

2. Waist, hip circumference and waist hip ratio of the subjects

The waist, hip circumference and waist hip ratio of the subjects is given in Table IX.

TABLE IX

WAIST, HIP CIRCUMFERENCE AND WAIST HIP RATIO OF THE SUBJECTS

Group	Subject number	Waist circumference (cm)	Hip circumference (cm)	Waist hip ratio
I (n=6)	1	89.0	101.5	0.87
	2	96.5	107.0	0.90
	3	91.5	101.5	0.90
	4	91.5	107.0	0.86
	5	96.5	107.0	0.90
	6	91.5	107.0	0.86
II (n=6)	1	94.0	107.0	0.88
	2	94.0	104.0	0.90
	3	91.5	107.0	0.86
	4	91.5	104.0	0.88
	5	96.5	109.0	0.89
	6	91.5	109.0	0.84
III (n=6)	1	91.5	107.0	0.86
	2	94.0	107.0	0.88
	3	91.5	107.0	0.86
	4	94.0	107.0	0.88
	5	91.5	101.5	0.90
	6	94.0	109.0	0.86

A cut off of 39 inch (99 cm) or higher for both men and women has been suggested as a level above which risk of various chronic diseases rises significantly

(Pouliot et. al., 1994). It is clear from the table that all the subjects had waist circumference below 97 cm.

The recommended value of waist to hip ratio is less than 0.95 (Folsom, 1993). All the subjects had waist to hip ratio below 0.9.

3. Blood pressure of the subjects

The blood pressure of the subjects is given in Table X.

TABLE X
BLOOD PRESSURE OF THE SUBJECTS

Group	Subject number	Systolic pressure (mm/Hg)	Diastolic pressure (mm/Hg)
I (n=6)	1	130	90
	2	140	90
	3	135	90
	4	130	85
	5	130	85
	6	130	80
II (n=6)	1	130	80
	2	125	80
	3	130	90
	4	130	85
	5	150	90
	6	135	90
III (n=6)	1	135	90
	2	125	90
	3	140	90
	4	150	90
	5	140	90
	6	130	80

All the subjects were in the high normal range of blood pressure (130-139 mm/Hg systolic and 85-89 mm/Hg diastolic pressure). Optimal blood pressure with respect of cardiovascular risk is less than 120 mm/Hg systolic and less than 80 mm/Hg diastolic (Fifth report of the Joint National Committee on Detection, Evaluation and Treatment of high blood pressure, 1993).

4. Age of onset of hyperlipidemia

The age of onset of hyperlipidemia among the subjects is given in Table XI.

TABLE XI
AGE OF ONSET OF HYPERLIPIDEMIA

Age (years)	Group I (n=6)	Group II (n=6)	Group III (n=6)	Total(n=18)
41-45	1	1	-	2
46-50	2	1	1	4
51-55	3	2	4	9
56-60	-	2	1	3

All the subjects were aware that they were hyperlipidemic. Out of the18 subjects, nine had the onset of hyperlipidemia between the age of 51-55 years. Epidemiological studies in India have revealed that the incidence of cardio-vascular is on the increase particularly the peak period during 51-60 years (Krause, 1996).

5. Symptoms of hyperlipidemia

The symptoms experienced by the hyperlipidemic subjects is given in Table XII.

TABLE XII
SYMPTOMS OF HYPERLIPIDEMIA

Symptoms	Group I (n=6)	Group II (n=6)	Group III (n=6)	Total(n=18
Weakness	3	2	4	9
Giddiness	1	3	1	5
Tiredness	1	1	1	3
Chest pain	1	-	1	2

Weakness has lead nine out of 18 subjects to the doctor. The other symptoms complained by them were giddiness, tiredness and chest pain.

6. Familial tendency of hyperlipidemia

The familial tendency of hyperlipidemia among the subjects is given in Table XIII.

TABLE XIII
FAMILIAL TENDENCY OF HYPERLIPIDEMIA

Familial relations	Group I (n=6)	Group II (n=6)	Group III (n=6)
Father	-	1	-
Mother	2	-	1
Grand father (p)	-	2	1
Grand Mother (P)	1	-	-
Grand father (M)	1	-	-
Grand Mother (M)	1	1	1
Total	5	4	3

P- Paternal M-Maternal

It is clear from the above table that five in group I, four in group II and three in group III had familial aggregation of hyperlipidemia. In group I and group III, the familial tendency of hyperlipidemia seems to have been inherited from mother than the father.

D. Dietary pattern of the subjects

1. Type of diet

The type of diet consumed by the subjects is given in Table XIV.

TABLE XIV
TYPE OF DIET OF THE SUBJECTS

Type of diet	Group I (n=6)	Group II (n=6)	Group III (n=6)	Total (n=18)
Vegetarian	-	1	1	2
Non-vegetarian	4	3	5	12
Ova-vegetarian	2	2	-	4

Non vegetarianism prevailed. It was found out that 12 (both from control and experimental group) out of 18 subjects were non-vegetarians. Wen har pan et al.,(1993), demonstrated that the risk of cardiovascular disease in vegetarians is lower than in omnivores.

2. Meal pattern of the subjects

All the 18 subjects had three meals a day (except for one who had two meals a day).

3. Frequency of consumption of food items

The frequency of intake of food items is given in Appendix V and the pattern is discussed.

a. Cereals

Rice (parboiled, milled) was consumed daily by 11 out of 18 subjects. Wheat flour and maida flour were consumed twice a week. Rava and vermicelli were included weekly in their diets. Other cereals such as ragi, rice flakes and raw rice were consumed occasionally.

b. Pulses

Red gram dhal and black gram dhal were consumed daily by the subjects. Red gram dhal was either used for rasam or sambar and black gram dhal was used for iddli. Bengal gram dhal and green gram dhal were included weekly. All other pulses were used by them occasionally.

c. Green leafy vegetables

Any one kind of green leafy vegetable was included only once a week by the subjects.

d. Roots and tubers

Onions are part of Indian recipes, so they were used daily. Carrot and potato were taken by them twice a week. Beetroot and yam were used occasionally.

e. Other vegetables

Brinjal, ladies finger and gourd vegetables were included weekly by majority of the subjects. Beans, cauliflower and drumstick were consumed by them occasionally.

f. Nuts and oil seeds

Coconut was consumed daily by 14 out of 18 subjects. It was used either for chutney, poriyal or kolambu. Groundnut, cashewnut and almonds were consumed occasionally.

g. Fruits

Tomato was included daily in the diets of all the subjects, because it is used in many recipes. Banana was consumed daily by eight out of 18 subjects. All other fruits were consumed during the season.

The quantum of intake of selected foods like milk, egg, fleshy foods and fats and oils was studied because their intake may have an association with the incidence of hyperlipidemia (Dietschy, 1998).

4. Quantum of consumption of milk

The quantum of consumption of milk by the subjects is given in Table XV.

TABLE XV
QUANTUM OF CONSUMPTION OF MILK

Quantum		Group I (n=6)	Group II (n=6)	Group III (n=6)	Total (n=18)
ml	Cups				
0-200	0-1	-	-	1	1
200-400	1-2	3	2	2	7
400-600	2-3	3	3	2	8
>600	>3	-	1	1	2

From the table it was found that all the subjects except one subject (in group III) consumed more than 200 ml (one cup) of milk. One subject in each of the experimental group II and group III, consumed more than 600 ml (three cups) of milk.

5. Frequency and quantum of consumption of egg

The frequency and quantum of consumption of egg by the subjects is indicated in Table XVI.

TABLE XVI

FREQUENCY AND QUANTUM OF CONSUMPTION OF EGG

Quantum (number)	Group I (n=6)	Group II (n=6)	Group III (n=6)	
	Weekly	Weekly	Weekly	Fortnightly
1-2	4	4	3	1
3-4	2	1	1	-

None of the subjects consumed egg daily. It was found that all the 16 subjects consumed egg weekly except one subject in group II who took egg fortnightly.

6. Frequency and quantum of consumption of fleshy foods

The frequency and quantum of consumption of fleshy foods by the subjects is given in Table XVII.

