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EFFECT OF ORAL FAT LOAD ON SERUM LIPIDS IN NON-INSULIN-DEPENDENT DIABETES MELLITUS SUBJECTS

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Abstract:
Background and Objective: Modest elevation of plasma triglycerides and lowering of high-density lipoprotein cholesterol are the characteristic features of dyslipidemia in non-insulin-dependent diabetes mellitus and insulin resistance. This study aim of the present study is to assess the effect of oral fat load on serum lipids in NIDDM subjects
Materials and Methods: This study was conducted at the Darbhanga Medical College and Hospital, Laheriasarai, and comprised 21 controlled diabetes and 11 age-sex-matched controlled subjects. All diabetes were the cases of non-insulin-dependent diabetic patients with no history of Ischemic Heart Disease, non-hypertensive, and controlled either on oral hypoglycemics or only on diet control. The institutional research committee approved the research protocol.
Results: Fat load is inefficiently metabolized in diabetic patients, even when the disease is mild and controlled. The study also confirms earlier observations that different factors influence post-prandial lipemia in different subsets of individuals.
Conclusion: Based on the previous studies and based on the results of the present study it can be concluded that such a protocol might help in detecting persons in high-risk groups even when their fasting values are within normal range.

Keywords: non-insulin-dependent diabetes mellitus, Incremental AUC, triglyceride

Introduction:

Diabetes mellitus is specified by hyperglycemia composed of biochemical alterations of glucose and lipid peroxidation (1). Increased activity of free radical-induced lipid peroxidation and buildup of lipid peroxidation products are linked to some diabetic problems (2). Lipid peroxidation is a free radical-related process that can be damaging because it causes membrane, lipid, and other cell components to be disrupted. It has been linked to several diseases, including carcinogenesis, atherosclerosis, and hypertension (3). It's also implicated in oxidative stress, which plays a big part in diabetes mellitus etiology (4). Antioxidant enzymes play a vital function in scavenging reactive oxygen species, which helps to keep lipid peroxidation under control (3). Furthermore, dyslipidemia

in non-insulin-dependent diabetes mellitus (NIDDM) and insulin resistance is characterized by a slight increase in plasma triglycerides and a decrease in high-density lipoprotein (HDL) cholesterol. These anomalies are also identified in glucose-tolerant first-degree relatives of NIDDM patients, which is very intriguing (5). Both of these characteristics are thought to play a role in the well-documented elevated risk of coronary artery disease (CAD) in people with NIDDM (6-8). The underlying mechanisms, however, are still being debated. The key question is whether low HDL and/or high triglycerides are causative variables in their own right. The interplay between very-low-density lipoprotein (VLDL) and HDL metabolism has recently been recognized as tight, and these two components are physiologically dependent on each other. Although there are as yet no interventional trials with lipid-lowering diets or drugs in diabetic patients to judge the impact on vascular disease. National and international bodies have furnished guidelines for the identification and treatment of lipid disorders in diabetes in the hope of reducing the huge toll of vascular disease in these patients. Thus, the aim of the present study is to assess the effect of oral fat load on serum lipids in NIDDM subjects

Material and methods:

This study was conducted at the Darbhanga Medical College and Hospital, Laheriasarai, and comprised 21 controlled diabetes and 11 age-sex-matched controlled subjects. All diabetes were the cases of non-insulin-dependent diabetic patients (NIDDM) with no history of Ischemic Heart Disease, nonhypertensive, and controlled either on oral hypoglycemics or only on diet control. Normal healthy subjects as a control were included from the general population and were matched for age with the Diabetic patients. Written consent was taken from enrolled subjects after being informed about the purpose and objective of the study. The institutional research committee approved the research protocol and procedures applied in the study.

Procedure

Patients with the above-mentioned criteria were identified and a complete history was taken. A physical examination was done including a fundus examination. Then the patients were subjected to the following investigations:

- Complete Haemogram
- Blood sugar Fasting and Post-prandial
- Serum urea and creatinine

Sample collection

All the subjects were asked to have their dinner at 7 PM on the previous night and not to take anything after this. The next morning, 14 hours of fasting samples were taken in recumbent posture without producing venous stasis. By the same prick blood was also taken out for the estimation of blood sugar and blood urea, TLC, DLC, Hb, ESR. The serum was separated from the blood within 4 hours by centrifuging the sample and the following tests were done.

