POST-PRANDIAL HYPERTRIGLYCERIDEMIA IN PATIENTS WITH TYPE 2 DIABETES MELLITUS WITH AND WITHOUT MACROVASCULAR DISEASE

By

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Under the guidance of

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2013

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I hereby declare that this dissertation titled **"POST-PRANDIAL HYPERTRIGLYCERIDEMIA IN PATIENTS WITH TYPE 2 DIABETES MELLITUS WITH AND WITHOUT MACROVASCULAR DISEASE"** is a bonafide and genuine research work carried out by me under the guidance and supervision of **Dr. PRABHAKAR K.** MD, Professor, Department of General Medicine, Sri. Devaraj Urs Medical College, Tamaka, Kolar.

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LIST OF ABBREVIATIONS

ANOVA	→	Analysis of Variants
APO	→	Apolipoprotien
BMI	→	Body Mass Index
BSA	→	Body Surface Area
CAD	→	Coronary Artery Disease
CETP	→	Cholesterol Ester Transfer Protien
CHF	→	Congestive Heart Failure
CHO	→	Cholesterol Oxidase
CI	→	Confidence Interval
CVD	→	Cerebro Vasular Disease
ECG	→	Electrocardiograph
EDRF	→	Endothelium Derived Growth Factor
EDTA	→	Ethylene Diamine Tetra Acetic Acid
ELISA	→	Enzyme Linked Immuno Sorbant Assay
FC	→	Free Cholesterol
FMD	→	Flow Mediated Dilation
GHB	→	Glycosylated Hemoglobin
GOD	→	Glucose Oxidase
GPO	→	Glucose Phosphate Oxidase
HDL	→	High Density Lipoprotien
HOD	→	Head of the Department
HTGL	→	Hepatic Triglyceride Lipase

HRT	→	Hormone Replacement Therapy
IMT	→	Intimal Medial Thickness
ICAM	→	Intercellular Cell Adhesion Molecules
IGT	→	Impaired Glucose Tolerance
IDL	→	Intermediate Density Lipoprotiens
LDL	→	Low Density Lipoprotiens
LCAT	→	Lecithin Cholesterol Acyl Transferase
LPL	→	Lipoprotien Lipase
MARS	→	Monitored Atherosclerotic Progression Study
MVD	→	Macro Vascular Disease
NO	→	Nitric Oxide
OGTT	→	Oral Glucose Tolerance Test
PAI	→	Plasminogen Activator Inhibitor
POD	→	Peroxidase
PP	→	Post Prandial
RLJH	→	R.L.Jalappa Hospital
RC	→	Research Centre
SVCAM	→	Soluble Vascular Cell Adhesion Molecules
TG	→	Triglycerides
TMT	→	Tread Mill Test
VCAM	→	Vascular Cell Adhesion Molecules
VLDL	→	Very Low Density Lipoprotien
WHO	→	World Health Organization

ABSTRACT

BACKGROUND AND OBJECTIVES: Dyslipidemia that accompanies type 2 diabetes plays an important role in the pathogenesis of accelerated atherosclerosis in a population. The most important components of this dyslipidemia are an elevated very low density lipoproteins (VLDL) and total triglycerides (TGs) and a decreased high density lipoproteins (HDL) concentration in the serum. While fasting hypertriglyceridemia may be a risk factor for atherosclerosis, particularly in the presence of diabetes mellitus, this association has not been consistent and fasting HDL-C appears to be a far more significant risk factor. However, when TGs are studied in postprandial state, they emerge as stronger and independent coronary risk factors than HDL-C.

Postprandial hypertriglyceridemia have been linked with asymptomatic and symptomatic macrovascular disease in both normal and hypertriglyceridemic subjects. and such abnormalities have been reported in type 2 diabetics,the increased risk of atherosclerosis among them, might therefore be related to the higher postprandial lipemia in them.

Earlier studies clearly demonstrate the presence of postprandial hypertriglyceridemia among diabetic subjects, irrespective of fasting triglyceride levels. however it is not clearly known whether diabetic patients with macrovascular disease have greater abnormalities of postprandial TG metabolism and their clearance than those without Thus, this investigation on the postprandial lipid abnormalities and the triglyceride clearance in patients with and without macrovascular disease is being conducted.

