Effect of Administration of Mushroom on Blood Lipids of Selected Hyperlipidemic Adult Population of Coimbatore City

By

Kala Lingam

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE OF 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 - 1998

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Introduction

I. INTRODUCTION

Health has been defined as the freedom from disease and maximum well being. Health is considered to be a vital process that involves the whole person and his or her unique pattern of living. A person is healthy when he is in harmony with his out and inner environment.

Health promotion is the science and art that helps pepole change their life style. Healthy population is extremely crucial as poor health will affect the quality of human beings and productivity of the country. With economic betterment, lifestyle and diet pattern, vascular and metabolic diseases are affecting increasing proportion of people thus increasing the burden of health care.

Hyperlipidemia is an upcoming health problem all over the world. Today hyperlipidemia and its related disease are not only a health issue, they have become a social problem with tremendrous consequences for well being of the individual and the world they live in.

Increase in the blood lipid level is otherwise called hyperlipidemia, a condition in which serum cholesterol and/ or triglycride level go above 95th percentile of the level found in a comparable healthy population (carlson etal,1990).

Hyperlipidemia paves way for other coronary heart diseases which in turn is the main factor in increasing the mortality rate. Reeves (1997) revealed that about 35 percent of the mortality occuring all over world, has hyperlipidemia as the main cause.

Reports show that in India the prevalence of hyperlipidemia is higher in North India than in south India. This is because of high intake of saturated fat by North Indian population (Kesaniemi, 1989). The hyperlipidemic persons found in urban population (35.7) exceed the rural hyperlipidemic population (20.8) (Agarwal, 1997).

Nissen (1990) revealed that the prevalence of hyperlipidemia is higher in the industralised countries like USA than developing countries like India.

According to Mohan et al (1997) in Tamil nadu, the prevalence of hyperlipidemia is 45.1 percent in males and 54.8 percent in female diabetic patients and also triglyceridemia is more common than hypercholesterolemia.

Various dietary and non-dietary factors contribute to occurance of Hyperlipidemia. Non-nutritional factors like alcohol, when consumed in moderation, would not force health hazard, but beyond a certain level (3 drinks a day and more) shall lead to hypertriglyceridemia (subet et al 1992).

Not only alcoholic beverages but beverages like coffee also contribute to raise in lipid profile level. Bruke, (1991) showed that boiled coffee consumption(5-6 cups a day) raises the cholesterol level more than filtered coffee.

Smoking is a major risk for hyperlipidemia and coronary heart disease. The prevalence of hyperlipidemia and coronary heart disease among the smokers is 55 percent more than in non smokers.

Obesity is often associated with increased LDL and VLDL and decreased level of HDL cholesterol. Weight reduction is usually associated with increased plasma HDL concentration and improved lipoprotein profile (Manson et al, 1990).

Diet either increases or decreases the serum lipid level. The diet has a direct effect on hyperlipidemia. It is a simple theory that by lowering the dietary intake of cholesterol, the serum cholesterol could be lowered saying that endogenous cholesterol production is less than cholesterol absorption. Consumption of lower kilocalories and low intake of dietary cholesterol shall lower the total serum cholesterol and LDL level in most hypercholesterolemic patients (Johnsan, 1990).

Proteins which we consume also lead to hypercholesterolomia. Animal protein generally leads to high cholesterol level than plant proteins. Apart from all these risk factors, the frequency of eating also contributes to the increased lipid profile. Richardson, (1990) reports that fibre, independent of fat intake is an important dietary component for the prevention of coronary heart disease and hyperlipidemia.

As hyperlipidemia are in increase in man due to various reasons, in future cheap food components which will have therapeutic value have great potential. Mushroom can form such a food component.

Mushroom has been paid attention as an alternate food source because of the taste and fleshy construction and it is a

source which provides adequate nutrition to world's increasing population (Bobek etal 1992).

Mushroom containing diet reduces the cholesterol levels in animals. Mushroom are also good protein source for vegetarians and contain amino acids which lacks in cereals, could be helpful in climbing dependence on animal protein to overcome the amino acid deficiency (lysine) (Mushincount, 1996).

As food the nutritional value of mushroom lies between meat and vegetable and has been rightly called "Vegetable meat". Mushroom contains a moisture content of 89.5 percent, ash;1.25percent, protein; 3.94percent, fat; 0.19percent and crude fibre 1.09percent. Tryptophan and lysine are present in high concentrations. Apart from high protein content, the digestibility of mushroom protein is as high as 72 to 83percent.

Mushroom also contain plently of ascorbic acid which prevents the blocking or clogging of arteries. The great advantage of mushroom is that they have the capacity to convert nutritionally invaluble substance into high protein food due to its essential amino acid content (Kegel's, 1990).

In a country like India where vegetarians dominate, every attempt must be made to popularise a vegetable protein like mushroom. There is dearth of information on human experiments with mushroom to test its lipid lowering effect. Hence with the existing details about mushroom, the present study has been undertaken to experiment the lipid lowering effect of mushroom in

selected hyperlipidemic subjects. The study has been attempted with the following objectives

A. Standardise a mushroom recipe that can supplement the diet of hyperlipidemics

B. Administer mushroom to the selected hyperlipidemics for a period of one month.

C. Evaluate the impact of mushroom supplementation on blood lipid level of the selected samples.

Review of Literature

II. REVIEW OF LITERATURE

The review of literature befitting to the present study entitled "Effect of administration Mushroom on blood lipids of selected hyperlipidemic adult population of Coimbatre City" is presented under the following headings.

A. Prevalence of hyperlipidemia.

B.Etiology of hyperlipdemia

C.Role of dietary factors in hyperlipidemia

D.Types and effects of mushroom

E.Hypolipidemic effect of mushroom

A. Prevalence of hyperlipidemia

Hyperlipidemia is a good predictor of the development of coronoary heart diesease. In 1993, it was estimated that mortality from hyperlipidemia was 10 percent . In India, Edmond(1997) revelaed that blood cholesterol levels are generally lower in developing countries than in developed countries.

Studies by Mohan et al (1997) showed that the prevalence of hyperlipidemia was higher in women than men. A Study connduct in Tamil Nadu (Vellore) by Swaminathan (1996) Conformed that femal diabetic patients were more susceptible to incresed lipid profile than male dieabetic patients.

Gopinath et al (1996) found that prevalence of hyperlipidemia in Urban Delhi was over six times than the rual environs and that the victims were largely drawn from the most affluent section of the population.

It has been estimated that even at the lowest possible figure, the incidence is 1.04 percent or that 2.78 million people in India suffer from hyperlipidmia (Sincar, 1992).

According to Maya Gowri et al (1990) Hyperlipidemia was not seen much among the Indian rural high income but they were found in urban high income group. The disease was seen commonly above the age group of 30 years.

WHO (1997) study has indicated that the mean level of cholesterol in men were :

Japan- 170mg/dl, France-228mg/dl, USA-205mg/dl, Scotland-241mg/dl and Soviet Union 229mg/dl.

Wilson(1997) revealed that mediterranean population have higher HDL and hence lower late of mortality than the western population.

The prevalence of hyperlipidemia in western countries, due to consumption of alcohol is three times more when compared with the eastern countries. USA tops the list by 31.1 percent and is followed by Canada 22 percent and UK 18 percent (Genz et al, (1997).

Bruchi et al (1997) reported that surgical menopause leads to increased prvelanec of hypercholestrolomic women.

B.Etiology of hyperlipidemia

The main reason for the increase in lipoproteins in sedentary life style, Mitter(1990) revealed that sedentary life style is associated with a greater risk of hyperlipidemia.

Lack of excercise leads to increase in cholesterol level. Sharad (1996) has pointed out that walking and aerobic excercise

for 45 minutes /brisk walking is known to increase HDL cholesterol and reduce the total cholesterol and triglycerides.

The prevalence of hyperlipideia is related to BMI, as LDL, triglyceride levels were higher and high density lipoprotein level lower in those with higher BMI. BMI was strongly related to lipid abnormalities Reeder (1995). Thomspon et al (1991), stated that obesity is often associated with increased rates of cholesterol, elevated very low density lipoprotein levels and low concentration of plasma low density lipoprotein.