- Serum total cholesterol (STC)
- Serum triglyceride (STG) Kit methad (Acetyl acetamino method).
- Serum High Density lipoprotein HDL). This test was conducted by the kit system (provided by the Ethnor)
- Serum VLDL and LDL estimation. The test was done by formula given by Friedwald et al (1972).

Protocol

All subjects were called with overnight fasting and on their first visit single fasting sample for lipid estimations and other routine tests was drawn. Subsequently, patients were called the following week with overnight fasting and again single sample for lipid estimation was withdrawn. During the second week of the study patients were given Placebo tablets and on the final day of the study four blood samples at 0,4,8 & 24 hours were taken. After the fasting sample patients were given an oral fat load

consisting of 50g of Butter,40g of processed cheese, 50g of milk cream, and 4 slices of bread. Four hours following this another sample was drawn and subjects were asked to consume fat-free meals consisting of seasonal fruits and a soft drink. The normal meal was allowed between 8-hour and 24-hour periods. During the study period, patients were allowed to take their usual drug if any.
The mean response of oral fat load in each individual was assessed by calculating the Area Under Curve (AUC) for a 0 to 24-hour period. AUC was calculated by using the Trapezoid rule. For a single trapezoid. Area of Trapezoid =(Sum of parallel limbs x Distance between limbs)/2, Since we have fasting, 4-hour, 8 hour and 24-hour values with unequal time distance, the formula for Area Under Curve in the present study as derived from the above equation is as :
AUC 0-24 = 2x(fasting+4hrs) + 2x(8hrs +4 hrs) +8x (24hrs +8hrs)
After obtaining AUC, Incremental Area 0-24 was calculated by formula.
Incremental Area 0-24 =AUC - (fasting x 24). (9)

Statistical analysis:
Statistical analysis was done using MS Excel, Version. 7.0 software. Under descriptive statistics, mean and standard deviation were calculated for continuous data. The difference in the mean value between groups was analyzed using the test of significance. An Independent sample t-test was used for the statistical analysis of variables between all diabetic patients and Controls. P value <0.05 was taken as significant.

Results:
Age and BMI assessment between case and control were shown in Table 1. Average ages were 60.38 years and 55.18 years in case and control respectively. The variation of age in the case group was lower as compared to the control group. A statistically significant difference was found between age in both groups. The average BMI level was 23.2 kg/m2 in in case group. (table 1)

Table 1: Subjects baseline physical characteristics

Parameters	**Control (n=11)**	**Case (n=21)**	**P value**
	Mean ± SD	**Mean ± SD**	
Age (years)	55.18 ± 8.70	60.38 ±5.66	0.02*
BMI (kg/m2)	24.70 ± 1.87	23.2 ± 12.86	0.05*

*Independent t-test

Table 2 illustrates the average and variation of baseline clinical characteristics and comparative AUC 0-24 and Incremental AUC 0-24 between NIDDM and Control subjects. As is visible from Table 2, there is a marked difference between the two groups in both Incremental AUC and AUC of triglycerides. In the diabetics, Incremental AUC was much greater as compared to that of the control population. These changes between the two groups were statistically significant. Not much changes were observed in serum cholesterol and HDL levels. LDL showed the tendency to decrease with fat load. (Table 2)

Table 2: Subjects baseline clinical characteristics

Parameter	**Control (n=11)**				**Case (n=21)**				**AUC**		**Increment AUC**	
	Mean ± SD				**Mean ± SD**							
	Fasting	4Hrs	8Hrs	24Hrs	Fasting	4Hrs	8Hrs	24Hrs	Control	Case	Control	Case
TG	128.2±33.4	178.6± 25.4	210.0 ± 521	134.8± 29.3	139.1±46.4	199.5±103.4	257.1 ±110.1	169.5±128.3	4133	4992	1058.47	1654
CHO	149.1±16.0	169.5± 15.2	151.2 ±9.1	151.1± 10.3	153.2±27.2	161.5± 30.6	157.2 ±28.9	158.3± 25.3	3690.93	3786	125.34	105
HDL	42.1±7.0	43.5± 8.2	41.2 ±5.1	42.1± 5.8	38.1±6.5	38.5± 8.1	38.2 ±9.0	40.5± 6.8	1006	936	12.90	14
LDL	80.1±20.5	91.2± 19.2	68.3 ±10.6	82.8± 14.8	87.2±22.5	83.2± 29.3	68.2 ±24.3	84.6± 27.5	1858.27	1851	73.44	-240