METHODOLOGY

40 subjects divided into 2 groups, admitted to wards as inpatients under Dept. of MEDICINE in R.L.J.H and RC, with type 2 Diabetes Mellitus with or without MACRO VASCULAR DISEASE were included .

20 healthy controls who are non diabetic individuals whose age, sex and body mass index (BMI) is matched, who are non-smokers, non-alcoholic and do not have overt clinical evidence of coronary artery disease (CAD),cerebrovascular disease (CVD) or peripheral vascular disease (PVD) were recruited as controls.

Data was collected by using pre-tested proforma .The purpose of the study was carefully explained to the patients and informed consent was taken.

All the subjects in group and group II and 20 non diabetic healthy individuals recruited as controls were assessed by applying various clinical methods and investigations.

A detailed history and physical examination was carried out for every subject which includes assessment of vital parameters,anthropometry (height, weight, waist and hip measurement) and thorough systemic examination.

After 12 hours fast, during which water intake was allowed,blood was collected for various biochemical parameters (at zero hour). The subjects were then given a standardised fatty meal be in the form of whipped cream and fruits containing 729 kcal/m2 body surface areas (BSA) and with 5.3 gm protein,24.75 gm carbohydrates, 240 mg cholesterol and 65.2 gm fat was given over 10-15 minutes. Blood samples were drawn at baseline,2,4,6 and 8 hours after the oral fat challenge. Post prandial triglyceride clearance and lipid profile of the subjects of the various groups was compared.

RESULTS:

All the groups were age, sex and BMI matched. Waist circumference and waist: hip ratio was significantly higher in diabetic patients compared to controls. Duration of diabetes was comparable in both diabetic groups (groups1 and group 2).

Fasting and postprandial plasma glucose and glycosylated hemoglobin was higher in diabetic patients compared to controls.Glycemic control was comparable in diabetic subjects with macrovascular disease and diabetics without macrovascular disease.

Significant fasting dyslipidemia as indicated by a significantly higher TC, LDL and lower HDL cholesterol was found in diabetic subjects with macrovascular disease as compared to those without macrovascular disease and healthy controls. In addition fasting serum TG levels were higher in the diabetics with macrovascular disease as compared to other groups.

The current study found a significant PP lipid abnormality particularly PP hypertryglyceridemia and a delayed Triglyceride clearance after an oral fat challenge in diabetics with and without macrovascular disease as compared to controls.

CONCLUSIONS

It may be concluded from the present study that there is a significant fasting and postprandial dyslipidemia in patients with diabetes mellitus particularly in those who manifest macrovascular complications. This dyslipidemia is much greater postprandially than in the fasting state.

It was also found that there is a delayed Triglyceride clearance in subjects with MVD after an oral fat challenge compared to the other two groups.

An enhanced PP glycemic and lipaemic burden with delayed PP TG clearance following a high fat meal may be responsible for the aforementioned endothelial dysfunctions. The results of the study would thus support the view that repeated challenges with high fat meals in diabetic patients may lead to atherosclerotic vascular disease by causing recurrent and persistent endothelial dysfucntion.

KEYWORDS *: Dyslipidemia, Postprandial hypertriglyceridemia, Triglyceride clearance, Macrovascular disease.*

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INTRODUCTION

Diabetes is frequently associated with the development of premature atherosclerotic vascular disease.[1-4] Even in populations with a low incidence of coronary artery disease (CAD), diabetes still emerges as a coronary risk factor.[5] Not only are the clinical manifestations of CAD increased in diabetics, but also autopsy studies showed that atherosclerotic changes are present to a greater extent in them.[6] The arterial lesions are essentially similar to that found in non-diabetic populations, although they are generally more diffuse and wide spread and there may be an increase in the frequency of raised lesions in diabetics.[5] The relative risks of CAD & cerebrovascular disease (CVD) are 2 to 4 fold & 2 to 3 fold higher, respectively, than the risk in non diabetic subjects.[1-4] This increased risk has been attributed to the high prevalence of multiple atherosclerotic risk factors among diabetic patients.[1-4] However, studies have also shown that the excess of CAD in type 2 diabetics cannot be accounted for by the levels of four major risk factors identified for CAD viz. hypertension, smoking, total serum cholesterol & age, suggesting a role for other factors.[7,8]

The Dyslipidemia that accompanies type2 diabetes plays an important role in the pathogenesis of accelerated atherosclerosis in this population.[8-10] The most important components of this dyslipidemia are an elevated VLDL & total triglycerides (TG's) & a decreased HDL concentration in the serum.[11]

