DISCUSSION
DISCUSSION

The present study has been done in light of the evaluation of the changes of serum lipid lipoprotein profile induced by high cholesterol fat diet (HCPD). The changes, whether, can reflect any indication regarding subjects being prone of future hyperlipidaemia, is a matter of debate. Many studies have been done, among which Deynen and Katan (1985) suggested that the variability in lipid profile is not a feature over a span for at least 6 years. Whereas literature of Messinger et al (1950), Conner (1968), Mattson, Erickson and Kligman (1972), Flayen et al (1979), Patsch et al (1980), Key et al (1956), Ahrens et al (1967), Robert and McMurray et al, (1981), Sacks et al (1984), Minnesota Univ Study, reveals that fluctuation in lipid profile levels are dependent on diet pattern. Therefore, it may be quite impossible to decide whether changes are marked or stationary and thus it may be extremely difficult to make any one common consensus for same.

Whatever, may be the outcome, one has to select a reliable procedure for understanding the pattern of lipid profile level changes and its implications. In order to study the procedure, selection of subjects is very essential. Whether these subjects may be randomly picked up or selected under certain specified conditions is to be finalized.
SELECTION OF SUBJECT

In present study, the subjects were chosen with the idea, that the levels of lipid lipoproteins profile may be understood in light of previous studies (Arora et al., 1985, 1986, 1989 and 1989) and thus analysis may be made from these. Thus only those healthy subjects were chosen who were subjects of previous studies, whether only once or more. All the subjects selected were studied within last 6-7 years, with the idea, that they may be traced easily. Since selection was limited, we opted for any number of subjects which we could finally assess to within the campus of study of this medical institution. Many subjects who were previously studied and had left the institution, mainly medical graduates and post graduates were dropped out. This was done so because it have been impossible to keep a check over their regular ingestion of HCFD, and a proper method of collection from distant placed and analysis of their blood samples for lipid profiles also could not have been possible. Therefore any such type of factor which may induce doubt was thus dropped and then the selection was made under strict rules. More over in contrast to previous studies, we did not opt for those subjects i.e. any attendant of the patient admitted in wards since these subjects, may not have had been taking their regular diet because of mere availability of same. If they had
missed previous days diet due to any reason, it would then give us altered fasting lipid-lipoprotein values on next day, to our view this would have been false.

**CHOICE OF HCFD**

The high cholesterol fat diet was given to the individuals in the form which was palatable and therefore slight alteration were done, keeping in view that the total amount of cholesterol and/or fat was nearly equal to the original diet.

Now the biggest problem is to really choose a HCFD. How to define, then, a HCFD? For this we screened the normal routine diet of every subject and took out a total amount of cholesterol, the form in which cholesterol was present, the total amount of fat and the quality of fat, and thus formulated a diet containing these much higher than their usual diet pattern. Since majority of subject which we had studied were vegetarians and those who took nonvegetarian diet was only once or twice in fifteen days, thus we found the egg was one common denominator of highest cholesterol value in routine diet. This egg, too, was not taken regularly. Thus the total cholesterol value in our subjects ranged from 200-500 mg/dl/day. Thus we opted for a diet of at least 750 mg% of cholesterol in a single ingested diet which could easily be defined as a HCFD is the subjects, which was apart from the cholesterol/fat taken in diet in rest of the day.
The detailing of the fat content in usual diet of subjects had also been done. The quality of fat (Messinger et al, 1950; Ahrens et al, 1967; Shore, Kausand Butlerfield, 1981 and Beynen and Katan, 1985) was mainly studied, since saturated fat, tend to increase the STC & LDL in blood lipid profile while polyunsaturated fats tends to decrease these same profiles and tends to increase HDL. We opted for high fat content mainly of saturated fats in HCFD. This was mainly done with idea of influencing the lipid profile to the maximum extent within the amount of specified diet. Our study had also included similar quality of fat. Amul butter in HCFD, as in previous studies, but goes quite in contrast to study of Beynen and Katan (1985) who instead of giving HCFD had selected for cessation of HCFD and then studying the levels of lipid changes thus induced.