Table 3 shows compression between the Incremental AUC of the NIDDM group with a CHO/HDL ratio of >4.5 to that of the group with a CHO/HDL ratio of 4.5. As evident in the table, there is a

marked difference between the Incremental AUC of triglyceride in both groups, with high-risk patients i.e. CHO/HDL >4.5 have got steep rise in Incremental AUC as compared to that of the comparatively low-risk group amongst diabetics. The high-risk NIDDM group is showing markedly increased Incremental AUC of triglyceride as compared to that of the high-risk Control group. This difference was statistically significant with a P value of <0.05. There is also an evident difference between the two groups in the case of Incremental AUC of cholesterol and that of LDL. (Table 3)

Table 3: Comparison of NIDDM and control subjects with CHO/HDL less than and greater than 4.5 mg/dL

Incremental AUC	Case		Control
	CHO/HDL < 4.5 mg/dL	CHO/HDL > 4.5 mg/dL	CHO/HDL > 4.5 mg/dL
TG	1418.43	2657	1398
CHO	8.19	518.5	-168
HDL	-7.37	106.6	57
LDL	-268	-119.6	-504

Incremental AUC in the high-risk group having HDLc <30 Incremental AUC of triglyceride was markedly higher as compared with that in comparatively lower-risk group P value being < 0.05. Decrease in LDL Incremental AUC values are much more marked in the lower-risk group. The mean Incremental AUC of triglyceride in both the groups is different and in the group with initial fasting triglyceride >150 Incremental AUC is statistically and significantly higher as compared to the group with initial fasting values of <150. The number of patients in the group with triglyceride less than 150 was 14 and that with triglyceride more than 150 was 7. (Table 4)

Table 4: Comparison of mean incremental AUC of TG and HDL in both groups with initial fasting

Incremental AUC	The group having fasting HDL		The group having fasting TG	
	< 30 mg/dL	> 30 mg/dL	< 150 mg/dL	> 150 mg/dL
TG	3171	1495	1070.8	2821
CHO	784	34	104.6	107
HDL	162	-1	6.7	30
LDL	-12	-264	116.2	-487

Figure 1: Comparison of NIDDM subjects based on family history

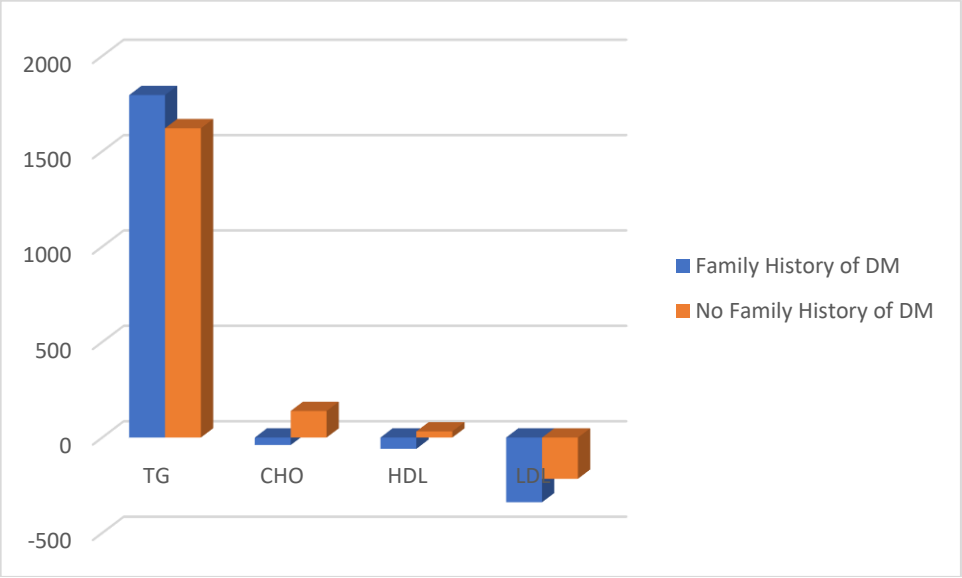

Figure 1 shows the Comparisons among the diabetic group based on Family history of Diabetes Mellitus and Ischemic Heart Disease. Since no patient had a positive family history of Ischemic Heart Disease, that correlation could not be obtained. Out of 21 diabetics, only four patients had a positive

family history of Diabetes Mellitus. Since the sample size was inadequate, no statistical significance could be obtained between the two groups. (Figure 1)