TABLE XVII

FREQUENCY AND QUANTUM OF CONSUMPTION OF FLESHY FOODS

Quantum of fleshy foods (g)	Group I (n=6)			Group II (n=6)			Group III (n=6)		
	Twice a week	Weekly	Occasionally	Twice a week	Weekly	Occasionally	Twice a week	Weekly	Occasionally
Mutton 50-100	1	2	-	1	1	1	3	-	-
100-150	1	-	-	-	-	-	1	1	-
Beef 50-100	-	-	1	-	-	1	-	-	1
Fish 50-100	-	1	2	-	-	3	-	2	1
100-150	-	-		-	-	-	-	-	1

Mutton was consumed by all the 12 non-vegetarian subjects. None consumed more than 150 g of mutton. Beef was consumed occasionally by one subject in all the groups. No one consumed more than 100 g of beef. Fish was consumed by three subjects in each of group I and group II and by four subjects in group III. Compared to beef, fish was consumed by many subjects. According to Ackmann (1986), eicosapentaenoic acid and omega-3 fatty acid present in fish decreases serum triglyceride in a majority of problem patients.

7. Quantum of consumption of fats and oils

The quantum of consumption of fats and oil by the subjects is tabulated in Table XVIII.

TABLE- XVIII
QUANTUM OF CONSUMPTION OF FATS AND OILS BY THE SUBJECTS

Quantum (ml)	Group I (n=6)	Group II (n=6)	Group III (n=6)	Total (n=18)
0-10	1	-	-	1
10-20	1	1	-	2
20-30	2	2	2	6
>30	2	3	4	9

Out of the 18 subjects, nine subjects took more than 30 ml of oil (two subjects from group I, three subjects and four subjects from experimental group II and group III, respectively). According to Achaya (1989), excess oil in the diet gets deposited in the body as athermatus plaque which occlude the blood vessel and cause infarction.

8. Diet prescription from dietician

All the subjects got diet prescription from the dietician. The foods advised to be included liberally and limited by the subjects are tabulated in Table XIX.

TABLE XIX
FOODS ADVISED TO BE INCLUDED LIBERALLY AND LIMITED

Foods	Group I (n=6)	Group II (n=6)	Group III (n=6)
Food to be included liberally			
Vegetable oils	4	5	4
Mixed cereal	1	2	1
Fibre rich foods	2	3	3
Egg white	3	2	2
Food to be limited			
Fleshy foods	3	2	2
Egg yolk	2	4	3
Butter	2	4	2
Salt	3	1	2

It was found from the above table that vegetable oils were advised to be included liberally in the diets of the subjects. They were advised to take egg white

only and egg yolk was contra indicated. Three subjects were asked to limit salt intake in control group (group I) and three subjects in experimental group (one in group II and two in group III) were also advised to limit salt.

9. Awareness of foods rich in fibre

Only one subject in each of group I (control) and group II (experimental) were aware of fibre rich foods whereas three subjects in group III were aware of fibre rich foods. The fibre rich foods mentioned by the subjects are tabulated in Table XX.

TABLE XX
FIBRE RICH FOODS

Fibre rich foods	Group I (n=6)	Group II (n=6)	Group III (n=6)
Green leafy vegetables	1	1	2
Plantain stem	-	-	2
Fruits with skin	-	1	-

The above table indicate that the awareness of fibre rich foods were poor among the subjects. Green leafy vegetables was mentioned by one subject in control group and three subjects in experimental group (one in group II and two in group III), plantain stem was mentioned by two subjects in group III and fruits with skin was mentioned by one subject in group II.

10. Awareness of foods rich in fat

Five out of six subjects in each of group I and group III were aware of foods rich in fat whereas in group II four subjects were aware. The foods rich in fat mentioned by the subjects are given in Table XXI.

TABLE XXI
FOODS RICH IN FAT

Foods rich in fat	Group I (n=6)	Group II (n=6)	Group III (n=6)
Fleshy foods	4	3	5
Oils	3	4	4
Milk	1	-	1
Butter	1	1	1

Fleshy foods was mentioned by four subject in group I, three subjects in group II and five subjects in group III as foods rich in fat. Butter was mentioned by one subject in all the groups.

11. Awareness of cholesterol rich foods

Four subjects in each of group I and group III and three subjects in group II were aware of cholesterol rich foods. The cholesterol rich foods mentioned by the subjects are tabulated in Table XXII.

TABLE XXII
CHOLESTEROL RICH FOODS

Cholesterol rich foods	Group I (n=6)	Group II (n=6)	Group III (n=6)
Egg yolk	4	3	4
Red meat	1	-	2

Four subjects in each of group I (control group) and group III (experimental) and three subjects in group II, mentioned egg yolk as cholesterol rich food.

12. Awareness of sodium rich foods

In group I, three subjects and in group III, two subjects were aware of sodium rich foods. In group II, no one was aware. The foods mentioned by the subjects as sodium rich foods are given in Table XXIII.

TABLE XXIII
SODIUM RICH FOODS

Sodium rich foods	Group I (n=6)	Group III (n=6)
Proprietory foods	1	1
Canned foods	2	1
Papad	1	-
Pickle	1	2
Salt	2	2
Baking soda	-	1

No one in group I was aware that that baking soda is a sodium rich food whereas in group III no one was aware that papad contains sodium.

E. Impact of spirulina on

1. Body weight

The impact of supplementation of spirulina on body weight was found out by noting the weight of the subjects after one month. The change in the mean body weight of the subject is presented in Table XXIV and the individual body weight and BMI of the subjects at initial and final stage of the study are given in Appendix VI

TABLE XXIV

IMPACT OF SPIRULINA ON MEAN BODY WEIGHT OF THE SUBJECTS

Groups	Mean body weight (kg)			Groups compared	't' value
	Initial	Final	Difference		
I	73.66 ±2.88	72.8 ± 2.64	-0.83 ± 0.4	IVS II	0.39^{NS}
II	71.5 ± 0.90	71.2 ±0.75	-0.7 ± 0.6	IVS III	0.47^{NS}
III	71.8 ± 5.5	70.8 ± 5.78	-1 ± 0.7	II VS III	0.72^{NS}

NS - Not significant

There was no significant decrease in body weight when two grams and three grams of spirulina was supplemented to the subjects for a period of one month whereas Becker **et al.** (1986), supplemented five grams of spirulina for one month and found reduction in body weight by 1.4 kg.

The changes in mean BMI values of the subjects is illustrated in Table XXV.

TABLE XXV

IMPACT OF SPIRULINA ON MEAN BMI VALUE OF THE SUBJECTS

Groups	Mean BMI			Groups compared	't' value
	Initial	Final	Difference		
I	25.5 ± 0.26	25.3 ± 0.28	-0.2 ± 0.15	IVS II	0.14^{NS}
II	25.4 ± 0.18	25.3 ± 0.26	-0.21 ± 0.21	I VS III	0.26^{NS}
III	25.5 ± 0.33	25.2 ± 0.5	-0.35 ± 0.26	II VS III	0.94^{NS}

NS - Non significant

There was no significant reduction in BMI because there was not significant reduction in body weight.

The waist, hip circumference of the individual subjects before and after supplementation are given in Appendix VI. There was not much decrease in the waist, hip circumference of the subjects after the supplementation of spirulina at the end of one month.

2. Blood pressure

The impact of supplementation of spirulina on blood pressure of the subjects was evaluated by noting down the systolic and diastolic pressure of the subjects in all the groups, before and after supplementation.

The changes in mean systolic pressure of the subjects is presented in Table XXVI and the individual values of systolic and diastolic pressure of the subjects at the initial and final stage of the study is given in Appendix VII.

TABLE XXVI

IMPACT OF SPIRULINA ON MEAN SYSTOLIC PRESSURE OF THE SUBJECTS

Groups	Mean systolic pressure (mm/Hg)			Groups compared	't' value
	Initial	Final	Difference		
I	132.5 ± 4.18	129.2 ± 0.49	-3.33 ± 2.58	I VS II	0.56^{NS}
II	133.3 ± 8.75	129.2 ± 6.64	-4.16 ± 2.04	IVS III	1.7^{NS}
III	136.6 ± 8.25	130.5 ± 7.34	-5.83 ± 2.74	II VS III	1.29^{NS}

NS-Not significant.

There was no significant decrease in the systolic pressure of the subjects after the supplementation of two grams and three grams of spirulina for one month.

The changes in mean diastolic pressure of the subjects is given in Table XXVII.