Every subject was given diet nearly with same protocol as in previous studies. Those subjects who had been studied many times previously, were given diet quite similar to the oldest protocol. In case, subject was reluctant then the choice was given in other forms of diet. Individual who routinely took eggs previously but presently not so, was not forced to take diet rich in eggs. Thus finally we could group all individuals by giving three types of diet, HCFD-I, HCFD-II and HCFD-III under strict supervision.
The diet in some studies was a multidose (prolonged feeding) for seven days in contrast to study of Arora and Mangal et al (1989) who gave only single dose. The individuals who are at risk of hyperlipidaemia are constantly taking HCFD rather than occasionally (as it would amount to be the same as multidose feeding trial).

The protocol which was finally used in this study was an unique one i.e. combination of single dose feeding as well as multidose (prolonged feeding). This was just to compare the post prandial (1 hour and 3 hour) to previous ones and those of prolonged feeding. It had an upper hand over any of the previous studies, since it gave us whole pattern of understanding of both post prandial and prolonged feeding which none other could do so. The individual subjects response could be understood as a whole.

Our protocol goes quite in contrast to those of Beynen and Katan (1985) who instead of giving HCFD had studied by cessation of HCFD. We think that the basic mistake which this study can make is to make conclusion of lipid levels reproducibility which may not give the indication of true risk subjects. This comment has been made because cessation of HCFD may give stable STC, LDL, HDL and STG levels which may be the lowest possible in any given individual. Thus those with no significant fall of STC and LDL in this study may mark subjects of
high risk. But this study bears a lot of chance to skip those subjects who may show this fall and still may be potent pre-hyperlipidaemic. Thus instituting HCFD may give a better response in various individuals and withdrawal of HCFD would give the fall patterns, which may be quite similar to Beynen and Katan study. Thus our protocol of HCFD covers all aspects and covers the pitfalls of all these quoted studies.

Any drastic changes in protocol could not be made because the results we opted for were based on previously studied subjects on similar protocols. Any change in protocol could have induced changes which may be quite difficult to explain and thus the study of reproducibility would have had been futile. Moreover, our goal of screening those subjects who were prone to be pre-hyperlipidemics and its consequent complication might not have been reached for.

REPRODUCIBILITY

Reproducibility is to obtain, through a test procedure to the results of any study under similar protocols, matches to previous result or not. If it matches then the inference may be the same as in previous one, but if it changes then this change may be a pointer of progression towards any unforeseen disaster in future in those subjects.
Any claims of the results of reproducibility for screening risk subject at this stage may be bogus. The sensitivity and specificity of this mode can only be assessed by following a patient for decades in order to correlate the previous findings to development of the disease process. Well it is a long term follow up, yet not done. But with optimistic view we may hope this reproducibility to screen individuals at risk for future CAD and thus may point towards various factors, which if interfered at this appropriate stage, may prevent from any catastrophe leading to morbidity or mortality.

The study of Beynen and Katan (1985) for reproducibility has not involved any point for screening high risk individuals. Instead the study proposed to seen the pattern of change in a group of individuals to cessation of HCFD, to lipid profiles, and thus seems to be incomplete in itself. The purpose of reproducibility test does not end just to study changing pattern but also in infer from change the probable outcome to any disease process which may presently be progressing subclinically. Moreover, any of the previous studies from which we had compared results also never proposed to screen these individuals. All these studies were done to simply evaluate the change in lipid profile in healthy young old or diseased individuals. In diseased individuals any change encountered may be quite difficult to explain in light of disease itself while in
healthy ones, the change may simply depict a normal biochemical process of diet induced changes and nothing more. Moreover those subjects who had been restudied over these years (1985-89) were never evaluated in light of risk but instead explanation were given for change of lipid profile with changing factors i.e. age, weight, dietary habits etc, which again seems to be incomplete in itself.

This present study for reproducibility has tried to highlight the results in view of any cause responsible for changing of pattern if any, as well as the probable outcome of this pattern. Thus we have indicated high risk group individuals, low risk group which completes the purpose of study as a whole. Whether the results thus made, fit true in subjects in future is a matter of debate and its validity in due course of time.