Figure 2: Comparison of NIDDM subjects based on their Dietetic habit

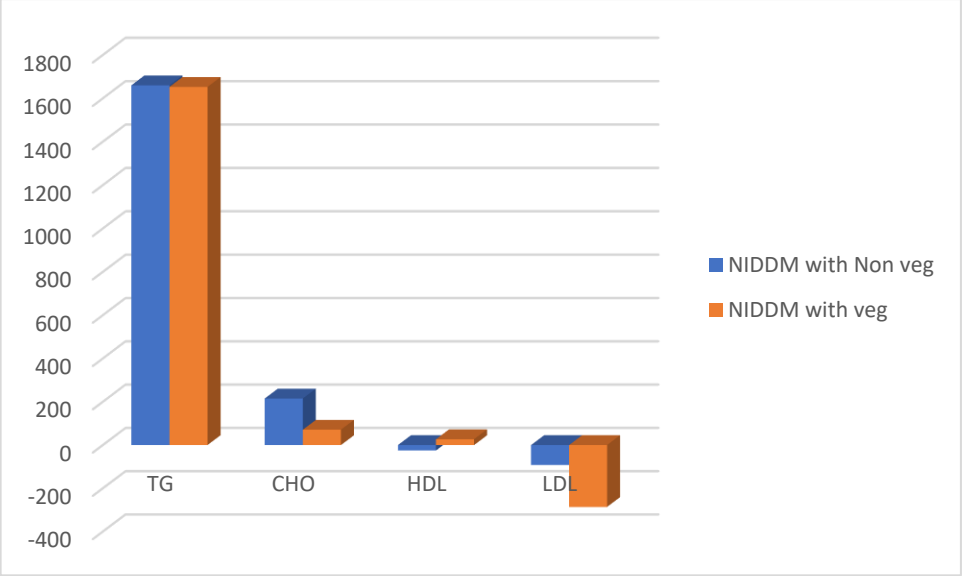

Figure 2 shows that the NIDDM subjects compared based on their Dietetic habit revealed no statistical significance, except in the case of serum cholesterol which was comparatively higher amongst the red meat eaters. (figure 2)

Figure 3: Comparison of NIDDM subjects based on their BMI

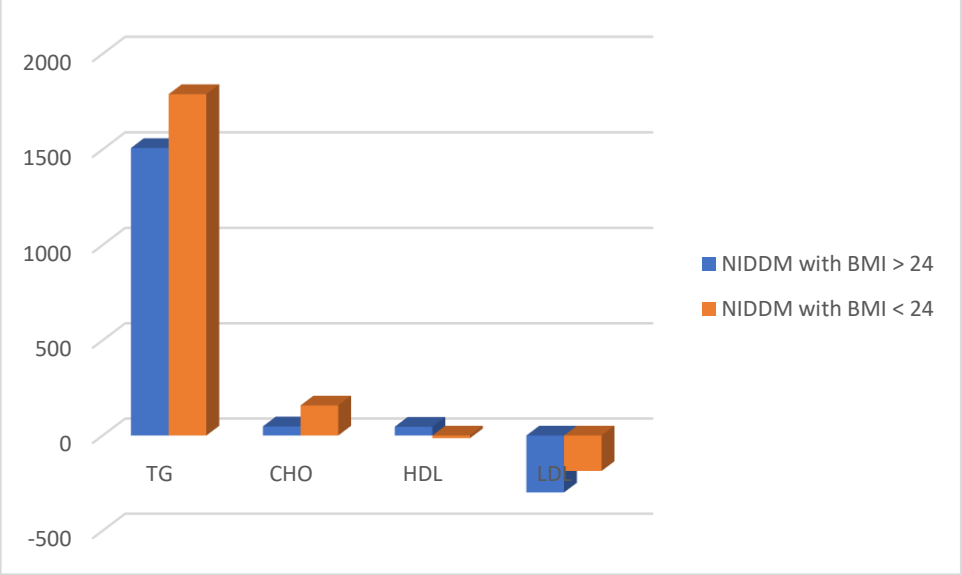

Figure 3 shows the comparison of NIDDM subjects based on their BMI. It is well known that obese persons whether diabetics or nondiabetics show a tendency towards hyperlipidemia. In our study, we could not find the same correlation between BMI and hyperlipidemia. This could be because of an inappropriate sample size. (figure 3)

Discussion:

In diabetes detection surveys, we take the help of glucose tolerance tests to diagnose diabetics. It is done either by measuring fasting plasma sugar level or by putting the glucose metabolism of suspected diabetics under stress as the glucose metabolism remains compromised, the plasma glucose level starts

behaving abnormally. In the same way, can there be any possibility to identify future hyperlipidemics either by measuring fasting lipid levels or if fasting lipid levels are found normal then by stressing the lipid metabolism of that high-risk person, by giving a high-fat cholesterol load? The concept of fat tolerance test is not new and has been largely overlooked in the past. The available on it indicates that estimation of post-prandial lipid levels may prove to be equally effective or rather better screening procedure as the fasting levels. Moreover, various metabolic ward studies have shown that a single fat load does not affect serum cholesterol levels appreciably though significant changes have been observed in triglyceride levels. There are some studies to suggest that prolonged high-fat and high-cholesterol feeding considerably affects all lipid and lipoprotein subfractions. Earlier studies using fat tolerance tests in non-diabetics suggest that a direct relationship exists between postprandial lipemia and fasting triglyceride levels and peak fat absorption is observed 3-4 hours after acute fat load, and levels of plasma triglyceride fall to fasting values within 8 hours (10). Post-prandial lipema is negatively correlated with plasma HD levels suggesting that atherogenic risk is reduced with increased triglyceride clearance. Post-prandial lipemia may be influenced by various (such as diabetes) in different subsets of individuals. In light of all these facts, the present study was carried out on Non Insulin Dependent Diabetic patients and on the subjects who did not have any disease as such. The results were interpreted to know whether these diabetics were at increased risk of CAD because of some lipid abnormalities at the basal stage and whether their response to oral fat load was different and unfavorable. Another important aspect of the present study was to develop some criteria to diagnose prelipidemics. In addition, Abayomi Akanaji et al (1990) reported no difference in fasting plasma total cholesterol and LDL between NIDDM and controls (11). The metabolism of HDL is very closely associated with the catabolism of TAG-rich lipoprotein (chylomicrons and VLDL)(12). Furthermore, a recent study on French diabetic patients showed that hypertriglyceridemia most potentially predicted coronary heart disease mortality (13). There may be two possible mechanisms for the increase of VLDL in type-2 diabetes. A clearance defect has been postulated with a decreased fractional catabolic rate for VLDL triglyceride (14). It is known that lipoprotein lipase is under hormonal regulation by insulin the activity of the enzyme is reduced in adipose tissue and muscles in uncontrolled diabetes (15). Clearance of triglycerides from the plasma is also impaired. The later effect is thought to be the result of decreased activity of the enzyme lipoproteins lipase which requires the presence of insulin for maintenance of adequate tissue level (17) We could not establish any defined significant relationship between STG of VLDL in alcoholics/non-alcoholic, vegetarians/nonvegetarians, and with persons having comparatively higher Body Mass Index. Since we had not included NIDDM patients taking Insulin a correlation with insulin could not be established, but researchers like Lewis et al (1991) have shown that STG was also reduced by insulin (18). Results from the present study indicate a significantly higher TC/HDLc ratio in diabetics as compared to the control group. The lipemic response was characterized in all subjects via several triglyceride measures which included peak triglyceride, percent increase in triglyceride Area Under the triglyceride curve, and the Incremental AUC above fasting triglyceride. In separate studies demonstrated in both healthy and cardiac populations that the magnitude of the lipemic response was directly related to the fasting triglyceride concentrations (19). However, Dutta observed that the hyperlipidemia seen in cardiac patients following a high-fat meal was not always associated with elevated fasting triglyceride (20). In our study, we found a significant positive correlation between fasting triglyceride concentration and peak triglyçeride values, supporting the findings of Denborough and Nestel (19). As is visible in the present study, the response of oral fat load in Diabetics (in the case of serum triglyceride) is much higher as compared to controls. It also depicts variability amongst the diabetic group. This post-prandial present study design used an 8-hour time point for monitoring the plasma post-prandial lipid peak, which consists of both intestinally derived chylomicrons and their remnants and hepatically derived VLDL. Studies have shown that this post-prandial lipid peak varies from 2-8 hours. The 9-hour point time was shown to be effective in measuring an individual's capacity to catabolize potentially atherogenic post-prandial lipoproteins (21,23). In addition, since the study was performed on an outpatient basis, this blood collection time did not interfere to any great degree with the normal life styles of this population or with the post-prandial protocol requirements of the

study. Lastly, our results indicate that a fat load is inefficiently metabolized in diabetic patients, even when the disease is mild and controlled. The study also confirms earlier observations that different factors influence post-prandial lipemia in different subsets of individuals.

Conclusion:

Based on the previous studies and based on the results of the present study it can be concluded that such a protocol might help in detecting persons in high-risk groups even when their fasting values are within normal range.

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