TABLE XXVII
IMPACT OF SPIRULINA ON MEAN DIASTOLIC PRESSURE OF THE SUBJECTS

Groups	Mean diastolic pressure (mm/Hg)			Groups compared	't' value
	Initial	Final	Difference		
I	86.6 ± 4.08	85.8 ± 3.76	-0.83 ± 2.04	I VS II	1.09^{NS}
II	85.8 ± 4.92	83.3 ± 4.08	-2.5 ± 2.74	I VS III	1.70^{NS}
III	88.3 ± 4.08	85 ± 3.16	-3.33 ± 2.58	II VS III	0.49^{NS}

NS - Not significant

The decrease in diastolic pressure was not significant when two grams and three grams of spirulina was supplemented for one month.

Thus there was only little effect on blood pressure of the subjects, if two grams and three grams of spirulina was supplemented for one month whereas a study conducted by Gayathri and Anuradha (1999) showed a significant reduction of blood pressure on three gramsof spirulina supplementation for a period of two months.

3. Lipid levels

The various lipids in the blood including serum - cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol are the important predictors of coronary heart disease. Hence, the concentration of these lipids were estimated in all the selected subjects before and after the supplementation of spirulina and the impact was assessed.

a. Total cholesterol

The changes in the mean cholesterol levels of the subjects is presented in Table XXVIII and illustrated in Fig. 1 and the individual blood lipid levels of the subjects at the initial and final stage of the study are given in Appendix VIII.

TABLE XXVIII

IMPACT OF SPIRULINA ON MEAN TOTAL CHOLESTEROL LEVELS OF THE SUBJECTS

Groups	Mean total cholesterol (mg/dL)			Groups compared	't' value
	Initial	Final	Difference		
I	256 ± 3.74	255.3 ± 4.27	-2.33 ± 1.36	IVS II	8.45*
II	273.5 ± 2.88	257.8 ± 4.21	-15.7 ± 3.26	I VS III	13.31*
III	283.5 ± 2.59	258.8 ± 2.4	-24.7 ± 3.5	II VSIII	4.2*

* - SIGNIFICANT AT ONE PERCENT LEVEL.

As per the National Cholesterol Education Program, greater than 240 mg/dl of serum cholesterol requires treatment. In this study, subjects whose cholesterol levels above 240 mg/dl were selected and spirulina was supplemented. There was a significant decrease in total cholesterol levels in both the experimental groups (group II and group III) when compared to control group (group I). There was also a significant decrease in group III (where the subjects received three grams of spirulina) when compared to group II (in which two gram of spirulina was given to the subjects).

b. Triglycerides

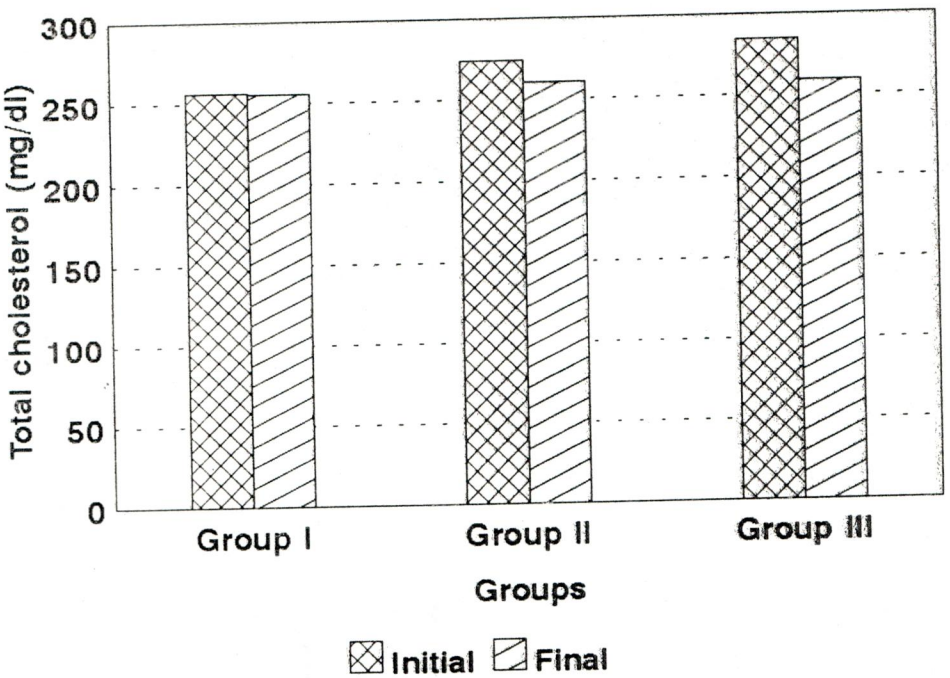
The changes in mean triglyceride levels of the subjects is tabulated in Table XXIX and illustrated in Fig. 2..

TABLE XXIX

IMPACT OF SPIRULINA ON MEAN TRIGLYCERIDE LEVELS OF THE SUBJECTS

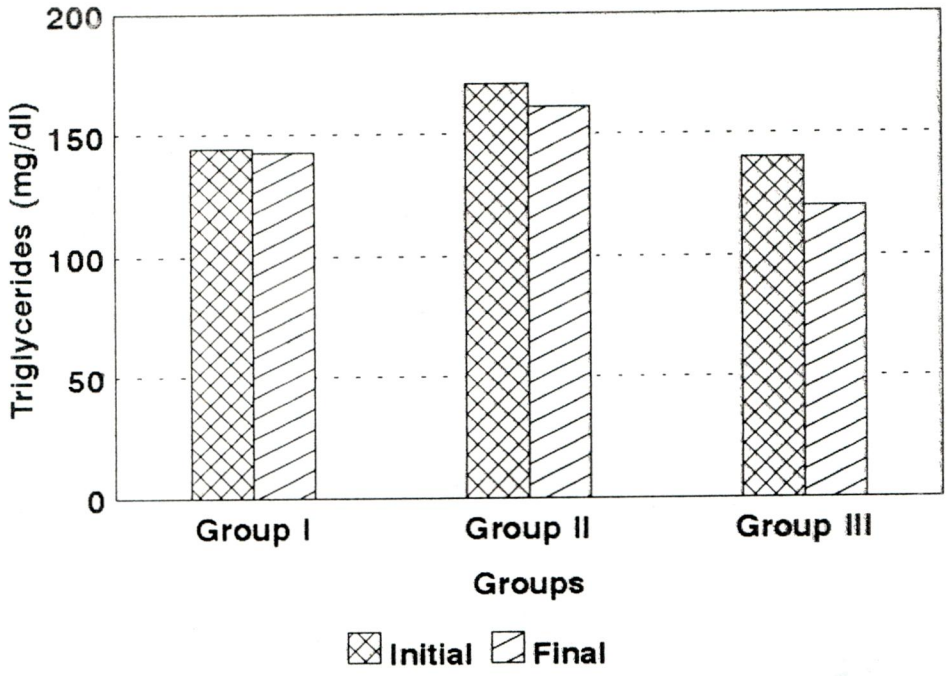
Groups	Mean triglycerides (mg/dL)			Groups compared	't' value
	Initial	Final	Difference		
I	144.2 ± 36.66	142.7 ± 35.83	-2.17 ± 1.33	I VS II	4.16*
II	171 ± 11.13	161 ± 13.91	-10 ± 4.0	I VS III	12.44*
III	140.3 ± 27.06	119.8 ± 28.69	-20.5 ± 3.02	II VS III	4.69*

* - Significant at one percent level.



Impact of spirulina on mean total cholesterol levels of the subjects

Fig. 1



Impact of spirulina on mean triglycerides levels of the subjects
Fig. 2

According to Patsch et al. (1992), there was positive relationship between coronary artery disease and plasma triglyceride. There was a significant reduction in serum triglyceride levels in group II and group III (experimental groups) when compared to group I (control group). When compared between the experimental groups, the reduction was significant in group III..

c. HDL - cholesterol

The changes in mean HDL - cholesterol levels of the subjects is given in Table XXX and illustrated in Fig. 3..