Eventually, this reproducibility, has been chosen with expectation that the individual over a reasonable period of time, may give us similar evolving pattern of lipid profiles induced by MCFD as well as an indications to this changing pattern attributable to any associated factor and thus point towards any disease process. Moreover changes in real basal values may also be helpful to assess the same. Therefore the study of pattern of change thus differs from study of real values
of lipid profiles, meaning thereby, that the real values may be elevated while changing pattern after ICFD be same (rise or fall or mixed). Moreover real values may be similar to previous studies but changing patterns may differ widely. Therefore the change in individual parameters of weight, height, dietary patterns, fat consumption etc. may be better studied in light of these changes. The parameters of the subjects are bound to change over time, whatever it may be and thus any subject to be studied over a time period frame would have these essential changes to variable extent.

**CHANGES IN LIPID LIPOPROTEIN PROFILE**

One of the greatest questionare remains that why, if all, the change should occur. The studies of Messinger (1950), Ahrens (1967), Packard (1983), Sacks (1984), Beynen and Katan (1985), Williams, Conner, Cook Karvinen, Lin and Cook, Riddle showed that hign doses of crystalline cholesterol (300mg/day) when fed to subjects failed to show any significant change in serum elevations of lipid. While in contrast to this the cholesterol rich cream and egg yolk induced remarkable change (10–20%). This study indicates that the quality of food rather than quantity has greater effect on the serum lipid profiles. The similar observation was studied by Williams and Conner et al (1964). The study of Keys et al (1956) had results that alteration in diet by doubling and
halving their cholesterol intake failed to show any response in serum cholesterol level in 4-12 months while rest of their diet was more or less constant.

The changes in our study were having quite similar pattern of changes as in old studied rather than any major change in absolute values excepting in two subjects which we could explain by use of oral contraceptives in one female, and use of tobacco for chewing as well as smoking in the other.

Beynen and Katan (1985) from their studies of reproducibility of variation between humans and changes in serum cholesterol by cessation of egg consumption concluded that part of the cholesterolemic response to dietary cholesterol in man is individually determined and stable for at least 6 years. Our study showed similar pattern in most subjects. But this reproducibility conclusion has not ended here.

In our study, changes did occur because of the quality of HCFD ears such that alimentary absorption was maximum (Edward Cook and Riddel).

One drawback in the analysis of this study had been of not of using proper statistical methods to standardise the results and compare from the previous statistical data. Thus the results, lacking a concrete base of statistical argument may not acceptable partially or wholly by other worker in the same field worldwide. However, it was also impossible to put these statistical methods on individual subjects and study the changes in
this light.

However, the changes in the lipid profile in this study can be attributed to those factors which are well known to favour hyperlipidaemia over a course of time in any individual. These factors which induces risk have changed in those healthy individuals, who had shown, notable changes in lipid profile. To explain this for e.g. the change of marital status eventually has led to marked decrease in physical activity due to increased mobility on petrol vehicles, increase in the standard and the quality of diet, increase in the outings along with taking rich dinner in hotels or restaurants, use of oral contraceptives etc. Thus the change of factors has shown linear relationship to the changes in lipid profile of these individuals. Those who during the period of past and present study, had started smoking, chewing tobacco for 1-2 years have shown similar change. Another drawback in the present study was that the analysis was based on just 15 days trial of HCPD. (Ingestion and withdrawal 7 days each). This short period may not be conducive for the proper interpretation of the destined true risk in the individual. Moreover this period is too short to freely accept any major change in the normal routine diet of the individual during study period. It may influence more over the lipid profile in comparison to the study over many months duration.
Now let us come to argue the sense of getting changes of post prandial (single dose trial) or of multidose (prolonged feeding) trial.

It may be well remembered that atherosclerosis may even be a post prandial phenomenon (Zilversmit, 1983) and can be best judged by post prandial studies. However, one or two studies over an individual may not suffice and thus the series of studies over many years (probably more than a decade of every individual may be required to finally conclude the risk suggested by post prandial change.

The prolonged feeding would always necessarily indicate the persistent rise of fasting basal values which are primarily dependent on sustained high cholesterol fat intake. This is only when the routine diet of the subject has HCFD intake and thus becomes the victim of the risk. Thus a combined form of analysis for both post prandial and prolonged feeding induced changes would be a better marker of the ominous pointer towards the future risk.