Whereas fasting hypertriglyceridemia may not be an independent risk factor for atherosclerosis in non-diabetics, it has been consistently shown to be associated

with a greater risk for CAD & atherosclerosis in those with type2 diabetes.[10-14] In this population, elevated TG levels may be a better predictor of CAD than elevated LDL cholesterol levels.[15]

One of the challenges with accurately determining the role of TG's, in the development of atherosclerosis is its characteristic rapid fluctuation, according to the metabolic state of the individual. Serum TG's are generally increased maximally 3-6 hours & 5-10 hours postprandially in nondiabetics & diabetics respectively.[16-19] Once postprandial hypertriglyceridemia occurs, it is exacerbated by the next meal & persists for the entire day. In 1979, Zilversmit, proposed that postprandial accumulation of 'Triglyceride Rich Lipoproteins (TRL's) resulted from a reduction in the rate of clearance of the TG rich dietary remnant particles at the endothelial surface.[20] Therefore, measurement of serum triglycerides in the postprandial state may provide a more reliable and sensitive estimate of triglyceridemia especially amongst diabetic subjects.

It has been hypothesized that postprandial TRL's promote the development of atherosclerosis.[20] The elevated postprandial TG levels may lead to an alteration in oxidative stress and consequent endothelial dysfunction that may finally lead to atherosclerosis and MVD in diabetic patients.[21,22] Various studies in support of this hypothesis have provided evidence of increased postprandial TRL's in those with angiography proven CAD as compared to normal controls.[15,23,24] The peak postprandial triglyceride levels have been linked with carotid atherosclerosis in healthy normo-& hypertriglyceridemic middle aged men using carotid intimal-medial thickness (IMT) as a marker of atherosclerosis.[25-27] Similarly, there is a strong

association between the post-prandial triglyceridaemia and endothelial dysfunctions in non-diabetic and healthy subjects.[28] It has been proved that the endothelial dysfunction is an early marker of atherosclerosis.[29]

There are very few studies comparing the post prandial lipaemia in patients with type 2 diabetes mellitus with and without macrovascular disease and healthy subjects.

With this background the present study was planned, to assess the post prandial lipaemia and the post prandial triglyceride levels and their clearance in patients with type 2 diabetes mellitus, with and without macrovascular disease and healthy subjects.

OBJECTIVES OF THE STUDY

1. To study post-prandial lipid abnormalities in patient with type 2 Diabetes Mellitus with and without macrovascular disease.

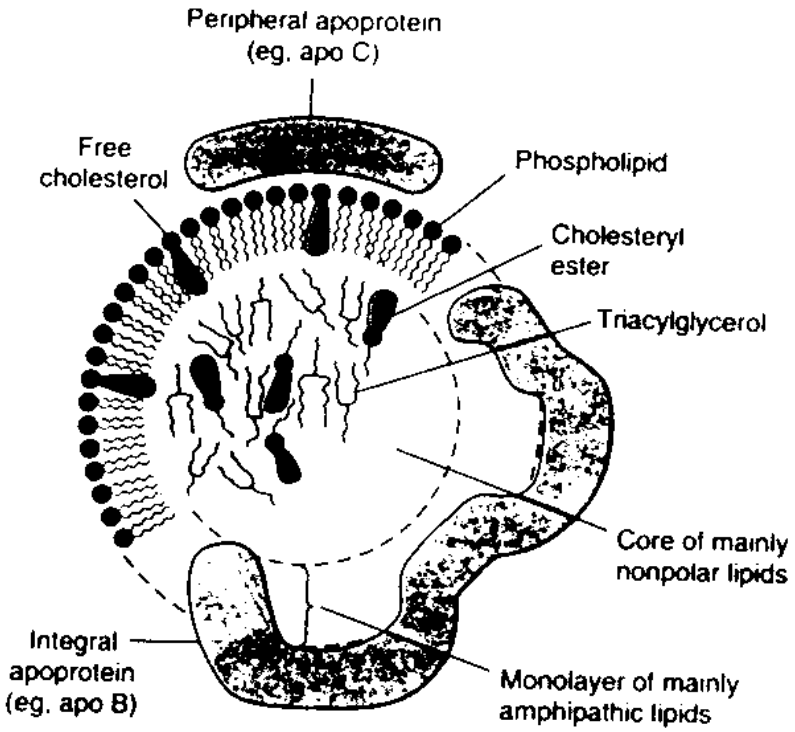
2. To study the post prandial triglyceride clearance in patients with diabetes mellitus , with and without MVD after a standard oral fat challenge, and the relevance of MVD to post prandial triglyceride levels and clearance.