TABLE XXX
IMPACT OF SPIRULINA ON MEAN HDL-CHOLESTEROL LEVELS OF THE SUBJECTS

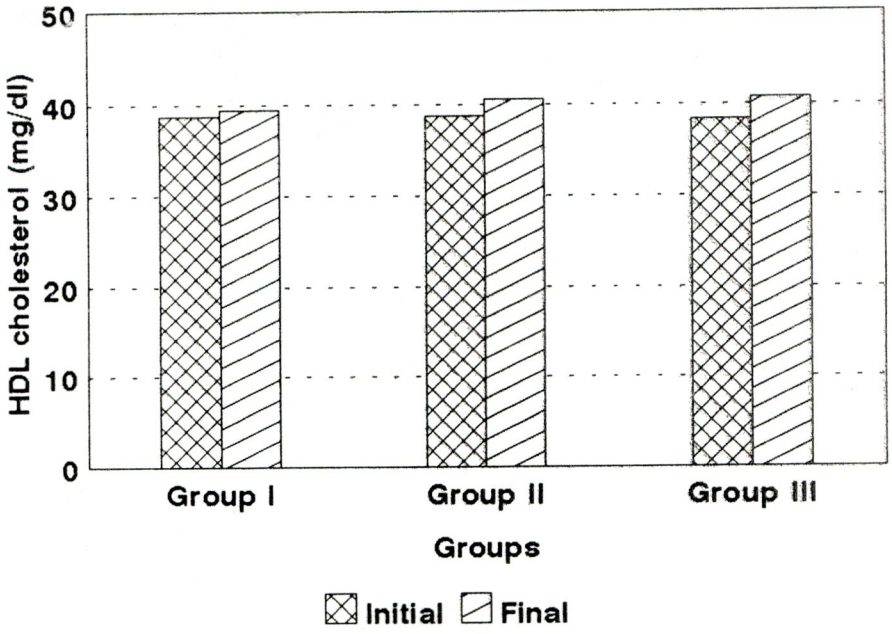
Groups	Mean HDL cholesterol (mg/dL)			Groups compared	't' value
	Initial	Final	Difference		
I	38.8 ± 5.81	39.5 ± 5.61	1.33 ± 0.82	I VS II	0.72^{NS}
II	38.8 ± 2.71	40.6 ± 2.5	1.83 ± 1.33	I VS III	1.97^{NS}
III	38.3 ± 3.44	40.8 ± 3.37	2.5 ± 1.05	II VS III	0.89^{NS}

NS - Not significant.

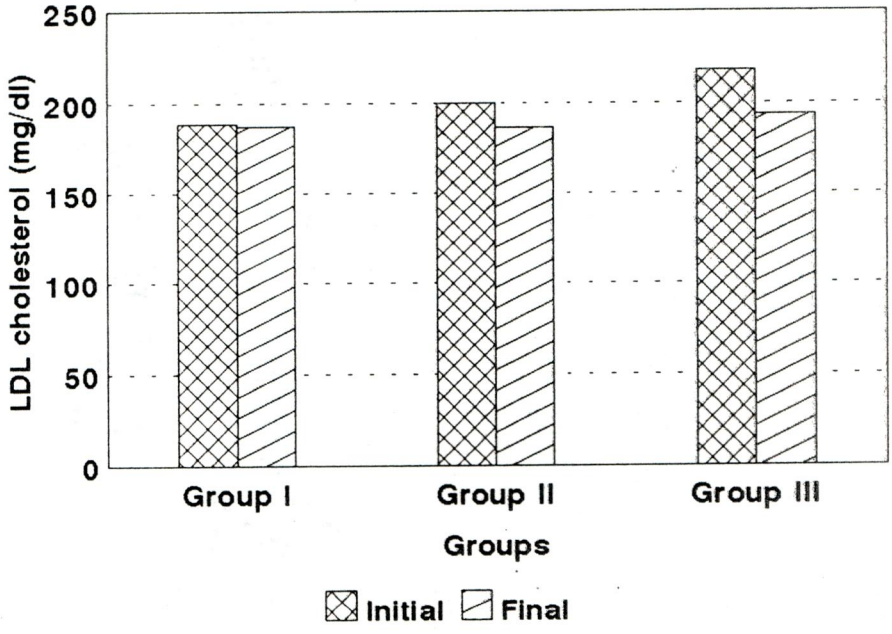
There was no significant increase in HDL cholesterol levels in group II and group III when compared to group I (control), after the supplementation of spirulina. The increase was not significant when the experimental groups (group II and group III) were compared..

d. LDL - Cholesterol

The changes in the mean LDL-cholesterol levels of the subjects is given in Table XXXI and illustrated in Fig. 4.



Impact of spirulina on HDL-cholesterol levels of the subjects
Fig. 3



Impact of spirulina on LDL-cholesterol levels of the subjects
Fig. 4

TABLE XXXI

IMPACT OF SPIRULINA ON MEAN LDL-CHOLESTEROL LEVELS OF THE SUBJECTS

Groups	Mean LDL- cholesterol (mg/dL)			Groups compared	't' value
	Initial	Final	Difference		
I	189 ± 9.05	187.5 ± 8.62	-1.6 ± 1.38	I VS II	6.48*
II	200.5 ± 5.09	186.8 ± 5.98	-13.7 ± 3.98	I VS III	9.04*
III	218 ± 7.86	194.2 ± 6.49	-24.7 ± 5.57	II VS III	3.59*

* - Significant at one percent level.

In both the experimental groups (group II and group III), there was a significant reduction in LDL-cholesterol levels when compared to control group (group I). There was a significant reduction when the experimental groups (group II and group III) were compared.

e. VLDL - Cholesterol

The changes in the mean VLDL-cholesterol levels of the subjects is presented in Table XXXII and illustrated in Fig. 5.

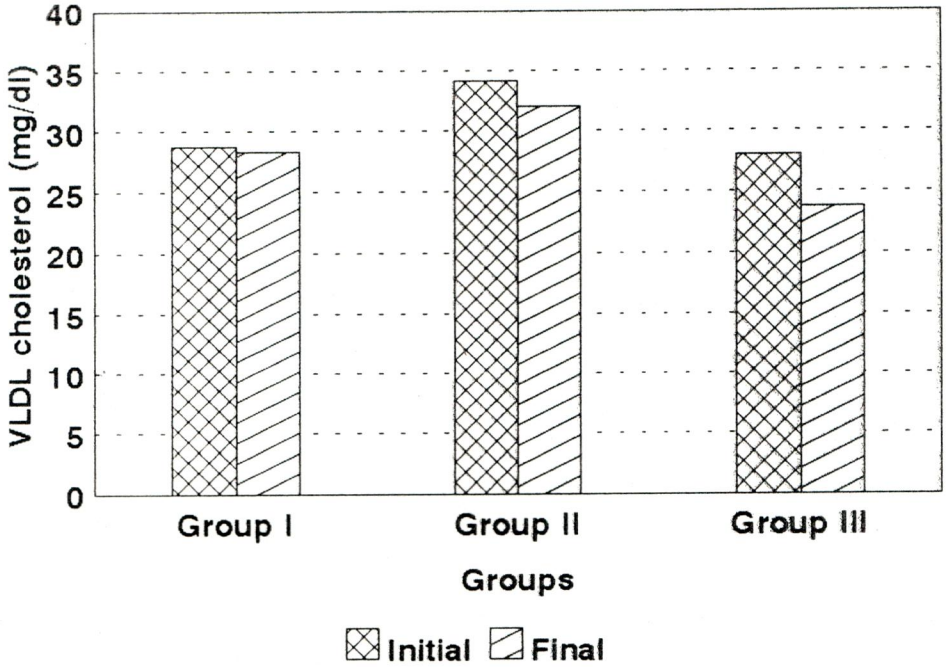
TABLE XXXII

IMPACT OF SPIRULINA ON MEAN VLDL - CHOLESTEROL LEVELS OF THE SUBJECTS

Groups	Mean VLDL -cholesterol			Groups compared	't' value
	Initial	Final	Difference		
I	28.8 ± 7.33	28.3 ± 7.09	-0.5 ± 0.55	IVS II	3.38*
II	34.2 ± 2.23	32 ± 2.83	-2.2 ± 0.98	I VS III	8.89*
III	28 ± 5.18	23.8 ± 5.78	-4.2 ± 0.75	II VS III	3.61*

* - Significant at one percent level.

When compared with control group (group I), there was a significant decrease in VLDL - cholesterol levels in both the experimental groups (group II and group III). There was a significant decrease in VLDL-cholesterol when compared between group II and group III.



Impact of spirulina on VLDL-cholesterol levels of the subjects
Fig. 5

A study conducted by Nayaka **et al.** (1986), showed that spirulina had hypocholesterolemic effect since 4.2 grams of spirulina reduced total cholesterol by 4.5 per cent within four weeks.

It was revealed from the above tables, that the supplementation of spirulina reduced total cholesterol, triglyeride, LDL - cholesterol and VLDL-cholesterol to a significant level whereas HDL-cholesterol was not increased significantly.

F. Correlation between intake of selected nutrient and blood lipid levels.

The selected intake of the individuals are given in Appendix IX.