Other risk factors contributing to hyperlipidemic conditions are hypothyroidism and nephrotic syndrome. Beaumont (1970) substantiated further that increase in very low denisty lipoprotein can be due to advanced liver disease, renal failure and diabetes.

C.Role of dietary factors in hyperlipidemia

Numerous dietary factors are known to affect serum lipid levels. Proper diet can lower than blood cholesterol levle significantly, but improper intake of diet can reverse the condition and shall increase the blood lipid profile level.

1.Factors that lower blood lipid profile

Collins et al (1997) and Frost (1997) reported that isocaloric replacement of saturated fatty acids with complex carbohydrates for 10 percent of the total dietary caloric intake resulted in a fall in total blood cholesterol by 0.52 mmol/l.

Lower fat consumption generally coincides with decreased saturated fat intake. Changes in blood lipid may be related more to type of fat consumed rather than total fat. Monounsaturated

fats, previosuly believed to be neutral with respect to serum cholesterol levels can effectively lower plasma low density lipoprotein concentrations without reducing the high density lipoprotein level (Mattson,1989).

Plant fibres, especially soluble carbohydrates (Pectin, guar, gum, carageen or bran) found in apples, citrus fruites, legumes, oats and barely can have significant hypocholesterolemic efects.

Fibers bind with bile acids, leading to a reduction in both plasma low density lipoprotein and hepatic cholesterol availability.

Balasubramaniam (1990) concluded that oat bran effectively lower plasma cholesterol. Oat bran decreased hepatic low density lipoprotein receptor activity and increased high density lipoprotein binding activity.

According to Mansell(1991) the use of garlic is accompanied by favourable effects coagulation, platelet aggregation, vasodilation and serum lipid concentration. Large doses of fresh garlic (7 to 8 cloves a day) had beneficial effects.

Fish Oil rich in omega -3-fatty acids significantly lower triglyceride levels when substitued for saturated fatty acid (Darvidson,1991).

Vitamin E, reduced the low density lipoprotein cholesterol and very low density lipoprotein cholesterol in young and middle age subjects. There were significant decrease in total serum cholesterol by 7 percent (Truswell, 1997).

According to Rajeip guptak et al (1997) Ghee contains

predominantly short chain fatty acid and stearic acid and also has 32 percent monounsaturated fatty acid which have no influence total cholesterol. It was further verified by Guptha (1997) that rural men who consume mustard oil in their diet have higher density lipoportein cholesterol then urban men who consumed peanut oil, he also proved that safflower oil lowers the low density lipoprotein cholesterol level but does not have any efect on high density lipoportein.

Among the nutritinal factors, the triglyceride levels and hyperlipidemia are consistently lower among population who habitually consume a high percentage of Kilo Calories as Carbohydrates.

2. Factors that increase blood lipid profile

There are certain dietary factors, both non-nutritional and nutritional, which increase the blood lipid level and must be taken only in small amounts.

Alcohol when consumed in light to moderate amounts shall increase the high density liporpotein level but when consumed in excess (5 to 6 drinks per day) will increase the triglcides in blood (Suh et al, 1991).

Not only alcohol but smoking is also a major risk factor for artheroselerosis and hyperlipidemia. Cigraette smoking is associated with increase in plasma triglyceride and decrease in high density cholesterol. Kennel (1981) substantiated that presence of hyperlipideia in smokers is due to the nicotine which stimulates secretion of catecholamines leading to

activation of adenyl cyclase of adipose tissue resulting in increased concentration of plasma free fatty acids. Goyal (1997) reported that there was a transient rise in basal triglyceride level in patients on hogh carbohydrate diets.

Accoridng to Morill (1993) high amounts of saturated fat in the diet tends to increase the low density lipoprotein as saturated fats are main source of cholesterol synthesis. Fatty acids like palmitic acids rise the cholesterol level in blood.

Bruke, (1991) found that higher intake of coffee (5 to 6 cups a day) shall increase the triglyceride level and low density lipoprotein level in blood.

Nuts also increase the blood lipid profile level considerably. Peanuts especially increase the blood lipid level twice when substituted for walnuts. Peanuts increase the low density lipoprotein (Monsen, 1994).

D. Types and Effects of Mushroom

Besides the every day eating of mushroom we add to our salads and fries, there are many varieties of fungi that possess therapeutic values and benefits.

Shiitake is a variety of mushroom, noted for its strengthening impact on the immune system. Its therefore indicated for all conditions where immunity is affected. This includes allergies, infection, colds, flu, cancer and AIDS. Shiitake has also been found to help reduce stubborn cases of high cholesterol and soothe bronchial inflammatory problems (Horie,1994).

This has been further substantiated by chiara et al (1996)

that in addition to antitumour effect of mushroom, shiitake mushroom can help in preventing high blood pressure , artherosclerosis, kidney ailments, diabets, cataract, neurologia, gall stones, numbness of hands and feet, haemorrhoides and also improves sexual powers.

In China and Japan, the use of Reishi (a type of mushroom) goes back about 4000 years. Chen (1986), reported Reishi as a main mushroom fighting cancer, it is also a powerful cleanser and is applicable for treating liver and respiratory disease. Reishi is also known for its longivity promoting qualities.

Red Reishi is considered among the most powerful natural herbs in Asia. Red Reishi is rich in organic compounds such as polysaccharides, aminoacids, proteins, triterpense, ascorbic acids, sterols, lipids, alkoloides, riboflavin and more.This compound also have antitumour activity and positive effect on immune system (Waternabe, 1990).

Another mushroom known as dancing mushroom (maitake) is applicable for the allevation of high blood pressure immune frality and liver disorders.

Zhung et al (1993) stated that with regard to health benefits and medicinal values, edible fungi produces secondary values which are biologically active. These secondary metabolites get stored. Agaricus bisporus has been shown to contain antitumour, antiviral properties, which are due to eritadennine 2,3,di-hydroxy 4-9 adenyl butric acid (Chibate et al,1996). The antitumuor properties are due to the polysaccharideslentains and emitanin-1.

Hayes et al (1998) revealed that coprinus comatus mushroom was reputed to exert hypoglycemic effect and may benefit the treatment of diabetes mellitus.

Studies carried out by Ishida and Tsunoda, 1970 proved the antivirial principle or lentinus edodes was a polysaccharide fraction found to be active aganist influenza but not against paramiyxo.

The screening programme for antimalarial agent condeucted by the united states public health service during and after world war II includes few fungi. They were reported to be active against plasmodium gullinaceum (Morris, 1995).

Mushroom have antibacterial activity. Some basidimycetes whose structure have been elucidated including phenolic compound, purines or pyrimidines, quinones and tupenoids are the reasons for the antibacterial activity of mushrooms (vogoel et al, 1994).

Apart from all these properties of mushroom studies by Wilkins (1997) reveals that a Japanese mushroom called maitake can help inhibit HIV and can also restrain breast cancer. Tests demonstrated that a polysaccharide called glucan extracted from mushroom indicates action in helper T-cells. It remains unknown why glucan from maitake mushroom activates the body's immune function but given its effectiveness against symptoms of AIDS patients together with antiAIDS drugs.

The nutritive value of mushroom very nearly equals that of milk.Mushroom contains high levels of amino acids such as lysine.carbohydrates and vitamins with a mineral content exceeding that in fish and meat and twice that of most

vegetables. They are highly valued in intelligence, enhancing memory, healing, vision and smelling. It has been shown to bring down blood sugar levels. They have also been shown to be hepato protective, apart from liver regeneration, beneficial effects vs hepatic necrosis and hepatitis have been noted (Subramaniam,1995).

E. Hypolipidemic effect of mushrooms

Mushroom apart from its various other properties, has got one main property of reducing the blood lipid level. Many studies have been carried out to find the hypocholesterolemic effect.

According to Bobek(1993) Oyster mushroom isolates (B-glucan) has direct effect on lipid metabolism. Bobek proved that the diet containing whole oyster mushroom strikingly reduced cholerterol contant in serum and in liver by 27 percent and 46 percent respectively. In addition there was a decrease in VLDL by 70 percent and increase in HDL cholesterol ws noticed when fed with oyster mushroom for ten weeks.

Submerged fermentation of mushroom have been found out to contain hypolipidemic effect. According to Chung, (1997) The mycelial other cellular polysaccharides siginificantly decreased the concentration of serum total cholesterol, LDL cholesterol and liver cholesterol. There was no change in HDL cholesterol and triglycerides.