REVIEW OF LITERATURE

NORMAL LIPOPROTEIN STRUCTURE, METABOLISM AND TRANSPORT

Normal lipoproteins consist of a hydrophobic core and a hydrophilic outer coat (Fig. 1) Triglycerides and the esterfied forms of cholesterol (cholestryl esters) are non-polar and from the core lipoproteins. Phospholipids and a small quantity of free cholesterol (unesterfied), which are soluble in both lipid and aqueous environments, cover the surface of the particle where they act as an interface in between the two.

Fig. 1: Generalised structure of a plasma lipoprotein



Lipoproteins are classified on the basis of their densities into five major classes:

- Chylomicrons
- Very low Density Lipoprotein (VLDL)

- Intermediate Density Lipoproteins (IDL)
- Low density Lipoproteins (LDL)
- High Density Lipoproteins (HDL)

Table 1: Composition of the lipoproteins in the plasma of humans

Lipoprotein	Source	Diameter (nm)	Density (g/mL)	Composition		Main Lipid Components	Apolipoproteins
				Protein (%)	Lipid (%)		
Chylomicrons	Intestine	90–1000	< 0.95	1–2	98–99	Triacylglycerol	A-I, A-II, A-IV,[1] B-48, C-I, C-II, C-III, E
Chylomicron remnants	Chylomicrons	45–150	< 1.006	6–8	92–94	Triacylglycerol, phospholipids, cholesterol	B-48, E
VLDL	Liver (intestine)	30–90	0.95–1.006	7–10	90–93	Triacylglycerol	B-100, C-I, C-II, C-III
IDL	VLDL	25–35	1.006–1.019	11	89	Triacylglycerol, cholesterol	B-100, E
LDL	VLDL	20–25	1.019–1.063	21	79	Cholesterol	B-100
HDL	Liver, intestine, VLDL, chylomicrons					Phospholipids, cholesterol	A-I, A-II, A-IV, C-I, C-II, C-III, D,[2] E
HDL_1		20–25	1.019–1.063	32	68		
HDL_2		10–20	1.063–1.125	33	67		
HDL_3		5–10	1.125–1.210	57	43		
Preβ-HDL[3]		< 5	> 1.210				A-I
Albumin/free fatty acids	Adipose tissue		> 1.281	99	1	Free fatty acids	

Abbreviations: HDL, high-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low density lipoproteins.
[1]Secreted with chylomicrons but transfers to HDL.
[2]Associated with HDL_2 and HDL_3 subfractions.
[3]Part of a minor fraction known as very high density lipoproteins (VHDL).

APOLIPOPROTEINS

The Apolipoproteins (Apo) provide a structural stability to the lipoproteins and determine their metabolic fate.

- Apo I, II, IV are found primarily on HDL.
- Apo AI and A II are synthesized in the small intestine and the liver, Apo A IV is manufactured only in the intestine.
- Apo A I comprises about 70-80% of the HDL protein.

- The function of Apo A I is to activate the enzyme Lecithin : cholesterol Acyl Transferase (LCAT)
- Plasma levels of Apo A I and HDL are inversely related to the risk of coronary artery disease.
- Apo A IV may play a role in the activation of LCAT.
- Apo B 100 is the major Apo associated with VLDL, IDL and LDL comprising 30, 60 and 90% of the protein component of these lipoproteins.
- Apo B 100 is synthesized in the liver and Apo B 48 in the intestine even though they are encoded by the same gene.
- Apo B 100 is essential for the assembly and secretion of VLDL from the liver and acts as a ligand for the removal of LDL by the LDL receptors.
- Apo B 48 is necessary for the assembly and secretion of chylomicrons.
- Apo C is synthesized in the liver and is present in varying concentrations in all the lipoproteins.
- Apo C inhibits the removal of VLDL and chylomicrons from the liver.
- Apo C II is an activator of lipoprotein lipase (LPL)
- Apo C III is an inhibitor of LPL
- Apo E is produced in the hepatocytes and mediates the uptake of lipoprotein in the liver both by LDL receptor and by the LDL receptor-related protein.