The lipid lipoproteins profile changes have largely shown a definite pattern of changes, but in few erratic changes have occurred more in STG and LDL, which may not be probably explainable at present. The change can be assessed both qualitatively and quantitatively. Quantitative assessment has been done by studying the
patterns of changes in comparison to the previous studies. Whether this patterns of change after HCFD has been consistently the same or shown a rise or fall or admixture of two. Quantitative assessment has been done by comparing the absolute values of lipids and those which has changed after HCFD, and whether it showed similar or disimilar changes. Even this form of assessment has incorporated LDL/HDL ratio, which may suggests a greater importance in changes which otherwise does not seem to have.

In light of above arguments let us discuss the various changes in this study. All the changes have been compared with older studies and explanation been put forth in light of possible association of the various factors contributing for the same.

A. **SERUM TOTAL CHOLESTEROL**

In the present study all the values of basal STC in all the individuals were within the normal range of Lipid Research Clinics Data, 1980 and also to that of mathur et al (1960) and Dutta et al (1967).

In few individuals there was a drastic rise of STC after HCFD. However, the rise in fasting basal value was only in two cases. This could be explained because one subject had got married and thus leading to decreased physical exertion due to mobilization over petrol
vehicles (consistent with the findings of Hartong et al, 1983 and Siltanen et al, 1982), intake of rich chole-
terol/fat/dietary habit (consistent with finding in studies of Beynen and Katan (1982) and Packard et al, 1983). However, the quality of fat also changed (Ahrens et al, 1967) and the long chain saturated fatty acid were mostly part of edible fat in the diet. The change in the second subject has due to the smoking of 5-10 bidi/day along with chewing of tobacco.

Studies of Sacks and Castelli et al (1975), West and Hayes et al (1968) showed that the basal STC in vegetarians had been low, as compared to non vegetarian population. These similar results were found in subjects who were vegetarian in our study.

The results of STC were quite in contrast after 7 days of HCFB from those of Arora and Kumar (1988). Their eight day fasting value showed a decrease, while in our study all values showed a tendency to rise, since the previous study has incorporated crystalline cholesterol in HCFD while we included egg/butter with or without crystalline cholesterol. Moreover, on going through study of Williams and Conner et al (1964) found that the subjects who were fed high cholesterol egg diet showed a mean rise of 69% in STC while the change was only 19% in same individuals when given crystalline cholesterol diet two and half times the egg cholesterol diet, thus
it was inferred that egg cholesterol was better absorbed through the gut than crystalline cholesterol and the ratio of absorption of both was approximate 4:1. Thus our study showed changes consistent to that of Williams and Conner (1964) and also explained why the study of Kumar (1988) showed the decrease in STC value.

The study of Beynen and Katan (1985) also showed that the same individual who were asked to take low cholesterol free for 15 days followed by high cholesterol diet for another 10 days showed that in former the fall in STC was significant from 191±27 to 168±21 mg/dl while in latter the rise was also significant to 13%. Thus this study also confirms the patterns of rise in our study. However, the study of Sacks et al (1984) observed that adding egg, which increased dietary cholesterol from 97 to 318 mg per day induced changes in other lipid profile except STC which showed insignificant change.

In the study of Arora and Pandey (1989) the results were comparable to the present. This is because the protocol pattern is quite similar in both the studies. It is also thus concluded that the changes over this duration of three years are not variable and thus finds its truth to the results of study of Beynen and Katan (1985).
The study of Arora and Gupta G. et al (1985) also had similar pattern of change on all the three individuals as that of present study. But post prandial studies show that the change in STC was not present to suggest any real change. This could be explained that the endogenous synthesis of cholesterol may be inhibited by some neuronal or biochemical means when the elderly subjects were given HCFD. However, the shift of intravascular cholesterol towards the extravascular sites, and in the intimal layer of endothelial lining may also contribute to similar changes. The STC values in all individuals gradually touched baseline values after HCFD withdrawal whereas the previous study did not show similar pattern. It may be quite difficult to explain this contradiction. It seems that there may be no reasons why the value should be persistently raised after withdrawal of HCFD after 7 days. If the endogenous mechanism in elderly are so active as described above, then the changes in STC should be quickly governed by them inducing normalcy in a short period of time and thus explain the present findings.