Oyster mushroom had a significant effect on metabolic turnover of low density lipoproteins. The experimental group of rats when fed with 4 percent dried oyster mushroom had serum

cholesterol decreased by almost 55 percent. However the HDL was not affected. The acceleration of LDL metabolic trunover could be one of the important mechanism transmitting the hypocholerterolemic effect of oyster mushroom (Kuniak, 1997).

Gujing fan, (1996) examined the following foods which may influence the lipid metabolism. Cereals, oats, millets, wheat, gum, soyabeans, mussels, scallops, fungi. He mentioned agaricus bisporus as one of the hypolipidemic food which has immediate effect on blood lipids.

Chung (1996) compared the hypocholesterolemic effect of two edible mushroom - Agarcus bisporus and Tremella fuciformis and the results revealed that similar significant decrease in serum LDL cholesterol level was observed (24percent and 31 percent respectively). There was no change in HDL cholesterol. He concluded that mushroom had effective hypocholesterolemic effect.

According to Ozdin (1995) the ethanolic extract and extraction of oyster mushroom reduces the cholesterol level. Whole oyster mushroom and extract decreased serum cholesterol by 27 - 33 percent, decreasing cholesterol in VLDL by 78 - 55 percent and in HDL by 50 - 30 percent.

Methodology

III METHODOLOGY

The present study entitled "Effect of Administration of mushroom on blood lipids of selected hyperlipidemic adult population of Coimbatore city " was undertaken to explore the lipid lowering effect of mushroom. The methodology followed in the present investigation are presented under the following headings.

A. Selection of Area

B. Selection of Sample

C. Preparation of questionnaire

D. Standardisation of Mushroom recipe

E. Conduct of Study

1. Eliciting socio - economic and dietary data.
2. Recording anthropometric data
3. Estimation of lipid profile
4. Supplementation of mushroom
5. Evaluation

A. Selection of area

The area selected for the study was Coimbatore city. Three main hospitals namely Ramakrishna hospital, Kovai medical centre and hospitals and Kongunadu hospital were selected to identify the hyperlipidemic subjects needed for the study. These three hospitals were selected because

1. The hospitals had seperate cardiac unit and have many hyperlipidemic patients attended these hospitals for treatment.

2. The authorities of the hospitals were co-operative and helpful.

3. The hospitals had biochemical labs for analysis of blood and urine.

B. selection of sample

The selection of sample was done by random sampling method. Kothari (1996) defines random sampling as the "sampling technique in which each and every unit of population has equal oppurtunity of being selected in the sample.

The out patients visiting the hospital for treatment and master health checkup were interviewed and the suitable subjects were selected. Hyperlipidemic males belonging to the age group of 30 to 55 years were selected. A total number of 100 hyperlipidemic patients were interviewed to collect details on hyperlipidemia. From this 30 subjects were randomly selected as sub - sample for the supplementation study. The subjects selected to study were divided into two groups of 15 each. One group of 15 subjects were designated as exprimental group to receive mushroom supplementation and the other 15 subjects served as control group without supplementation.

C. Preparation of questionnaire

Keeping in view the objectives of the study, a questionnaire was framed to gather details regarding socio - economic status and dietary pattern of the selected subjects.

Interviewing a Subject.

According to Chaudhary (1991)"Questionnaire refers to a device for securing answers to questions by using a form which has set of questions.

The questionnaire framed by the investigator include socio - economic status, dietary pattern, anthropometric measurements, oil and fat consumption pattern, alcohol and coffee consumption, frequency of food consumption and also type of the oil used were included in the questionnaire. The questionnaire developed is presented in Appendix ID. Standardisation of mushroom recipe

The two types of mushroom which are popular at market are white button mushroom and oyster mushroom. Oyster mushroom was chosen because of their high protein content and minerals than button mushroom (Haynes, 1988). Also oyster mushroom were cheaper than button mushroom. Plate 1 shows the selected variety of mushroom.

A recipe using oyster mushroom (mushroom porriyal)was developed and standardised in the laboratory. The method of preparation is presented in Appendix II. This recipe can be used as a side dish for any meal.

1. Eliciting socio - economic and dietary data

The investigator personally interviewed the subjects and recorded the information using a schedule structured by the investigator. Details regarding age, sex, other socio - economic data and dietary data of the selected samples were collected.

Selected variety of mushroom

Plate II depicts the personal interview being conducted by the investigator.

2. Recording anthropometric data

The pattern of growth and the physical state of the body though genetically determined, are profoundly influenced by diet and nutrition. Hence anthropometric measurements are useful criteria for assessing nutritional status. (Swaminathan 1995).

i. Height

The height of the selected patients were measured by nonstrech fibre glass tape. The tape was fixed to the wall. The subjects were asked to remove footwear and made to stand straight with heels, buttocks and shoulders touching the wall and with feet parailel and placed together with arms hanging at the sides in a nutural manner. The subjects were requested to look straight, A scale was gently lowered compressing the hair and making contact with the head and the height was measured against the markings on the tape fixed to the wall (Jelliffe, 1984). Plate 3 presents the height being taken for one of the subjects.

ii. Weight

Weight is a measurement of body mass. It is the simplest anthropometric measurement with least individual error. Subjects were asked to remove the footwear and wearing minimum essential clothing, the weight was measured using portable bathroom scale. Care was taken to ensure that the weighing was not done after a

full meal or a full bladder (Jelliffe, 1984). Plate 4 shows weight measurement.

iii. Body Mass Index / quelets Index

Body Mass Index is very useful for assessment of nutritional status, since it is highly correlated with fat and body weight over all ages (Borken etal, 1985). The BMI/Quelets Index was calculated for all the patients using the formula

$$\frac{\text{Weight in Kg}}{\text{Height in m}^2} \quad \text{(WHO, 1995)}$$

iii. Estimation of Lipid Profile

The serum lipid profile i,e. the total cholesterol, high density lipoprotein, low density lipoprotein, very low density lipoprotein and plasma triglycerides were estimated by the following principles.

Serum triglyceride was estimated using the CHOD - PAP procedure (wernu etal, 1981).

HDL Cholesterol was estimated using Menagent cholesterol procedure (Grove, 1989).

LDL Cholesterol was computed from the total cholesterol triglyceride and HDL value by using the fried wald fourmula, (1993).

$$\text{LDL-C} = \text{TC} - (\text{HDL} - \text{C}) + \frac{[\text{Triglycerides}]}{5}$$

Height being taken for one of the subjects.

Weight being taken for one of the subjects.

Total cholesterol was estimated using the CHOD - PAD enzymatic procedure (Allain etal, 1994).

The procedures followed in the estimation of each fraction of blood lipids are presented in Appendix - IV

4. Supplementation of mushroom

For all the experimental group mushroom porriyal was supplemented for a period of one month. During the supplementation period, uniform calorie intake by all subjects in all groups was ensured by making their whole day's diet isocaloric. Isocaloric menu adopted by the experimental group of subjects is presented in Appendix - V. The control group of hyperlipidemic subjects were not given any treatment or supplementation.

5. Evaluation

The serum lipid profile of all the subjects were estimated before and after supplementation. The values obtained were statistically analysed to evaluate the effect of mushroom on serum lipids.

Results and Discussion

IV.RESULTS AND DISCUSSIONS

The results of the study entitled "Effect of Administration of Mushroom on Blood Lipids of Selected Hyperlipidemic Adult Population of Coimbatore City". have been tabulated, statistically analysed and dissussed under the following heading.

A.Socio economic status of selected subjects

B.Dietary pattern of the selected subjects

C.Prevalence of diseases among the selected subjects

D.Body Mass Indices (BMI) of the selected subjects

E.Blood lipid profile of the selected subjects

A.Socio-economic status of the selected subjects

The socio economic details of the selected 100 subjects were tabulated and the relevant disscussions are presented in the following.