LIPOPROTEIN METABOLISM

ENZYMES

1. **Lipoprotein lipase (LPL):** LPL is synthesized in fat and muscle cells. After secretion it binds to proteoglycans on the luminal surface of the capillary beds. LPL mediates the hydrolysis of the triglycerides in chylomicrons and VLDL to

generate free fatty acids (FFA) and glycerol. Insulin stimulates synthesis and secretion of LPL, and reduced activity of LPL in diabetics can lead to impaired triglyceride clearance.

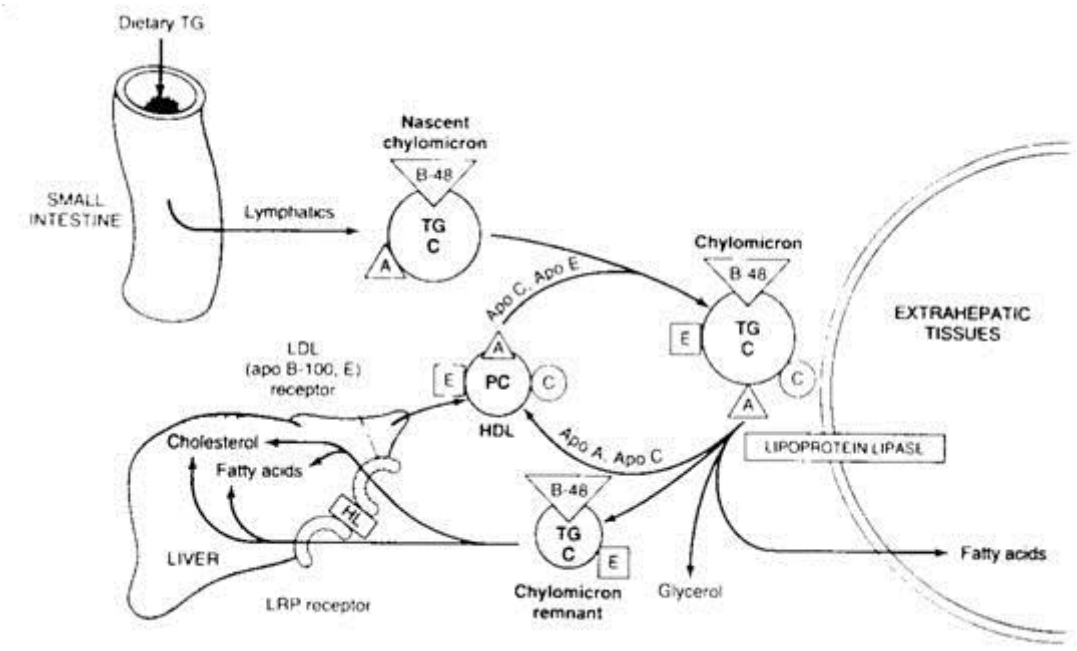
2. **Hepatic Triglyceride Lipase (HTGL):** this enzyme is synthesized in the liver and interacts with the lipoproteins in the hepatic sinusoids. HTGL can remove triglycerides from VLDL remnants, thus promoting the conversion of VLDL to LDL, and may also play a role in the clearance of chylomicrons and in the conversion of HDL 2 to HDL 3.
3. **Lecithin: Cholesterol Acyl Transferase (LCAT):** this enzyme is synthesized in the liver and secreted in the plasma where it is bound to HDL. LCAT mediates the transfer of linolate from lecithin to free cholesterol on the surface of HDL to form cholestryl esters that are then transferred to VLDL and eventually LDL.
4. **Cholesterol Ester Transfer Protein:** This is synthesized in the liver and circulates in blood, associated with HDL. It mediates the exchange of cholestryl esters from HDL with triglycerides of chylomicrons or VLDL.

EXOGENOUS LIPOPROTEIN PATHWAY (FIG. 2)

- Dietary fat and cholesterol are absorbed from the small intestine, where they are reesterified into triglyceride and cholestryl esters, and incorporated into the lipid core of the chylomicrons. These chylomicons are secreted from the intestine only when associated with Apo B 48.
- Chylomicrons acquire Apo C/E on entering the circulation (from HDL).
- The triglycerides and cholestryl esters are metabolized to fatty acids and cholesterol, which are subsequently deposited in the adipose tissue, muscle and liver.