The correlation between the intake of selected nutrientand blood lipid levels of the subjects in group I is presented are in Table XXXIII.

TABLE XXXIII
CORRELATION BETWEEN INTAKE OF SELECTED NUTRIENT AND BLOOD LIPID LEVELS OF GROUP I SUBJECTS

Blood lipids / Nutrients	Total cholesterol	Triglycerides	HDL - cholesterol	LDL - cholesterol	VLDL - cholesterol
Energy	0.88	0.32	-0.19	0.11	0.32
Protein	0.57	0.27	-0.25	0.18	0.27
Fat	0.75	-0.37	-0.22	0.13	-0.34
Fibre	-0.75	-0.49	-0.28	-0.1	-0.49

There was a positive correlation between energy, fat, protein and total cholesterol and LDL-cholesterol and there was a positive correlation between energy, protein and triglyceride and VLDL - cholesterol whereas fat and fibre had a negative correlation with triglyceride and VLDL - cholesterol. There was a negative correlation between all the nutrients and HDL - cholesterol.

The correlation between the intake of selected nutrientand blood lipid levels of group II subjects is given in Table XXXIV.

TABLE XXXIV

CORRELATION BETWEEN INTAKE OF SELECTED NUTRIENT AND BLOOD LIPID LEVELS OF GROUP II SUBJECTS

Blood lipids / Nutrients	Total cholesterol	Triglycerides	HDL - cholesterol	LDL - cholesterol	VLDL - cholesterol
Energy	0.98	-0.24	-0.50	0.49	-0.35
Protein	0.14	0.02	-0.47	0.37	-0.04
Fat	0.65	-0.09	-0.08	0.31	-0.12
Fibre	-0.74	-0.45	-0.42	-0.45	-0.53

Energy protein, fat had a positive correlation with total cholesterol and LDL-cholesterol. There was a negative correlation between all the selected nutrients and triglyceride, HDL-cholesterol and VLDL-cholesterol except correlation of protein and triglyceride.

The correlation between the intake of selected nutrientand blood lipid levels of group III subjects is given in Table XXXV.

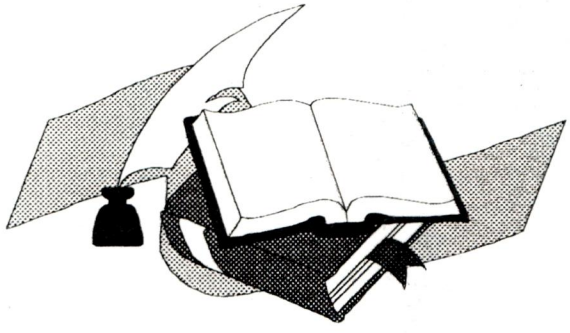
TABLE XXXV

CORRELATION BETWEEN INTAKE OF SELECTED NUTRIENT AND BLOOD LIPID LEVELS OF GROUP III SUBJECTS

Blood lipids / Nutrients	Total cholesterol	Triglycerides	HDL - cholesterol	LDL - cholesterol	VLDL - cholesterol
Energy	0.80	-0.42	-0.64	0.51	-0.36
Protein	0.68	-0.33	-0.61	0.49	-0.23
Fat	0.72	0.01	-0.79	0.24	0.01
Fibre	-0.81	-0.4	-0.56	-0.59	-0.39

The correlation of energy, protein and fat with total cholesterol and LDL cholesterol was positive whereas with HDL-cholesterol was negative. There was a positive correlation between fat and triglyceride, VLDL - cholesterol.

Thus in all the groups there was a positive correlation between energy, protein, fat and total cholesterol and LDL - cholesterol and there was a negative correlation between all the selected nutrients and HDL-cholesterol It was revealed that fibre had a negative correlation with the blood lipid levels.



Summary and Conclusion

V SUMMARY AND CONCLUSION

Cardio-vascular disease is one of the chronic disease and it accounts for half of the death by chronic diseases. The three major risk factors for cardio-vascular disease are hyperlipidemia, hypertension and cigarette smoking. The hyperlipidemias may occur from increased levels of cholesterol and triglycerides in a variety of lipoproteins. The serum cholesterol is a potent indicator of risk of coronary heart disease. Hyperlipidemia should be managed properly in order to reduce the risk of heart disease. Spirulina - a health food has got a hypocholesterolemic effect. The present study is undertaken to see the impact of supplementation of spirulina on hyperlipidemic subjects.

A total of 18 hyperlipidemic male subjects were selected in the age group of 40-60 years. Among them six of them were in the control group (group I) and the remaining 12 were divided into two groups, to one group (Group II), two grams of Spirulina was supplemented and to the other group (group III), three grams of Spirulina was supplemented. The impact of spirulina was noted by estimating the lipid levels after one month.

The salient findings of the present study are:

Background information of the hyperlipidemic subjects

1. Fourteen subjects out of 18, were in the age group of 51-60 years.
2. All the subjects were literate.
3. Out of the 18 subjects, eight were doing business and two were engaged in agricultural work.
4. The total monthly income of the families of the subjects ranged from Rs.6000/- to Rs.11000/-.

Life style pattern of the subjects

5. Out of 18 subjects, 16 drank coffee. Among them, only one subject drank less than one cup. Instant coffee consumption was more and none took boiled coffee.
6. Thirteen out of 18 subjects smoked and among them six subjects smoked more than one pack.

7. Out of the 18 subjects, four subjects chewed pan and one subject in group III chewed pan with tobacco six times a day.
8. Nine out of 18 subjects took alcohol and among them two subjects took toddy occasionally. Five out of nine subjects took 120 ml of alcohol which is not a moderate amount.
9. Out of the 18 subjects, only five subjects did exercise.

Clinical status of the subjects

10. All subjects had a BMI in the range of 25-26, they are in the borderline for overweight.
11. All the subjects had waist circumference and waist hip ratio below the cut-off point.
12. All the subjects were in the high normal range of blood pressure (130-139 mm/Hg systolic and 85 - 89 mm/Hg diastolic pressure).
13. The incidence of hyperlipidemia was in the age of 51-55 years for nine out of 18 subjects.
14. Nine out of 18 subjects experienced weakness which lead them to the doctor.
15. There was a familial tendency among 12 out of 18 subjects which came from the grandparents.

Dietary pattern of the subjects

16. Non-vegetarianism prevailed.
17. Rice was consumed daily by 11 out of 18 subjects. Red gram dhal and black gram dhal were commonly consumed by the subjects. Green leafy vegetables was used once a week by all the subjects. Since onion is used in many recipes, it was used daily. Brinjal, ladies finger and gourd vegetables were included weekly by majority of the subjects. Coconut was used by majority daily. Tomato was included daily in the diet of all subjects because it is included either in sambar or rasam or any curry.
18. The quantum of intake of selected foods like milk, egg, fleshy foods and fats and oils was noted. Except one, all subjects consumed more than 200 ml of milk. None of the subjects consumed egg daily, 16 out of 18 consumed egg weekly.

Mutton was consumed by all the 12 non-vegetarians, but none consumed more than 150 g. Ten out of 12 subjects consumed fish. Beef was consumed occasionally by one subject in all the groups. Out of the 18 subjects, nine subjects took more than 30 ml (2 tablespoons) of oil.

19. All the subjects were asked to liberally include vegetable oils in their diet and they were advised to avoid egg yolk and limit salt. Subjects were not very much aware of fibre rich foods. Majority of the subjects were aware of foods rich in fat but only one subject was aware that butter is to be avoided. All the subjects in experimental group III were aware that egg yolk and red meat are rich sources of cholesterol. No one in group III (experimental) were aware of sodium rich foods.

Impact of spirulina on body weight

20. There was no significant decrease in body weight due to impact of supplementation with spirulina for one month hence there was no significant reduction in BMI and in waist, hip circumference of the subjects.

Impact of spirulina on blood pressure

21. There was no significant decrease in systolic and diastolic pressure of the subjects after one month of supplementation of spirulina.

Impact of spirunila on lipid levels

22. There was a significant reduction in total cholesterol, triglyceride, LDL - cholesterol and VLDL - cholesterol after supplementation with spirulina for 30 days. There was significant reduction between groups for all the above mentioned lipid levels. But there was no significant increase in HDL - cholesterol after supplementation.