1. Age

Distribution of the selected samples according to their age are presented in Table -I

TABLE - I

DISTRIBUTION OF SELECTED SUBJECTS ACCORDING TO AGE
(N=100)

Age (Years)	Number
35 - 40	15
40 - 45	22
45 - 50	24
50 - 55	39

From Table I, it is evident that as age increases incidence of hyperlipidemia also increases Thirty nine out of 100 selected subjects were in the age group of 50 to 55 years. Incidence of hyperlipidemia was comparatively less in 45-50 years and 40 to 45 years age group. In 35 to 40 years class there were only fifteen subjects. This trend of more incidence in older age group may be because of the decreasing physical activity and excercise combined with faulty dietary habits. The extra calories consumed are converted to fat and stored in the body leading to various forms of hyperlipidemia.

2. Income

Distribution of the selected subjects according to their monthly income as classified by HUDCO (1991)is given in Table II.

TABLE - II

DISTRIBUTION OF SELECTED SUBJECTS ACCORDING TO THEIR INCOME
(N=100)

Income group *	Number
Low income group (Rs.1250-2650)	20
Middle Income group (Rs.2650-4450)	57
High Income group (Rs.4450 and above)	23

* HUDCO (1991)

The data presented in Table II indicate that the prevalence rate of hyperlipidemia is greater in middle income (57) than in the low (20) and high income groups (23). This may be because of

the fact that subjects in middle income group do less physical work and lead a sedentary life. At the same time there are less number of subjects from high income group. This may be because of the awareness prevaling among these subjects and because of attending various aerobic training programmes.

3. Occupation

The Occupational status of the selected subjects is presented in Table - III

TABLE - III

OCCUPATIONAL STATUS OF THE SELECTED SUBJECTS (N=100)

Occupation	Number
Mechanic	6
Business	24
Teaching	11
Doctors	4
Engineers	7
Clerks	28
Manager	16
Agriculturist	2
Lawyers	2

Table III indicates that out of 100 selected subjects the prevalence of hyperlipidemia in the subjects doing clerical job (28) and business (24) was higher when compared with other occupations. In both the cases there was less physical activity. This was followed by managers(10), teachers (11) and engineers

(7). Although these are also white collor jobs, the amount of physical activity done here is more than the business and clerical people. The prevalence is least among mechanics (6), doctors (4), agricultrists (2) and lawyers (2). This may be attributed to high amount activity and awareness among the doctors and lawyers.

B. Dietary pattern

The dietary pattern of the selected subjects are presented under the following headings.

1. Nature of diet
2. Type and frequency of animal foods consumed
3. Meal pattern
4. Consumption of snacks
5. Consumption of beverages
6. Type of alcoholic beverages consumption
7. Fat and oil consumption

1. Nature of diet consumed by the selected subjects

The nature of diet to the 100 subjects are presented in Table IV.

TABLE - IV

NATURE OF DIET CONSUMED BY THE SELECTED SUBJECTS (N=100)

Dietary Pattern	Number
Pure vegetarians	9
Ova vegetarans	4
Non Vegetarians	87

The data presented in Table IV with regard to the nature of diet of the subjects revealed that 9 subjects were vegetarians and 87 subjects were non-vegetarians. Four subjects were Ova vegetarians. The fact that majority of the subjects were non vegetarians are in line with the results of Vergrosen, (1996)that animal food consumption leads to higher blood cholesterol level than plant foods. Analysis of the diet of the vegetarians also indicated that they were consuming more saturated fats in the form of ghee and butter.

2. Type and frequency of animal foods consumed by selected subjects.

The type of animal foods consumed by the subjects and the frequency of their consumption are presented in Table V

TABLE - V

TYPE AND FREQUENCY OF ANIMAL FOODS CONSUMED BY SELECTED SUBJECTS (N=100)

Animal food	Daily	Weekly	Monthly
Egg	20	5	30
Chicken	5	16	12
Meat	10	6	14
Seafoods	2	4	35
Egg and Chicken	-	36	7
Egg and Meat	-	3	3
Egg and Seafoods	-	10	11
Egg + Chicken,meat and seafoood	-	3	2
Beef	-	4	2

Table V indicate that egg and chicken were the most favoured animal foods. Fifty six subjects consumed egg at least once in a

week and twenty subjects consumed daily and thrity subjects consumed egg monthly.

This was followed by chicken consumption. Sixteen subjects consumed chicken weekly,5 consumed daily and 12 subjects consumed monthly. Meat and sea foods are consumed by less number as compared to chicken and egg. Meat was consumed by 10 subjects daily, six subjects weekly and 14 subjects monthly. The seafoods are the least consumed. Only 2 subjects consumed daily, four weekly and thirty five monthly. Egg and chicken together was consumed by 36 subjects weekly and 7 monthly. Egg and meat were consumed by 3 subjects weekly and 3 subjects monthly and sea foods plus egg was consumed by 10 subjects weekly and 11 monthly.

All the animal foods except beef was consumed by 3 subjects weekly and 2 subjects monthly. Beef was consumed by only 4 subjects once in a week and 2 subjects consumed once in a month. Sea foods are consumed only by minimum number of subjects . In general the consumption of non-vegetarian foods by the selected hyperlipidemics was high. The high saturated fat content and fat added during cooking are more harmful and responsible for hyperlipidemia. At the same time sea foods consumption was less. This goes parallel with the results of Davidson etal (1991) that omega - 3 - fatty acid in fish significantly lowers the triglyceride level whereas meat and chicken increase the triglyceride level.

3. Meal pattern of selected subjects

TABLE VI

MEAL PATTERN OF THE SELECTED SUBJECTS
(N = 100)

Pattern of consumption	Number
Rice preparation thric a day	38
Wheat preparation thric a day	14
Rice preparation once and wheat preparation twice a day	21
Wheat preparation once and rice preparation twice a day	11
Rice preparation twice a day	1
Rice preparation once a day	15

Table VI indicates that out of 100 selected subjects 38 subjects consumed only rice or rice preparations for all the 3 meals daily. The incidence of hyperlipidemia was seen higher among the subjects who consumed rice thrice a day than those subjects who consumed wheat thrice a day (14). Rice has low fibre content than wheat thus the absorption of nutrients especially the carbohydrates is more. Increased intake of rice is a important factor in the development of hyperlipidemia. Subjects who consumed rice or rice preparation twice a day and wheat preparation once was 21 which is again higher than those subjects who consumed wheat preparation twice a day and rice preparation once a day.

Table VI also indicated that 84 subjects took all the 3 meals a day and one subject took meals only twice a day. He consumed lunch and dinner alone. 15 subjects out of the 100

subjects consumed meals only once in a day. They consumed dinner alone. These 15 subjects skipped both breakfast and lunch. This goes parallel with the results of Truswell (1997), that frequency of meal consumption is inversly propotional to the body fat absorption.

4. Consumption of snacks by the selected subjects

The details regarding the type and frequency of consumption of snacks by the selected 100 hyperlipidemic subjects are tabulated and discussed in the following.

TABLE VII

FREQUENCY OF CONSUMPTION OF SNACKS BY THE SELECTED SUBJECTS
N = 100

Snack items	Number	Daily	Weekly	rarely
Fried foods	3	--	2	1
Pastries / sweets	15	7	6	2
Biscuits	7	--	6	1
Fried foods and pastries / sweets	6	--	6	--
Fried foods and pastries / sweets and biscuits	57	43	9	5
Fried foods and biscuits	13	10	3	--

Table VII indicates that 57 subjects out of 100 selected samples consumed all types of snacks. Out of this 43 subjects consumed snacks daily, 9 weekly and 5 rarely. The fat consumption and carbohydrate consumption increase when the snacks are consumed, thus increasing the blood lipids. This is followed by

15 subjects who consumed pastries and sweets. 7 subjects consumed sweets daily, 6 of them weekly and 2 rarely. Thirteen subjects consumed fried food and biscuits both contributing to extra calories. Ten subjects consumed both fried foods and biscuits daily. Fried foods and sweets are consumed by six subjects weekly. Biscuits are consumed by 7 subjects frequently. The data presented in table VII bring out the fact that all the 100 subjects had the habit of snacking, which is one of the leading causative factors for hyperlipidemia. These foods increase the consumption of fats and oils and calories through preformed sugar.

