MATERIAL AND METHODS
MATERIAL AND METHODS

The case material for the present study consisted of 31 healthy male and 17 healthy female volunteers of 15-46 years of age. The subjects were randomly selected from among the junior doctors and students of M.L.B. Medical College, healthy attendants of the patients attending OPD and wards of Medical College Hospital, Jhansi and domestic servants.

Informed consent was taken from every case. Detailed history taking, thorough clinical examination and relevant investigation were done to exclude any cause of hyperlipoproteinemia. Detailed dietary history was also elicited to assess the amount of different constituents - fat and cholesterol, protein and carbohydrates in the usual diet. Daily fat consumption (with its type egg, ghee, oil, milk and milk products, eggs and food additives) of the majority of the subjects were consuming less than 300 mg of cholesterol and 7/3 ratio of the usual diet ranged from 0.40 to 0.35. No female subject of the study was using oral contraceptive at the time of study or 3 months prior to it.

DESIGNS OF TEST DIET

As this study was taken to assess the changes in serum lipid profile after ingesting different amount and types of cholesterol fat diet (and other types of
diets: protein, carbohydrates), 14 different protocols were used.

All the subjects were asked to have their dinner at 6.00 PM on the previous night and not to take anything except water till the next morning. Fasting blood samples were taken at 8 AM in recumbent posture without producing venous stasis (Keenselman et al., 1961). After this they were given a test meal which differed in different protocols.

Postprandial blood samples were taken at different time intervals in different protocols. All subjects were confined to bed at the time of test and were not allowed to take anything except water. Five millilitres of blood was collected for each test sample and plasma was separated from the blood within four hours. Following tests were performed in each blood sample.

1. **SERUM TOTAL CHOLESTEROL (STC)**

Cholesterol estimation was done by one step method of Wybenga and Pillegi (1970) utilizing commercial kits supplied by EMDER.

2. **SERUM TRIGLYCERIDES (STG)**

Serum triglycerides was estimated by acetyl acetone method, utilizing kits provided by HI-TECH Laboratories.
3. **SERUM HIGH DENSITY LIPOPROTEIN (HDL)**

Quantitative estimation of HDL cholesterol in serum was done by kit supplied by EIMCO, using the precipitating method.

4. **SERUM LOW DENSITY LIPOPROTEIN (LDL)**

This fraction of lipoprotein was estimated by formula given by Friedwald et al. (1972).

\[
LDL = STC - (STC/5 + HDL) \text{ mg/dl.}
\]

5. **SERUM VERY LOW DENSITY LIPOPROTEIN (VLDL)**

This too was estimated by above mentioned formula which is valid till STC values are less than 500 mg/dl.

Statistical analysis of the data was done by using different tests of significance (Paired 't' test and student 't' test).

**PROTOCOL 1**

Number of subjects = 10 (6 males and 4 females).

**TEST DIET:** 100 gms butter smeared over 4 average sized breads and 300 ml of boiled sweetened milk. The provided about 300 mg cholesterol and 35 gms of fat.

Postprandial blood samples were taken at one hour interval upto five hour interval in most of the subjects.
PROTOCOL 2

Number of subjects: 5 (4 males and 1 female).

**Test Diet**: 50 gm butter smeared over 4 breads with 200 ml of milk. This provided about 160 mg of cholesterol and 55 gm of fat.

Postprandial blood samples were taken at one hour interval up to five hours.

PROTOCOL 3

Number of subjects: 8 (4 males and 4 females).

**Test Diet**: Single boiled hen egg, supplying about 300 mg of cholesterol and 6 gm of fat.

Postprandial blood samples were taken at one and three hour.

PROTOCOL 4

Number of subjects: 2 (1 male and 1 female).

**Test Diet**: 2 boiled eggs, providing about 600 mg cholesterol and 12 gm of fat.

Postprandial samples were taken at one and three hour.

PROTOCOL 5

**Subjects**: 2 (1 male and 1 female).

**Test Diet**: 3 boiled eggs plus 230 ml of milk (The same test diet was used in work done by Aare et al., 1989 when concept of cholesterol tolerance test was proposed).

Postprandial samples were taken at 15 minutes interval up to 1 hour and one sample at 2nd hour.
PROTOCOL 6

Subjects : 2 male subjects.

TEST DIET : 4 boiled eggs, providing about 1200 mg cholesterol and 24 gm fat.

Postprandial samples were taken at 1 hour interval upto 3 hours.

PROTOCOL 7

Number of subjects : 2 male subjects.