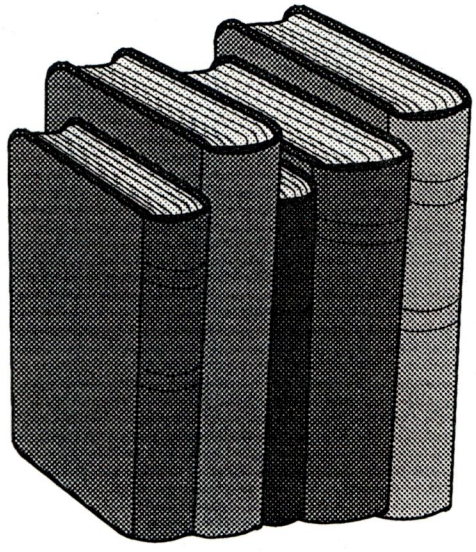
Correlation between intake of selected nutrients and blood lipid levels

23. There was a positive correlation between energy, protein, fat and total cholesterol and LDL - cholesterol in all the three groups (control and experimental groups). There was a negative correlation between intake of all the selected nutrients and HDL- cholesterol. It was found that fibre had negative correlation with the blood lipid levels.

Thus, supplementation of spirulina brought about significant reduction in blood lipid levels in 30 days. One month of spirulina supplementation had no significant effect on weight reduction and blood pressure. Perhaps, an extensive study may have an effect on the above parameters.

Recommendations for future research

1. Impact of supplementation of spirulina on the blood lipid levels in different pathologic conditions.
2. Impact of supplementation of spirulina on improvement of skin rashes and allergies.



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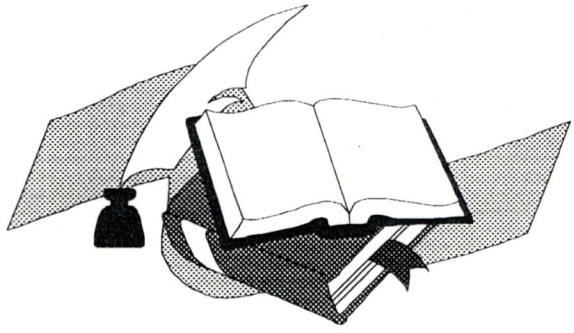

Appendix

APPENDIX - IA
BACKGROUND INFORMATION

1. Name of the interviewee :
2. Address :

3. Age :
4. Sex :
5. Education :
6. Occupation :
7. Income of the interviewee :
8. Total income of the family :

APPENDIX - IB
LIFE-STYLE PATTERN

1. Do you have the habit of drinking coffee?

Yes ☐ No ☐

If yes, a. indicate quantity and frequency of consumption per day

b. Indicate whether you are taking

i. Filter coffee
ii. Instant coffee
iii. Both

2. Do you have the habit of smoking?

Yes ☐ No ☐

If yes, indicate whether it is

i. Cigarette ☐
ii. Cigar ☐
iii. Beedi ☐

b. Indicate the number of smoking per day

3. Do you have the habit of chewing pan?

Yes ☐ No ☐

If yes, indicate the frequency of chewing

b. Do you chew pan with tobacco?

Yes ☐ No ☐

4. Do you take alcohol?

Yes ☐ No ☐

If yes, mention the type and frequency

Type of alcohol	Daily	Twice a week	Thrice a week	Weekly	Occasionally
Beer					
Whisky					
Brandy					
Rum					
Wine					

b. Indicate the amount consumed

i. Half a peg ☐ ii. 1 peg ☐ iii. 2 pegs ☐ iv. 3 pegs ☐

5. Do you have the habit of exercise?

Yes ☐ No ☐

If yes, mention the type and frequency

Type	Daily	Twice a week	Thrice a week	Weekly
Walking				
Jogging				
Yoga				
Aerobics				

APPENDIX - IC
CLINICAL STATUS

1. Height : (cm)
2. Weight : (kg)
3. Body mass index :
4. Waist circumference :
5. Hip circumference :
6. Waist / Hip ratio :
7. Blood pressure
   - Systolic : mmHg
   - Diastolic : mmHg
8. Total cholesterol :
   - HDL cholesterol :
   - VLDL cholesterol :
   - LDL cholesterol :
   - Triglycerides :
9. Are you aware that you are suffering from hyperlipidemia?

   Yes ☐ No ☐
10. Mention the age of onset of your disease

11. Mention the symptoms that lead you to a physician

12. Familial tendency

Familial relations	Hyperlipidemia
Father	
Mother	
Brother	
Sister	
Paternal	
Grand father	
Grand mother	
Uncle	
Aunt	
Maternal	
Grand father	
Grand mother	
Uncle	
Aunt	

APPENDIX - ID
DIETARY PATTERN

1. Are you a a. Vegetarian ☐
   b. Non-vegetarian ☐
   c. Ova-vegetarian ☐
2. Meal pattern a. 2 meals/day ☐
   b. 3 meals/day ☐
   c. 4 meals/day ☐
3. Mention your dietary pattern
   Early morning :
   Breakfast :
   Mid morning :
   Lunch :
   Tea :
   Dinner :
4. Mention the frequency of the consumption of the following:

Food items	Daily	Twice a week	Thrice a week	Weekly	Occasionally
I. Cereals					
Ragi					
Rice (Parboiled, milled)					
Rice (raw)					
Rice flakes					
Wheat flour					
Maida flour					
Vermicelli					
Bread					
II Pulses					
Whole bengal gram					
Bengal gram dhal					
Red gram dhal					
Black gram dhal					
Green gram dhal					
Green peas					
Dry peas					
III. Leafy vegetables					
Agathi					
Amaranth tender					
Sirukeerai					
Arai keerai					
Cabbage					
Drumstick leaves					
Manathakkali leaves					
IV. Roots and Tubers					
Beet root					
Carrot					
Onion big					
Onion small					
Potato					
Yam					

Food items	Daily	Twice a week	Thrice a week	Weekly	Occasionally
V. Other vegetables					
Gourd vegetables					
Beans					
Brinjal					
Cauli flower					
Drumstick					
Ladies finger					
VI. Nuts and Oil seeds					
Almond					
Cashewnut					
Coconut					
Groundnut					
VII. Fruits					
Apple					
Banana					
Grapes					
Guava					
Mango					
Orange					
Pineapple					
Tomato					
Lemon					

5. Mention the frequency of consumption and amount consumed for the following

Food items	Amount	Daily	Twice a week	Thrice a week	Weekly	Occasionally
1. Milk	0-200 ml					
	200-400 ml					
	400-600 ml					
	>600 ml					
2. Egg	1-2 Nos					
	2-4 Nos					
3. Meat	0-50g					
a. Lamb	50-100 g					
	100-150g					
b. Port	0-50g					
	50-100 g					
	100-150g					
c. Beef	0-50g					
	50-100 g					
	100-150g					
4.. Fish	0-50g					
	50-100 g					
	100-150g					
5. Fats & Oils.	1-2 tsp					
	3-4 tsp					
	> 4 tsp					

6. Do you get any diet prescription from your doctor?

Yes ☐ No ☐

If yes

a. Have you been advised to take the following foods liberally?

i. Mixed cereal ☐ ii.Plenty of food with fibre ☐
iii. Mixture of vegetable oil ☐ iv. Egg white only ☐
b. Have you been advised to limit the following foods?
i. Fleshy foods ☐ ii. Egg yolk ☐
iii. Butter ☐ iv. Salt ☐
7. Have you been advised a low fat, low calorie diet?
Yes ☐ No ☐
8. Are you aware of food rich in fibre?
Yes ☐ No ☐
If yes, list some foods

9. Are you aware of fat rich foods?
Yes ☐ No ☐
If yes, list some foods

10. Are you aware of foods rich in cholesterol?
Yes ☐ No ☐
If yes, list some foods

11. Are you aware of foods rich in sodium?
Yes ☐ No ☐
If yes, list some foods

APPENDIX - II

Estimation of total cholesterol - ZAK's method

Principle:

Cholesterol reacts with ferric chloride in the presence of concentrated sulphuric acid to give a red colour. The intensity of the colour developed is directly proportional to the amount of cholesterol present and is read at 540 nm in a spectro colorimeter.

Reagents:

1. **Stock ferric chloride solution.**

840 mg of pure, dry ferric chloride is weighed and dissolved in 100 ml of glacial acetic acid in a standard flask.

2. **Ferric chloride precipitating Reagent.**

10 ml of the stock ferric chloride solution is placed in a 100 ml standard flask and made upto the mark with pure glacial acetic acid.