When our study is compared for five subjects with that of Arora and Mangal et al (1989) no difference of pattern could be elicited in post prandial values. This again is in consistent with finding of Beynen and Katan (1985). While comparing with those of Arora and
Gupta VK (1986) for 8 subjects we found a contradictory finding for basal fasting values. It was that our values for STC were high than that of previous studies. This could be explained by hormonal effect on puberty (Beagtehole et al, 1980). Values of the STC rises on puberty while in previous years studies of those individual below puberty group this showed lower fasting values. Moreover, the physical activity is much more in the prepubertal age group which might again explain these lower values in 1986. Those subjects of this group who were previously unmarried, again showed higher fasting STC value after marriage, the explanation for which had earlier been given. Moreover the habit for tobacco chewing has also come up as individual grew to marriage age group thus contributing for same.

B. **HIGH DENSITY LIPOPROTEINS (HDL)**

All the values in our study were well within normal range as in Lipid Research Clinics data (1980).

Our study over three subjects who had earlier been studied showed changes of HDL fasting basal values to minimal and the pattern of change was also quite similar to Arora and Gupta G et al (1985). The rise was not enough to account for and again favour the findings of Beynen and Katan (1985). Why HDL values should not show any change by HCPD diet. at this elderly age, is appealing. One must also be very clear in itself that the
protective mechanism comes to a stand still at this elderly age and thus diet induced changes are minimum for this protective factor. Moreover any factor at this age do not change to appreciable amount since every routine or work/diet/habit is set and is least vulnerable to any deviation and also had been same since last many years.

The study of Arora and Gupta VK (1986) showed that HDL values showed a high rise which goes against to present study findings. Why this should be so become a matter of discussion? The age at that time, for eight subjects ranged from 8-16 years and in present stage is from 14 to 23 years. There was marked changes in weight and height along with changes in dietary habits, decreased physical activity especially in those who had left studies in between and took up jobs of driver, electrician etc. Thus HDL changes after HCFD was towards a low rise in present study in comparison towards drastic rise in 86 study. However, the basal HDL values did show rise in present studies being in accordance with the data of Lipid Research Clinic (1980). The HDL has not been reported to have any definite correlation to change in height, weight and other factors but above do show relation to changes.

In rest of studies of Kumar (1988) Pandey (1989) and Mangal (1989) which included the remaining subjects, in whom few were repeated, showed variable changes to HDL. This indicated that HDL is in itself is incapable of
giving access to the understanding of the importance of any change. Whatever may be so the changes of HDL is dependent by the type of HCFD. This because in the study where crystalline cholesterol has been given the HDL basal values are higher than in those where egg cholesterol forms the main part of protocol. Since HDL is endogenously produced in gut/liver and peripheral break down of VLDL, thus diet induced changes are not easily appreciable. This is an very interesting fact to remember and would thus highlight that HDL values alone may not be of any use to study and screen the risk ones. Since every individual has his own level of basal lipid profile. The HDL in different individual show a large variation.

C. **SERUM TRIGLYCERIDES (STG)**

All the serum triglyceride values are well within normal levels as in lipid research Clinics data (1980).

The serum triglyceride values in earlier studies showed that it decreased by HCrD where crystalline cholesterol has been used in diet protocol but increased in protocols using egg cholesterol. In post prandial studies the values increased but was variable in degree of rise. In present study the protocol was such that the post prandial showed marked increase and multidose trial (prolonged feeding) showed only slightly higher
levels than basal. Is STG levels sensitive to dietary changes. If yes, it is quite easy to understand since STG is the main mode of transport of triglyceride and cholesterol from gut to site of metabolism, thus is, bound to show extreme changes post prandially after HCFD. If gut absorption is proper and the material is conducive for being absorbed, STG changes reflect accordingly. Thus for crystalline cholesterol diet, the STG may not show large variations in its level or may even fall since crystalline form is very poorly absorbed and is largely passed through stools. Those individual prone for hyper triglyceridemic shows tendency for basal STG to be high and post prandial rise may be drastic. Since STG is main substrate for LDL and VLDL, the changes in STG reflects similar changes in LDL which is a bad marker in any individual if it shows any rise in basal fasting levels.

D. **SERUM LOW DENSITY LIPOPROTEINS (LDL)**

All the levels of LDL are within normal range as specified by data of Lipid Research Clinics (1980).