5. Consumption of beverages by the selected subjects

TABLE VIII

TYPE AND CONSUMPTION OF BEVERAGES BY THE SELECTED SUBJECTS
N = 100

Type of Beverage	No.of Subjects	Early Morning	Mid Morning	Tea Time	Bed Time
Coffee	28	28	21	27	--
Tea	16	16	12	16	2
Milk	8	8	--	6	8
Butter Milk	5	--	5	2	5
Fruit Juice	7	1	7	7	--
Tea &Coffee	32	32	27	32	2
Milk &Juice	4	4	4	4	4
Aerated drinks	25	--	24	1	--
Juice and areated drinks	8	--	8	--	--

Table VIII indicates that coffee consumption was higher than consumption of other beverages. Twenty eight subjects consumed coffee daily and they consumed a minimum of four cups per day. Bruke (1991) reported that coffee consumption (3 cups per day) shall increase the low density lipoprotein. Sixteen subjects out of hundred took only tea daily, 12 subjects took tea for mid morning and 2 subjects consumed at bed time. Milk was consumed by only eight subjects, fruit juice by 7 subjects. Tea and coffee was consumed by 32 subjects and milk and juice were consumed by only four subjects. Aerated drinks were consumed by 25 subjects and both fresh juice and aerated drinks were consumed by 8 subjects.

In general tea and coffee consumption was more prevalent than other drinks.

6. Consumption of alcoholic beverages by the selected subjects

The amount of alcoholic beverages consumed by the selected subjects are presented in table IX

TABLE IX

CONSUMPTION OF ALCOHOLIC BEVERAGES BY THE SELECTED SUBJECTS

N = 100

Beverage	60 ml	120 ml	180 ml
Rum	7	17	12
Whisky	12	32	17
Vodka	12	32	17
Beer	38	14	42
Brandy	14	7	23
Gin	12	7	9

Table IX clearly shows that 99 out 100 selected samples consumed one or the other type of alcoholic beverage. Thirty six subjects consumed rum, 61 subjects consumed whisky, 61 consumed vodka. Consumption of beer is high as 94 subjects consumed beer. Brandy and gin were less popular when compared with other alcoholic beverages. Consumption of alcoholic drinks from light to moderate level does not pose any serious health hazard but heavy consumption (above 60ml) shall lead to decrease in the high density lipoprotein.

Alcoholic berverages which are harmful to health are consumed by 94percent of the selected subjects. This may be an important causative factor.

7. Fat and oil consumption of selected subjects

The information recorded for the oil and fat consumption of the selected subjects are presented in table X.

TABLE - X

TYPE, AMOUNT AND FREQUENCY OF FAT AND OIL CONSUMPTION OF SELECTED SUBJECTS (N = 100)

Type of oil	Daily				Weekly				Rarely			
	No	<10 g	10-25 g	>25 g	No	<10 g	10-25 g	>25 g	No	<10 g	10-25 g	>25 g
Gingelly Oil	28	3	5	21	48	10	13	25	7	--	5	2
Groundnut Oil	56	4	10	42	--	--	--	--	9	--	--	9
Dalda	16	--	16	--	21	--	19	2	42	2	2	38
Butter	12	3	9	--	10	2	7	1	68	14	42	12
Palm Oil	12	--	8	4	--	--	--	--	--	--	--	--
Refined Oil	2	--	--	2	37	3	65	26	18	--	3	15
Coconut Oil	2	--	--	2	7	--	--	7	84	--	62	22
Ghee	2	--	--	2	17	--	17	--	72	--	62	10

Table X indicates that gingelly oil and groundnut oil were the most popular oils used by maximum subjects daily (56 and 28 respectively). About 42 subjects consumed gingelly oil about 25g each day and 10 subjects consumed 10 - 25g and four subjects consumed below 10g. Most of the subjects consumed above 25g of gingelly oil. Groundnut oil consumption was also high and 21 out of 28 consumed about 25g daily. Twenty five subjects out of 48 consumed above 25g weekly. Dalda, which is a highly saturated fat is consumed by 16 subjects daily and the amount of consumption is also high (10-25g). Nineteen subjects consumed dalda weekly to about 10-25g and 38 subjects consumed rarely to a high level of above 25g. Butter consumption was also high, 12 subjects consumed

daily, 10 subjects weekly and 68 subjects consumed rarely. Palm oil and refined oil were the least popular oils. Only 12 and 2 subjects consumed then daily. Coconut oil was consumed by 2 subjects only to above 25g, 7 subjects weekly and 84 subjects consumed coconut oil rarely. Ghee was consumed by 2 subjects daily to above 25g. Seventeen consumed weekly and 72 subjects consumed rarely.

Coconut oil is used rarely by 84 subjects but the amount used by them was also more, 62 subjects consumed 10-25g of oil and 22 subjects consumed above 25g of coconut oil. Ghee which is a saturated fat was consumed by 57 subjects weekly and 54 of them consumed less than 10 g and 10-25g is consumed by 2 subjects.

Ghee was rarely used by 11 subjects and the amount used is also less than 10g. After the diagnosis of hyperlipidemia most of the subjects switched on to gingelly oil and refined or sunflower oil.

From the table it is clear that consumption of saturated fats was high among the selected subjects. Almost all subjects consumed butter, or ghee, which are essential factors in the development of hiperlipidemia.

C. Prevalence of diseases among the selected subjects

Prevalence of diseases among the 100 selected subjects are presented in table XI.

TABLE XI

PREVALENCE OF DISEASES AMONG THE SELECTED SUBJECTS (N = 100)

Major illness	Number
Hypertension	48
Heart diseases	14
Diabetes	9
Hypertension and heart diseases	12
Diabetes and Hypertension	7
Tuberculosis	4

Data presented in table XI indicates that 48 percent of the subjects had hypertension. This large incidence may be because hyperlipidemia cause the constriction of the blood vessels leading to hypertension. This is followed by heart diseases. Fourteen subjects suffered from heart diseases and 12 subjects had both hypertension and heart diseases. Heart diseases and hypertension mutually complement one another. Incidence of diabetes was 9. Seven subjects suffered from both diabetes and hypertension. Seven out hundred selected subjects had tuberculosis which does not have any relation with hyperlipidemia.

E. Body Mass Index (BMI)

The height and weights of all the subjects were measured and the body mass indices (BMI) were calculated. Table XII presents the distribution of subjects according to the BMI

classification given by Garrow, 1995. The individual height, weight and BMI are presented in Appendix V.

TABLE XII

DISTRIBUTION OF SELECTED SUBJECTS ACCORDING TO BODY MASS INDEX

N = 100

BMI	Number
18.5 - 20	7
20 - 25	53
25 - 30	37
30 and above	3

18.5 - 20 - Low weight

20 - 25 - Normal

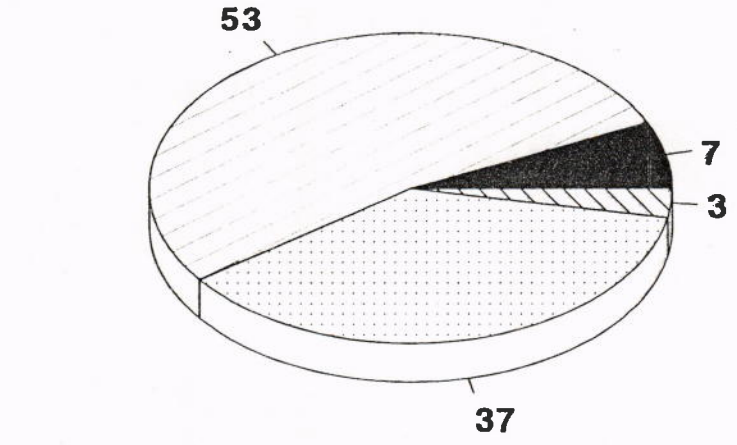
25 - 30 - Obese grade I

30 and above - Obese grade II

The data presented in Table XII indicate that BMI of 7 subjects were below normal and 53 were normal 37 subjects out of 100 were classified as Ist grade obese and 3 subjects belonged to II grade obesity. In general 40 percent of the subjects were obese. Obesity is one of the main causes which predispose to hyperlipidemia. This is in line with the results of Tannguchi (1991) that increase in the Body Mass Index is a risk factor of CHD and hyperlipidemia.

F.Blood lipid profile of the selected subjects

The blood lipid profile of the selected subjects (30) before and after supplementation of mushroom are presented in table XIII. The individual values are indicated in appendix VII. Figure1 also depicts the variation in blood lipid profile before and after supplementation.