TEST DIET : 6 boiled eggs, providing about 1800 mg of cholesterol and 36 gm of fat.

Postprandial samples were taken at one hour interval upto three hours.

PROTOCOL 8

Subjects : 2 (1 male and 1 female).

TEST DIET : 4 eggs albumin providing about 24 gm of fat and 24 gm of protein.

Postprandial samples were taken at one hour interval upto 3 hours.

PROTOCOL 9

Number of subjects : 3 (1 male and 1 female).

TEST DIET : 75 gm of glucose dissolved in water.

Postprandial samples were taken at 1, 2 and 3 hours.
PROTOCOL 10

Number of subjects: 2 male subjects.

**Test Diet**: 50 gm of pure (deai) ghee with 4 breads.

Postprandial samples were taken at first, second, third and fifth postprandial hours.

PROTOCOL 11

Number of subjects: 2 (both males).

**Test Diet**: 50 gm of saffola (Kardi) oil with 4 breads.

Postprandial samples were taken at one and three postprandial hours.

PROTOCOL 12

Number of subjects: 1 male subject.

**Test Diet**: Alcohol in the form of 100 ml whisky 43.0% v/v, 750 proof.

Postprandial samples were taken at one and three hours.

PROTOCOL 13

Number of subjects: 9 (male: female = 1:1).

**Test Diet**: 1 gm of crystalline cholesterol dissolved in 200 ml of milk.

Postprandial samples were taken at 1 & 3 hours.
PROTOCOL 14

Number of subjects: 2 (males; these were same subjects which were studied in protocol 3).

These subjects were asked to have 2 eggs plus 200 ml milk daily in their breakfast after single dose 'cholesterol tolerance test'. The second study was conducted after 15 days of first study.

TEST DIET: 3 eggs plus 250 ml of milk.

Two postprandial samples were taken at one and three hours.

For the case of study, subjects in these protocols were grouped as follows:

GROUP A: Subjects studied under protocol 1 and 2 (Test diet in the form of different amounts of butter).

GROUP B: Subjects studied under protocol 3 (Test diet in the form of single egg).

GROUP C: Subjects studied under protocols 4, 5, 6, and 7 (Test diet in the form of different amounts of egg cholesterol).

GROUP D: Subjects studied under protocols 10, 11, and 12 (Test diet in the form of miscellaneous food articles).

GROUP E: Subjects studied under protocol 13 (Test diet in the form of crystalline cholesterol).
Subjects in protocol 9 and 14 were the same and they were put in group C.

ASSESSMENT OF THE INDIVIDUAL RISK

Individual risk for developing atherosclerotic related disorder was also assessed by studying both basal lipid lipoprotein profile and test diet induced changes in HDL, LDL and VLDL.

1. Assessment of risk by studying fasting (basal) profile: For this fasting STC and LDL/HDL ratio in each individual was assessed and subjects were categorised in following groups.

a. Low Risk Group
   STC level less than 220 mg/dl and
   LDL/HDL ratio less than 3.

b. Border Line or Moderate Risk
   1. STC level more than 220 mg/dl or
   2. LDL/HDL ratio more than 3, if STC level is less than 220 mg/dl.

c. High Risk
   STC level more than 220 mg/dl and
   LDL/HDL ratio more than 3.

2. Evaluation of risk after giving test diet was also done: This was based on an arbitrary scale, where definite points were given on a definite change in quantum of LDL, VLDL and HDL. Following was the
<table>
<thead>
<tr>
<th>Percentage of rise in basal level of LDL, VLDL &amp; HDL</th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDL</td>
</tr>
<tr>
<td><strong>Upto 5</strong></td>
<td>0</td>
</tr>
<tr>
<td><strong>5 to 15</strong></td>
<td>+1</td>
</tr>
<tr>
<td><strong>15 to 20</strong></td>
<td>+2</td>
</tr>
<tr>
<td><strong>30 to 45</strong></td>
<td>+3</td>
</tr>
<tr>
<td><strong>45 to 60</strong></td>
<td>+4</td>
</tr>
</tbody>
</table>

| Percentage of fall in basal level in LDL, VLDL & HDL |
|---------------------------------|---------|
| **Upto 5**                      | 0       | 0     | 0     |
| **5 to 15**                     | -1      | +1    | -0.5  |
| **15 to 20**                    | -2      | +2    | -1.0  |
| **30 to 45**                    | -3      | +3    | -1.5  |
| **45 to 60**                    | -4      | +4    | -2    |