The LDL levels in study of 1985 showed a marked rise. The subjects were middle aged people who showed a greater change on ingestion of HCFD (including eggs and crystalline cholesterol). Since the total cholesterol value was high thus changes were also marked. The LDL levels in study of Arora and Gupta VK (1986) showed small variation and was variable from subject to
subject. This variability could be attributed to younger age groups and hyper or hyporesponders which contributed to these. Since LDL rise is a pointer towards unfavourable outcomes. The changes were distinctly marked in elderly subjects.

In present study LDL values were quite variable. Thus LDL alone would also not suffice to explain the risk of the individual.

For this reason, we finally picked up the role of LDL/HDL ratio in combination with changes of STC, LDL and HDL and finally grouped the twenty three subjects into three broad groups:

1. High risk group.
2. Low risk groups and
3. Moderate risk group.

LDL/HDL ratio was taken as follows:
if $\leq 2$ is normal.
if $7$ $2$ to $\leq 3$ is low risk.
if $7$ $3$ to $\leq 4$ is moderate risk.
if $\geq 4$ is high risk.

These groups have been made on basis of both qualitative and quantitative assessment of each individual, quantitative assessment has been done after studying changes of STC, HDL, LDL, LDL/HDL ratio. Qualitative assessment has been done by studying the
patterns of changes in comparison to previous studies. This overall mode of selection has thus made us possible to make real use of this study of the reproducibility of changes induced in lipid lipoprotein profile after high cholesterol fat diet in these healthy individuals.

**High Risk Group**

These included six subjects (Table 1, 2, 3, 10, 21 and 23). All these subjects showed drastic rise of STC after HCFD. LDL/HDL was more than 4.

**Low Risk Group**

These included eight subjects (Table 5, 13, 14, 16, 17, 18, 19 and 20). There was rise in STC after HCFD, but HDL showed rise in majority and LDL remains largely the same. The LDL/HDL ratio thus was less than 3 in all cases.

**Moderate Risk Group**

This included nine subjects (Table 4, 6, 7, 8, 9, 11, 12, 15 and 22). The STC value is variably changing in single dose trial but a consistent rise in multidose feeding LDL is largely the same. HDL presented variably. LDL/HDL ratio was such that older studies showed less than 2 while present studies showed higher ratio of 73.

Thus from afore mentioned details it may be quite clear that the risk for an individual for future possible catastrophes due to atherosclerosis is not
reflected by any single changing parameters of lipid lipoprotein profile. Instead a combination of these has specified it more clearly.

Any positive family history of CAD, sedentary life style, smoking, high fat cholesterol diet, and tobacco chewing adds to the risk of an individual.

To make an overall view, it may be reasonably understood that the great complexities of these lipid lipoprotein profile basal values and its changes in response to diet has varying claims by various studies. Moreover, the simple changes in individuals may not give us any fruitful result. However, analysis in light of linking the various profiles may guide us to screen the risk group subjects. This may be a petty claims of ours, which in order to be a righteous claim, has to stand to itself to the test of time and would require a decade or more for it.

The protocol used in this study, to over view is also not very satisfying. The protocol should use a still higher HCFD and the time frame of study should be extended from fifteen days, as in present work, to at least 3 months, so that monthly changes could be studied and a definite pattern may thus be ascertained. Secondly the number of subjects could be decreased so as to save the expenses of the state. However, if statistical integration is to be done then the number of subjects would
have to be increased within reasonable limits, so as to make concrete results.

Many fallacies may be there in this study. It is quite possible, that subjects, among whom few were medical students, might have had been waking till late night for studies and thus taking help of tea or coffee for the same and might be contributing to the false results. Moreover the set up of laboratory in our department is not conducive for these type of studies. Since these are very sensitive to many factors e.g. environmental temperature itself, thus airconditioned laboratory with full fledged automatic equipments, analysers etc, should be set up before embarking on it. The use of manual pipettes, fluctuation of voltage in main electric line may all contribute to fallacies. Since the measurements while testing samples ranges in micrograms, the accuracy of measurement should be repeatedly confirmed. The calorimeter would give in appropriate readings in course of voltage fluctuations, and thus servo control voltage stabilizers should be used for it. Yet in so much of compromised situations we have tried our level best to keep up the accuracy as much possible and put forth these results with confidence.