Distribution of selected subjects according to Body Mass Index

Fig. 2

TABLE - XIII

BLOOD LIPID PROFILE OF THE SELECTED SAMPLES

BEFORE AND AFTER MUSHROOM SUPPLEMENTATION

N = 100

Lipids	Experimental		't' value	Control		't' Value
	Initial	Final		Initial	Final	
Total Cholesterol	217.893+8.70	215.85+9.60	2.57 *	217.60+21.5	220.58+19.62	2.53 *
Low density lipoprotein	143.18+7.15	140.30+7.07	6.74 **	142.18+23.50	143.713+26.43	0.21 N.S
Very low density lipoprotein	28.70+4.14	27.986+4.37	1.37 N.S	29.07+3.67	30.98+4.34	1.46 N.S
Triglycerides	184.32+23.04	188.8+14.04	1.07 N.S	166.28+30.2	174.40+30.14	0.71 N.S
High density lipoprotein	46.0+4.49	46.44+4.61	1.86 N.S	48.97+4.63	33.33+9.26	6.48 **

** - Significant at one percent level

* - Significant at five percent level

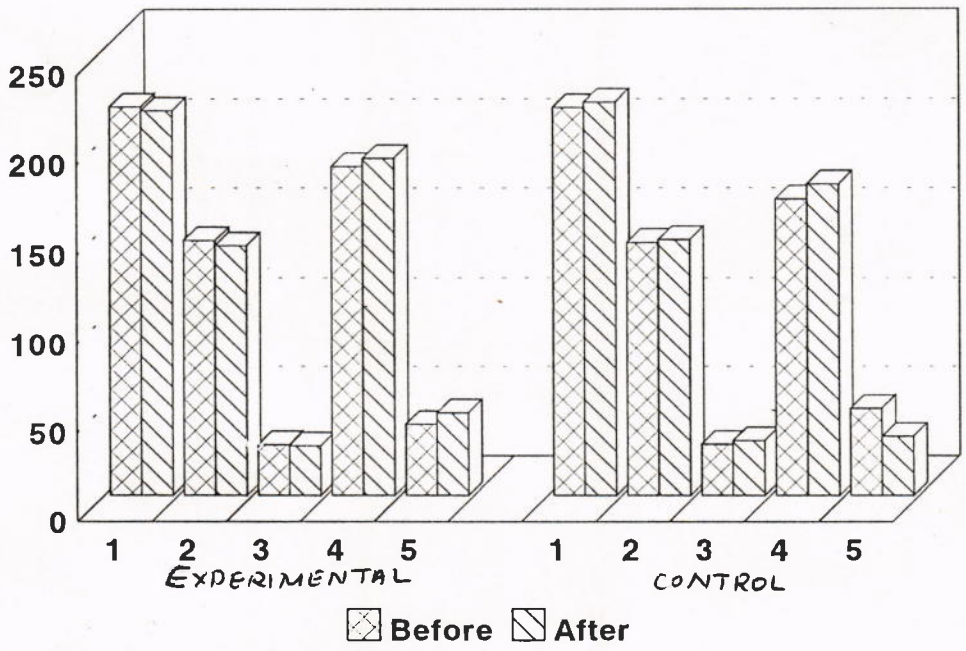
N.S. - Not significant .

The data presented in Table XIII indicates that there was a slight decrease in serum total cholesterol level of the experimental group after mushroom supplementation. The difference was statistically significant at one percent level.In the case of low density lipoprotein and very low density lipoprotein levels also there was a reduction in the values. The reduction in LDL-C was significant at five percent level whereas reduction of VLDL was not satistically significant . There was also a slight increase in the triglyceride level. The high density lipoprotein level was not much affected by mushroom supplementation. There was only a 0.44mg increase in HDL level which was insignificant statistically.

In the case of control gruop all the lipid fractions had increased after a period of one month. This group did not receive any special treatment. With regard to HDL level of the control group the values had decreased after one month period. The increase in the initial and final values of cholesterol and HDL of control group were statistically significant.

The results of the present investigation bring out the fact that the selected hyperlipidemic subjects had moderate income, higher fat consumption and low physical activity, which were the main causes for hyperlipidemia. Majority of the selected samples were non vegetarians, had the habit of smoking and used alcohol also.

When mushroom was supplemented daily for a period of one month, the experimental group showed fruitful results with lowered total cholesterol, LDL and VLDL and slight incrase in HDL levels. The control group which had not received any supplementation did not show positive changes.



Blood lipid profile of the selected subjects before and after mushroom supplementation
Fig. 1

1. Total serum cholesterol
2. Low density lipoprotein
3. Very low density lipoprotein
4. Triglyceride
5. High density lipoprotein

Summary and Conclusion

V SUMMARY AND CONCLUSION

Hyperlipidemia is an upcoming health problem all over the world affecting millions of populations of age groups 35 to 55 years. The present study "Effect of Administration of Mushroom on Blood Level of Selected Hyperlipidemic Adult Population of Coimbatore City", was conducted to test the effectiveness of mushroom in lowering blood cholesterol level. The objectives of the study were to standardise a mushroom recipe that can be supplemented to hyperlipidemics and then evaluate the impact of mushroom supplemention on blood lipid levels of selected hyperlipidemic subjects.

Three hospitals namely Ramakrishna Hospital, Kovai Medical Centre and Hospital and Kongunadu were selected to identify the hyperlipidemic subjects. One hundred hyperlipidemic samples were randomly selected from these three hospitals. Thirty subjects were selected as sub samples and were divided into two groups of fifteen subjects each. One group of fifteen hyperlipidemics were designated as an experimental group to receive mushroom supplementaion. The second group of fifteen hyperlipidimic did not receive any supplement and served as control.

A questionnaire was prepared to elicit information on the socio-economic status, dietary pattern and clinical histroy of all the 100 subjects.The height and weight of all the subjects were measured and body mass index calculated . After collecting the back information from all 100 subjects the experimental groups of 15 subjects were supplemented with mushroom for one month. The blood lipid profile of both the experimental and

control groups of subjects were estimated before and after supplementation. The findings revealed the following.

1. The socio-economic status of the selected subjects showed that majority of the subjects belonged to the age group of 50 to 55 years and that the incidence of hyperlipideimia increased as the age progreses. Income wise classification showed that the middle income group and high income groups were at a greater risk than the lower income group subjects. Classification according to occupational status showed that subjects with less physical work were more prone to the disorder than subjects with high mannual work.

2. Nature of diet of the selected subjects showed that maximum number of subjects consumed animal foods and eggs. Subjects who were vegetarians included more saturated fats in their diet.

3. The type of nonvegetarian item consumed by the subjects were egg, chicken and meat.The consumption of seafoods were less than the other animal foods.

4. The beverages consumed by the selected subjects showed that coffee,tea and aerated drinks were consumed by the large number of subjects, thus incrasing the calorie consumption. Fruit juices, buttermilk and milk were consumed by comparatively less number of subjects.

5. The meal pattern of the selected subjects showed that majority of the subjects consumed rice perparations thrice a day. Wheat perparation were consumed by only a fewer number of subjects. The number of subjects skipping meals were high.

6. Consumption of snacks by the selected subjects showed that almost half of the selected subjects consumed all varieties of snakcs. All subjects had the habit of consuming one snack or the other daily. This habit increased the fat and oil consumption of the subjects.

7. Alcohol consumption pattern indicated that 94 perecent of the sample consumed one type or other alcohol in moderate to heavy amounts.

8. The fat and oil consumption of the selected subjects revealed that gingelly oil and groundnut oil were more popular than refined and palm oil. The consumption of saturated fats like butter and ghee were also high among selected subjects.

9. The data collected on the prevalence of diseases among the selected subjects showed that hypertension and heart diseases were more prevalent among the selected subjects.

10. Forty percent of the subjects had grade I or grade II obesity.

11. Blood lipid profile of the selected subjects were on the higher side. After mushroom supplementation a positive change was noticed in LDL, VLDL and HDL levels of the experimental subjects. In the control group no such changes were noticed. Instead there was a slight increase in the other lipid levels and decrease in HDL levels.