3. **Ferric chloride diluting Reagent:**

8.5 ml of stock ferric chloride solution is diluted to 100 ml with glacial acetic acid in a 100 ml standard flask.

4. **Standard Cholesterol:**

100 mg, dry cholesterl is placed in a 100 ml standard flask and made upto the mark with pure acetic acid.

5. **Working standard:**

10 ml of stock cholesterol is made upto 100 ml with glacial acetic acid. One ml of this solution contains 100 μg of cholesterol.

Procedure:

0.5, 1.0, 1.5, 2.0 and 2.5 ml of working standard was pipetted out into clean dry test tubes and the volume was made upto 5.0 ml with ferric chloride diluting agent 0.1 ml of the serum was taken in clean dry centifuge tubes in duplicate. To this, added 4.9 ml of ferric chloride precipitating reagent and mixed well, allowed to stand for a while and then certifuged. Tranferred 2.5 ml of the clear supernatant into a dry test tube and added 2.5 ml of ferric chloride diluting reagent to this. This was done in duplicate. All the tubes were kept in cold water and to each tube added 4.0 ml concentrated sulphuric acid. The solutions were mixed well and the tubes were allowed to come to room temperature. A blank was prepared simultaneously by taking 5.0 ml of diluting agent and 4.0 ml of sulphuric acid. After 30 minutes the intensity of the colour developed was read at 540 nm using control as blank.

Observation:

Solution

Volume ml	Concentration µg	Volume of ferric chloride diluting reagent ml	Volume of concentrated sulphuric acid ml	Percentage transmission	Optical Density
Blank	-	5.0	4.0	-	-
Standard	-	-	-	-	-
0.5	50	4.5	4.0		
1.0	100	4.0	4.0		
1.5	150	3.5	4.0		
2.0	200	3.0	4.0		
2.5	250	2.5	4.0		
Sample					
2.5	-	2.5	4.0		
2.5	-	2.5	4.0		

Calculations:

From the graph plotted,

2.5 ml of the supernatant contains µg of cholesterol.

This 2.5 ml supernatant contains 0.05 ml of serum.

Therefore, 0.05ml of serum contains µg of cholesterol.

Then, 100 ml of the serum will contain $\frac{x \times 100}{0.05 \times 100}$ mg

= mg/100 ml sesrum.

Result:

The amount of cholesterol present in 100 ml of the serum was found to be mg.

APPENDIX - III

Estimation of triglyerides (Enzymatic method)

Principle:

Triglycerides from serum/plasma are hydrolyzed by lipase and the glycerol that is liberated is reacted enzymatically to give a highly coloured Quinoeimine dye which has an absorbance maximum at 546 nm. The intnesity of the color produced is directly proportional to the concentration of Triglycerides in the sample.

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

$$\text{Glycerol + ATP} \xrightarrow[\text{Kinase}]{\text{Glycerol}} \text{Glycerol-1- Phosphate+ADP}$$

$$\text{Glycerol-1-phosphate+}O_2 \xrightarrow{\text{GPO}} \text{DAP+}H_2O_2$$

$$H_2O_2\text{+4 AAP+P-Chlorophenol} \longrightarrow \text{Quinoeimine Dye.}$$

Reagents (Supplied in the kit):

Reagent 1: Triglycerides enzyme reagent.

Reagent 2: Triglycerides standard 250 mg%.

Preparation of working solution.

Solution 1: Dissolve the content of the vial of Reagent 1 in 6 ml of deionized/distilled water by gentle swirling. Do not shake.Avoid frothing.

Procedure:

For spectrophotometer:

	Blank (B)	Standard (S)	Test (T)
Solution 1	1.0 ml	1.0 ml	1.0 ml
Incubate at 37^0C for 30 minutes or 10 minutes at room temperature.			
Serum/Plasma Reagent 2:	-	-	0.04 ml
Triglyceride standard, 250 mg%	-	0.04 ml	-
Mix, incubate at 37^0C for 3 minutes or 10 minutes at room temperature.			
Deionized/Distilled water	2.0 ml	2.0 ml	2.0 ml

Mix well and measure the optic density (O.D) of blank (B), standard (S) and test (T) against distilled water at 546 nm or using green filter. The final colour is stable for 1 hour.

Calculations:

$$\text{Triglycerides in mg/100 ml} = \frac{\text{O.D. Test - O.D. Blank}}{\text{O.D. standard-O.D. Blank}} \times 200 \text{ mgs}$$

APPENDIX - IV
Estimation of HDL-cholesterol
(Enzymatic method)

Principle

Serum low density lipoprotein (LDL) and very low density lipoproteins (VLDL) are selectively precipitated by Mg^{++} ions and phosphotungstate and removed by centrifugation. Cholesterol associated with HDL fractions remaining in solution is measured by enzymatic procedure.

Precipitating Reagent
Serum/Plasma ----------> HDL-Cholesterol+ (Supernatant) LDL & VLDL Cholesterol (Precipitate)

Cholesterol
Cholesterol Esters ----------> Cholesterol+Fatty acids
Esterase

Cholesterol
$Cholesterol+O_2$ ----------> $Cholestenone+H_2O_2$
Oxidase

Peroxidase
H_2O_2+4-2, 2 Aminoantipyrine+Phenol ----------> Quinoeimine+H_2O (dye complex)

Reagents (Supplied in the kit):
Reagent 1 : Precipitating reagent
Reagent 2 : Cholesterol Enzyme Reagent
Reagent 3 : Cholesterol Diluent.
Reagent 4 : Cholesterol standard, 100 mg %
Reagent 5 : Cholesterol Buffer.

Preparation of reagent A: Transfer the entire contents of 1 bottle of Reagent 2 to the empty glass bottle provided in the kit and reconstitute with 22 ml of Reagent 5.

Preparation of working Cholesterol Reagent:Mix Reagent 4 and Reagent 3 in equal volume (1:1) as per requirement. Reconstituted Reagent 4 is stable for 31 days when stored at 2.8^0C.

Procedure:

Step A : Precipitation.

Into 3" X 1/2 ", glass tube, take 0.5 ml of serum/plasma and add 0.05 ml(50 µl) Reagent 1. Mix well. Leave it at room temperature for 10 min. Centrifuge it at 2000 r.p.m. for 15 min. Take the clear supernatant for HDL - cholesterol estimation.

Step B. Cholesterol Estimation.

	Blank (B)	Test HDL (TH)	Standard (S)
Supernatant from Step A Serum/Plasma	-	0.05 ml	-
Reagent 4 : Cholesterol standard 100 mg%	-	-	0.05 ml
Working cholesterol Reagent	3.0 ml	3.0 ml	3.0 ml

Mix well and incubate at 37^0C for 10 minutes or 30 minutes at room tempertaure. Read the colour intensity at 490-530 nm against distilled water. Final color is stable for 2 hours.

Calculations:

$$\text{HDL-Cholesterol in mg/100 ml} = \frac{\text{O.D. (TH)-O.D. (B)}}{\text{O.D. (S)-O.D. (B)}} \times 100 \times 1.1$$

APPENDIX - V

Frequency of consumptin of food items

FOO D ITEM	Group I					Group II					Group III				
	Daily	Twice a week	Thrice a week	Weekly	Occassi onally	Daily	Twice a week	Thrice a week	Weekly	Occassi onally	Daily	Twice a week	Thrice a week	Weekly	Occassi onally
Ragi	-	-	-	1	5-	-	-	1	-5	-	1	-	2	3	-
Rice (Parbolied milled)	4	-	-	-	2	4	-	-	-	2	3	-	-	-	3
Rice (raw)	2	-	-	-	4	2	-	-	-	4	3	-	-	-	3
Rice flakes	-	-	1	1	4	-	-	-	0	6	-	-	-	1	5
Wheat flour	1	3	2	-	-	1	2	3	-	-	-	3	3	-	-
Maida flour	-	-1	3	2	-	1	-	4	1	-	1	-	5	-	-
Vermicelli	-	-	-	2	4	-	-	-	2	4	-	-	-	3	3
Bread	-	-	1	4	1	-	-	-	4	2	-	--	-	4	2
Rava	-	-	-	4	2	-	1	-	4	1	-	2	-	4	-
Whole Bengal gram	-	-	-	1	5	-	-	-	-	6	-	-	-	2	4
Bengal gram dhal	-	1	3	2	-	-	3	-	3	-	-	2	1	3	-
Red gram dhal	4	-	2	-	-	5	-	1	-	-	4	-	2	-	-
Black gram dhal	5	-	1	-	-	4	-	2	-	-	5	-	-	-	-
green gram dhal	--	-	4	2	-	-	-	3	3	-	-	-	4	2	-
Green gram whole	-	-	-	2	4	-	-	-	1	5	-	-	-	1	5
Bengal gram roasted	1	2	3	-	-	1	3	2	-	-	1	2	2	1	-
Green peas	-	-	-	1	5	-	-	-	-	6	-	-	-	2	4
Dry peas	-	-	-	-	6	-	-	-	-	6	-	-	-	1	5
Agathi	-	-	-	2	4	-	-	-	2	4	-	1	-	2	3
Amaranth tennder	-	-	1	4	1	-	-	1	2	3	-	-	-2	4	-
Sirukeerai	-	-	1	1	4	-	-	-2	4	-	-	-	3	3	-
Arikeerai	-	-	-	1	5	-	-	-	1	5	-	-	-	4	2