The results of the present study indicated that mushroom has greater potential in reducing the blood lipid level. If consumed for a longer period of time, apart from supplying

essential amino acid, vitamins and minerals, mushroom can also reduce blood lipids and save the population groups from hypertension and other cardiovascular problems.

Further studies for a longer period of supplementation can be conducted to further discover the potentials of mushroom.

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Appendices

APPEMDIX I

QUESTIONNAIRE

TO ELLICIT INFORMETION ABOUT THE LIFESTYEE FOOD HABITS AND DIETARY PATTERN

1. Name of the interviewer :
2. Name of the interviewee :
3. Age:
4. Sex:
5. Educational status:
6. Address:
7. Family background:

Names of family members	Age	Sex	Education	Occupation	Income

8. Income from other properties :
9. Total income of the family :
10. Anthropometic measurement :
    Height :
    Weight :
11. Dietary pattern
    Pure vegeterian ova vegetarian
    Non vegeterian

11.1 if non vegeterian, type of non vegetarian item consumed

Type of Non-veg	Amount	Frequency of consumption		
		Daily	weekly	monthly
Egg				
Meat				
Chicken				
Beef				
Seafoods				
others				

11.2 Meal pattern

Early morning	
Breakfast	
Midmorning	
Lunch	
Evening	
Dinner	
Bed time	

11.3 Food halits

	Frequency of consumption		
	Daily	Weekly	levely
Fast foods			
Fried foods			
Pasties			
others			
Complete meals			
Twice / day			
Thrice / day			
Once / day			
Beverages			
Bottle drinks			
Fresh drinks			
Alcoholic drinks			

12. Medical history

Do you suffer from any major illness
Diabetes
Hypertension
Heat disease
Tuberculosies
Others
Do you suffer from any minor illness
Common cold
Fever
Stomach pain
Headache
Any other problem

13. Fat oil consumption

Type of oil used	Frequency and amount of consumption											
	Dai -ly	<10 g	10.25 g	>25 g	Wee -kly	10 g	10.25 g	>25 g	rar -ly	<10 g	10.25 g	>25 g
Gingelly oil												
Groundnut oil												
Coconut oil												
Ghee												
Refined oil												
Palm oil												

APPENDIX - II

Method of preparation of the recipe

Ingredients

Oyster mushroom - 50 grams

Onion - 5 grams

Pepper - 2 grams

salt to taste

Method

Heat the pon and add chopped onions to it and saute it. Add the washed and cut mushrooms and cover the pan with lid for five minutes add salt to taste and pepper and remove from fire.

APPENDIX - III

THE NUTRIENT CONTENT OF THE STANDARISED RECIPE

List of K.cal	Amount (g)	Carbohydrates (gm)	Protein (gm)	Fat (gm)	Fire (gm)	Energy (K.cal)
Oyster mushroom	50	11.2	1.55	0.4	0.2	21.5
Onions	3	0.33	0.036	0.003	0.018	1.5
Pepper	2	0.98	0.23	0.136	0.298	6.08
Total		12.51	1.816	0.539	0.516	29.08

APPENDIX IV
ESTIMATION OF CHOLESTEROL

PRINCIPLES OF THE REACTION

The meangent cholesterol 500 is fully enzymatic procedure with caloimetric determination at 500nm.
The reaction takes place as follows

$$\text{Esterified cholesterol} \xrightarrow[\text{esterase}]{\text{Cholesterol}} \text{4 - cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O \xrightarrow[\text{oxidase}]{\text{Cholesterol}} \text{4-cholesterol} + 4O$$

The optical density read at 500nm is propotional to the concenmtration of total cholestrol.

REAGENTS

1. ENZYMES
   Sodium cholate - 1.2 nm
   4 amino antipyrine - 0.12 m mol/l
   Peroxidase - 500 U/l
   Cholesterol Oxidase - 35 U/l
   Cholesterol Esterase- 200 U/l

2. BUFFER
   Phosphate buffer - 100 m mal/l
   Phenol - 2b mmol / l

Stroage - Store the package at 2 - 8 C
Specimen's - Use Unhaemolysed serum

Reagent preparation

Reconstitute a vial of engymes (Reagent 1) with one bottle of Buffer (Reagent 2). Mix gently until completely disolved. Do not shake. Shaking might cause engymes denaturation. The reconstituted reagent is stable for one month at 2 - 8 C

	Sample	Procedure standard	R.Blank
Reagent	3 ml	3 ml	3 ml
Serum	0.03 ml	--	--
Standard	--	0.03 ml	--
Distilled water	--	--	0.03 ml

Incubate 10 minutes at 37 C Read the optical density of the spcimen and the standard aganist the reagent blank.

Note the colour development is stable for 30 minutes at room temperature and volumes may be modified proportionally.

The test is linear upto 500 mg / dl

Far higher concentration repeat the test on a specimen diluted in saline solution and multiply the rest by the dilution factor.

Formula

Values are calcuated with the following farmula.

$$\text{Cholesterol mg/dl} = \frac{\text{O.D Specimen}}{\text{O.D Standard}} \times \text{Concentration standerad (mg / dl)}$$

The standard is available seperately

Expected values

150 - 260 mg / dl

ESTIMATION OF TRIGLYCERIDES

Contents

Reagent 1 (5 bottles)	:	Buffer
Store at 2 C to 8 C	:	Active ingredients piper buffer ESDAS
Reagent 2 (5 bottles)	:	Enzymes
Stroed at 2 C to 8 C	:	Active ingredients lipoprotein lipore glycerokinasl Glycerol-3 phosphate oxidase perioxidase 4 amino antipyline ATP.
Reagent 3 (1 bottle)	:	Standard
Store at 2 to 8 C	:	(Triglyceride 200 mg/ dl ready to use)

Preparation of working solution

Transfer and dissolve contents of one bottle of Reagent 2 with one bottle of reagent 1. Mix well and store at 2 C to 8 C. The engymes chromegent reagent.

Procedure

Pipette into test tubes

	Blank	Standard	Test
Enzyme chromagen	2.0 ml	2.0 ml	2.0 ml
Reagent	--	0.2 ml	2.0 ml
Standard	--	--	--
Sample	--	--	--

Mix and incubate at 37 C for 5 minutes. Read absorbance of the test (AT) Standard (AS) and the reagent blank (AB) aganist distiled water of 546 nm wavelength (530 to 570 nm) of with green filter.

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

Calculations

Triglycerides concentration (mg/dl)

$$= \frac{A}{A} - \frac{A}{A} \times 200$$

To convert mg/dl to mmol/l use equation mmol/l = mg/dl X 0.0114

Normal values of serum tiglycerides

Men = 60 - 165 mg /dl
0.68 - 1.88 mmol/l

Women = 40 - 140 mg/dl
0.46 - 1.60 mmol /l

Linearity

This method is linear upto 1000mg/dl. For sample values higher than 1000 mg/dl dilute the sample suitably with 0.9% saline and repeat the assay.

Principle

Serum triglyceride are hydrolyged to glycerol and free fatty acid by lipase. In the presence of ATP and glycokinase, the glycerol is converted to glycerol - 3 - phosphate. The glycerol -3- phosphateis then exidised by glycerol 3 phosphate oxidase to yeild hydrogen peroxide. Hydroxide reacts in the presence of peroxidase with ESPASCN - ethly - N - Sulfapropyl - n - anisidine) and 4 amino antipyrine to form a coloured comlex. The intensity of the colour so developed is proportional to

triglycevdes concentration and is measured photometrically at 546nm (530nm)or with green flter.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipoprotein lipase}} \text{glycerol} + 3RCOOH$$

Glycrol + fatty acids

$$\text{glycerol} + ATP \xrightarrow{\text{Glycerokinase}} \text{glycerol-3-phesphate} + ATP$$

$$\text{glycerol-3-phosphate} + O_2 \xrightarrow{\text{glycerol-3-phosphate oxidase + ADP}} \text{dihydroxy acetone phosphate} + H_2O_2$$

$$H_2O_2 + 4\text{ aminophenagonl} + 4\text{-chlorophenol} \xrightarrow{\text{peroxidasl}} 4\text{-(P-bengoquinone monomino) phenagono} + 2H_2O + Hcl$$

Calculated values of low density lipoprotain cholesstro (LDL -C and VLDL - C)

$$LDL - C = TC - (HDL - C + VLDL - C)$$

$$VLDL - C = \frac{\text{Triglycerides}}{5}$$

ESTIMATION OF CHOLESTROL HDL

Menagent cholesterol HDL allows detumination of lipoprotein HDL after precipitation of LDL and VLDL fractions with phosphotungstic acid and magnesium chloride.

Reagents		Composition
Reagent	3 x 10 ml	Phospholtungstic acid 13.9 m mol/l Magnesium Chloride 490 m mol/l
Standard 1	1x5 ml	20 mg/dl
Standard 2	1x5 ml	20 mg/dl
Standard 3	1x5 ml	40 mg/dl

Storage

Store the package at 25 C

Specimens

Serum

Reagent preparation

The precipitating reagent (reagent 1) is ready for use.

Procedure

In a centrifuge test tube

Serum 1ml

Precipitating reagent 0.1 ml

Mix and let stand 10 minutes at 18.25 C
Centrifuge 15 at 5000 - 6000 spm. Remove the supernatant and measure the HDL - Cholesterol using menagent cholesterol - 500.

Formula

Calculate the results using the menagent cholestrol 500 produce; multiply the value obtained by 11 to allow for dilution occuring with precetitation.

Expected values

Men = 30 - 60 mg/dl
Women = 40 - 70 mg/dl.

Storage and stability

Engolit triglyceride reagents are stable till the expiry date indicated on the lables when stroed at 2 to 8 C. The engymes chromogen reagent is stable for two weeks at 2-8 C when stored in amber coloured vial specimen collection and storage.

1. Serum from fasting blood sample is prefered
2. Plasma collection with use of heparin as anticoagulant may abo be used.
3. Plasma collected with use of anticoagertant containing flourde of oxalate should be aveoided.

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

Precautions

Enzokit triglycerides is for in vitro use only. Avoid contact with skin, eyes and clothes. Do not piptte by mouth.

Interfering substances

Haemolysis and high bilurubin content can inter fere with the test.

APPENDIX - V

ISO Caloric diet for the selected subjects

Meal pattern	cookedweight	Raw weight	carbohydrates(g)	Protein(g)	fat(g)	Kilocalories(K.Cal)
Early morning						
Coffee - Milk	1/2 Cup	100 ml	4	3.2	0.1	65
Sugar	2 Tsp	10 g	10	--	--	4.0
Breal fast						
Iddli	4 in numbers	260	58.6	11.2	0.58	276.4
Sambar	1 Cup	40	28.8	5.1	2.7	97
Onion Chutney	1/2 Cup	30	2.9	1.0	0.1	133.2
Mid morning						
Tea - Milk	1/2 Cup	100 ml	4	3.2	0.1	65
Sugar	2 tsp	10 g	10	--	--	40
Lunch						
Rice	225 g	75 g	60	4.8	0.3	255
Sambar	1 Cup	40 g	28.8	5.1	1.7	97
Rasam	1 Cup	--	--	--	--	--
Curd	1/2 Cup	100 ml	4	3.2	0.1	65
Vegetable A (leajy vegetable)	3/4 Cup	150 g	8	2	--	50
or						
Vegetable B (roots & Tubers)	1/2 Cup	100 g	10	2	--	50
Mushroom Curry	50 g	50 g	2.5	1.55	0.4	7.5
Tea time						
Tea - Milk	1/2 Cup	100 ml	4	3.2	0.1	65
Sugar	2 Tsp	10 g	10	--	--	40
Dinner						
Chappati	4 in Number	100 g	72	12	--	340
Vegetable A	1/2 Cup	50 g	6	1	--	30
Fruit	1	100 g	10	--	4	40
Milk	1/2 Cup	100 ml	4	3.2	0.1	65
Overall Oil Consump -tion for the day	3 table spoon	15 ml	--	--	15	135
Total			338.6	16.2	21.28	1956.1

APPENDIX - VI
INDIVIDVAL BMI VALUES OF SURVEYED PATIENTS

1	22.7	26	19.8	51	22	76	27.3
2	27.9	27	32	52	22.3	77	26.9
3	29.8	28	21.7	53	22.3	78	27.3
4	27.3	29	27.5	54	22.5	79	20.1
5	19.3	30	20.9	55	29.1	80	23.2
6	21.3	31	22.9	56	29.5	81	24.2
7	22.5	32	21.1	57	22.6	82	26.3
8	27.3	33	26.9	58	28.3	83	22.1
9	28.3	34	24.9	59	21.9	84	25.9
10	27.1	35	20.9	60	22.1	85	26.9
11	22.1	36	19.5	61	20.8	86	23.1
12	20.9	37	25.6	62	20.9	87	28.1
13	21.7	38	23.1	63	28.3	88	23.2
14	26.6	39	24.3	64	24.5	89	32.1
15	23.1	40	22.5	65	28.8	90	26.6
16	29.8	41	27.8	66	19.2	91	22.1
17	21.1	42	21.7	67	22.7	92	25.9
18	22.6	43	22.1	68	27.9	93	28.1
19	22.9	44	26.1	69	22.9	94	24
20	29.9	45	22.7	70	25	95	22.5
21	28.2	46	27.5	71	19.3	96	30.2
22	28.9	47	19.2	72	22.6	97	22.4
23	26.6	48	22.3	73	29.3	98	29.1
24	24.2	49	21.4	74	26.3	99	28.6
25	21.5	50	18.7	75	23.4	100	21.7

APPENDIX - VII

INDIVIDUAL LIPID PROFILE VALUES SELECTED PATIENTS

EXPERIMENTAL

Serum cholestrol (mg/dl)		low density (mg/dl) lipoprotein		Very low density (mg/dl) lipoprotein		Trig lycercle (mg/dl)		High density (mg/dl) lipoprotein	
Before	After	Before	After	Before	After	Before	After	Before	After
215	214.5	134	132.7	35	34	175.5	175	46	46
220.9	220.6	143.8	140.7	35.1	33.7	200.7	200	42	43
230	229.5	152.6	151.6	30.7	30.1	180	175	46.7	46
210	205	138.3	133.8	31.3	35.1	185	184.7	40.4	40.5
231.3	229	145.3	144.2	34	32	205.3	203.9	52	54
220.3	220	138	135.2	29	25.9	210.2	210	53	53
210	208	139.1	135.1	24.1	22.7	175	173	47	46
220	217.7	140.6	140	30	30.2	205.1	202	50.3	50.5
205	205.3	137.6	135.1	25.1	24.1	180.9	179.7	43	44
217	205	137.4	135	30.9	28.3	202.9	202	48.7	50
221.9	220	141.9	137	28	27.7	201.4	200	52	53
220.7	220	156	150	24.4	26.8	117.3	175	40.3	42
216	215.5	149.8	145	23.2	22.1	170	170	43	42
228	227.7	156.6	154.2	25.7	24.1	180	181	45.7	46
200.5	200	136.5	135	24	23	175.5	201	40	40.7

INDIVIDUAL LIPID PROFILE VALUES SELECTED PATIENTS

CONTROL

Serum cholestrol (mg/dl)		low density (mg/dl) lipoprotein		Very low density (mg/dl) lipoprotein		Triglycercle (mg/dl)		High density (mg/dl) lipoprotein	
Before	After	Before	After	Before	After	Before	After	Before	After
217	218	123	125	35	32	175	215	42.1	32
222.2	223	180.3	149.7	24.16	33.1	202.9	200	43	33.1
210	212	175.5	125	30	36	180	175.9	52	55
220.9	220.9	160.5	200	28.1	24.9	195.8	204	46.4	24.9
221	222	172.7	175	24.7	31	120	189	47.2	31
216	217	152	162	33	33	201.1	195	50.1	33
202	202.5	123.7	173	28.1	26	125	180	53.2	26
217	220	147	154	31.1	38	130.7	205.2	48.2	38
200.1	215	112.7	133	27.1	29	165	124	57.2	29
205.9	210	125.1	145	26	35	172	133	53	35
205	207.9	111.1	120	23.7	29	128.7	170	49	29
207.1	209.9	112.2	125	28	29	135.9	175	43.2	29
214.9	225	142.9	105	32	24	201.2	129	53.2	24
220.7	220	149	112	30.2	28	18.9	136	43.7	28
287	285	145	152	35	36.7	180	185	53	53