FOO D ITEM	Group I					Group II					Group III				
	Daily	Twice a week	Thrice a week	Weekly	Occassi onally	Daily	Twice a week	Thrice a week	Weekly	Occassi onally	Daily	Twice a week	Thrice a week	Weekly	Occassi onally
Cabbage	-	-	-	5	1	-	-	-	4	2	-	-	-	4	2
Drumstict leaves	-	-	-	6	-	-	-	-	4	2	-1	-	4	1	-
Manathakkali leaves	-	-	-	5	1	-	-	-	3	3	-	-	-	5	1
Betroot	-	-	-	3	3	-	-	-	4	2	-	-	-	4	2
Carrot	-	2	1	3	-	-	3	2	1	-	-	2	-	4	-
Onion.big	6	-	-	-	-	6	-	-	-	-	3	2	1	-	-
Onion.small	6	-	-	-	-	6	-	-	-	-	5	-	1	-	-
Potato	-	3	2	1	-	-	2	-	4	-	-	2	4	-	-
Yam	-	-	1	2	3	-	-	-	2	4	-	-	-	4	2
Hourd vegetables	-	-	-	4	2	-	1	-	3	2	-	-	-	6	-
Beans	-	1	-	4	1	-	-	-	3	3	-	2	-3	1	-
Brinjal	-	-	-5	1	-	-	-	4	2	-	1	-	3	2	-
Califlower	-	-	-	2	4	-	-	-	1	5	-	-	-	2	4
Drumstick	-	-	-	3	3	-	-	-	5	1	-	-	-	5	1
Ladies finger	-1	-	4	1	-	-	-	5	1	-	2	-	4	-	-
Almond	-	-	-	-	4	-	-	-	-	2	-	-	-	-	3
Cashewnut	-	-	-	1	5	-	-	-	-	4	-	-	-	-	4
Coconut	5	-	1	-	-	6	-	-	-	-	3	1	2	-	-
Groundnut	-	-	-	3	3	-	-	-	2	4	-	-	-	2	4
Apple	-	-	-	-	6	-	-	-	-	6	-	-	-	-	6
Banana	3	1	1	-	1	4	-	2	-	-	1	1	3	-	1
Grapes	-	-	-	-	6	-	-	-	1	5	-	-	-	-	6
Guava	-	-	-	-	6	-	-	-	-	6	-	-	-	-	6
Mango	-	-	-	-	6	-	-	-	-	6	-	-	-	-	6
Orange	-	-	-	-	6		-	-	-	6	-	-	-	-	6
Pineaple	-	-	-	-	6	-	-	-	-	6	-	-	-	-	6
Tomato	1	1	4	-	-	4	-	2	-	-	4	-	2	-	-
Lemon	-	-	2	4	-	-	1	2	2	1	1	1	4	-	-

APPENDIX - VI

Body Weight, BMI, Waist, hip circumference of the subjects

		Weight(kg)		BMI		Waist circumference (cm)		Hip circumference (cm)	
Groups	Subject number	Initial	Final	Initial	Final	Initial	Final	Initial	Final
I	1	72	71	25.5	25.2	89	89	101.5	101.5
	2	78	77	25.4	25.1	96.5	96.5	107	107
	3	75	74	25.9	25.6	91.5	91	101.5	101
	4	70	70	25.7	25.7	91.5	91.5	107	107
	5	75	74	25.3	25	96.5	96.5	107	107
	6	72	71	25.2	25.2	91.5	91	107	107
II	1	71.5	71	25.3	25.2	94	94	107	107
	2	71	72	25.4	25.8	94	94	104	104
	3	72	72	25.5	25.5	91.5	91.5	107	107
	4	72	71	25.5	25.2	91.5	91	104	103
	5	70	70	25.1	25.1	91.5	91.5	109	109
	6	72.5	71	25.6	25.1	96.5	96	109	108
III	1	62	61	25.8	25.4	91.5	91.5	107	107
	2	75	75	25.9	25.9	94	94	107	107
	3	71.5	70	25.3	24.8	91.5	90.5	107	106
	4	72	70	25.5	24.8	94	93.5	107	107
	5	78.5	78	25.6	25.5	96.5	96	101.5	101
	6	72	71	25.0	24.6	94	94	109	109

APPENDIX - VII

Blood Pressure of the subjects

		Systolic pressure mmHg		Diastolic pressure mmHg	
Groups	Subject number	Initial	Final	Initial	Final
I	1	130	130	90	90
	2	140	135	90	90
	3	135	135	90	85
	4	130	125	85	85
	5	130	125	85	85
	6	130	125	80	80
II	1	130	125	80	80
	2	125	120	80	80
	3	130	125	90	90
	4	130	125	85	80
	5	150	145	90	85
	6	135	135	90	85
III	1	135	130	90	85
	2	125	120	90	85
	3	140	135	90	85
	4	150	140	90	85
	5	140	135	90	90
	6	130	125	80	80

APPENDIX - VIII

Blood lipid levels of the subjects

Groups	Sub-ject No.	Total cholesterol (mg/dL)		Triglycerides (mg/dL)		HDL-cholesterol (mg/dL)		LDL-cholesterol (mg/dL)		VLDL-cholesterol (mg/dL)	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
I	1	256	254	175	172	34	36	187	184	35	34
	2	250	249	125	126	46	45	179	179	25	25
	3	256	257	105	104	45	47	190	189	21	21
	4	258	254	110	107	32	32	204	201	22	21
	5	260	262	155	156	36	38	193	193	31	31
	6	260	256	195	191	40	39	181	179	39	38
II	1	270	256	190	185	38	39	194	180	38	37
	2	271	254	170	157	42	44	195	179	34	31
	3	273	262	160	151	38	40	203	192	32	30
	4	274	254	160	145	42	42	200	193	32	29
	5	275	257	175	163	35	37	205	188	35	32
	6	278	264	171	165	38	42	206	189	34	33
III	1	281	256	134	115	43	46	221	187	27	23
	2	281	262	183	166	38	40	207	189	36	33
	3	282	260	120	102	42	44	216	196	24	20
	4	284	256	161	139	37	38	215	190	32	28
	5	286	259	110	85	35	39	229	203	22	17
	6	287	260	134	112	35	38	225	200	27	22

APPENDIX - IX

Mean intake of selected nutrients by the subjects

Groups	Subject No	Energy (kcal)	Carbohydrates (g)	Protein (g)	Fat (g)	Fibre (g)	Sodium (mg)
I	1	2412	450.5	57.1	42.4	7.9	412
	2	2473	447.2	58.3	50.1	8.1	459
	3	2512	459.0	60.5	48.2	6.8	433
	4	2638	466.6	65.1	56.8	8.6	483
	5	2825	505.2	70.3	58.1	9.4	517
	6	2797	522.5	68.1	48.3	10.5	461
II	1	2412	451.8	55.1	49.5	7.8	432
	2	2372	408.5	65.3	53.1	8.2	481
	3	2517	439.7	62.1	57.2	7.9	462
	4	2617	454.3	59.3	63.4	8.5	521
	5	2962	539.5	69.1	59.1	9.5	571
	6	2788	521.3	61.4	51.3	9.1	475
III	1	2451	440.5	62.1	48.9	7.8	432
	2	2725	499.3	65.3	52.1	8.5	459
	3	2513	442.3	65.5	53.5	7.5	413
	4	2912	530.8	70.5	56.3	9.5	478
	5	2811	512.9	62.7	56.5	9.1	471
	6	3005	548.2	72.8	57.9	10.3	513