- Chylomicron remnant particles which are depleted of triglycerides are taken up by the liver through a process mediated by Apo E.
- Normolipidemic subjects dispose off most of the dietary fat in the blood within 8 hrs of the last meal. But some individual with dyslipidemias, have a measurable level of intestinally derived lipoproteins as long as 24 hrs after the last meal.
- If the postprandial levels of chylomicrons are elevated or if their removal is prolonged, cholesterol delivery to the vessel wall may be increased. This may be one of the factors related to accelerated atherogenesis in this group.

Fig. 2: Metabolic fate of chylomicrons



ENDOGENOUS LIPID PATHWAY (Fig. 3 & 4)

- Hepatic triglycerides and cholesterol are secreted associated with VLDL (Apo B 100) from the liver.
- VLDL triglyceride is metabolized by Lipoprotein Lipase (LPL) and the Apo's returned to HDL.

- VLDL remnant may have two metabolic fates, they either may be directly removed by the liver or undergo progressive lipid depletion and form IDL and finally LDL.

- Hepatic lipase, an enzyme bound to the endothelial surface in the hepatic sinusoids, influences the conversion of IDL to LDL.

- LDL is the end product of VLDL processing. Apo B 100 and cholesterol are conserved in this processes.

- The half time of LDL in the plasma is determined mainly by its rate of uptake and metabolism via LDL receptors. Following binding to the LDL receptor, the regulation of cholesterol homeostasis is maintained.

- Approximately 60% of the LDL is taken up by the hepatic LDL receptors and the rest is metabolized by receptor independent pathways.

- HDL, which is the smallest of the lipoproteins, plays an important role in lipid metabolism.

- HDL is the major site for cholesterol esterification, as they accept cholesterol from peripheral cells and other lipoproteins.

- On being enriched with cholestryl esters and other Apo's, the HDL is converted to HDL_2, which is a substrate for hepatic lipase and finally gets converted to HDL_3. HDL, hence is important in the reverse cholesterol transport whereby free cholesterol is returned from peripheral cells to liver.

Fig. 3: Metabolic fate of VLDL and LDL

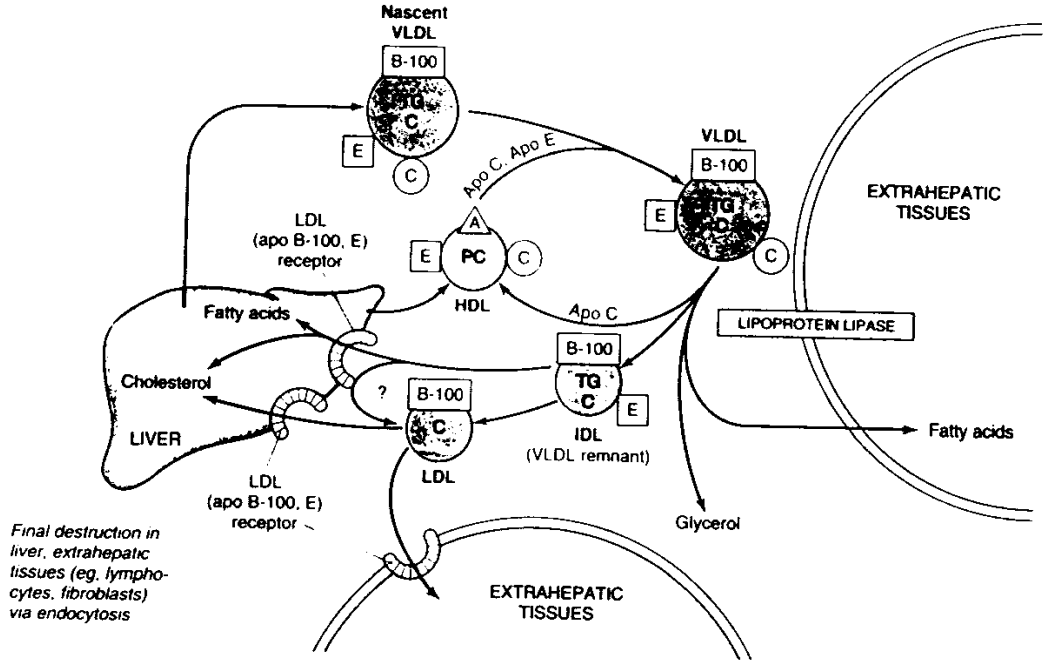
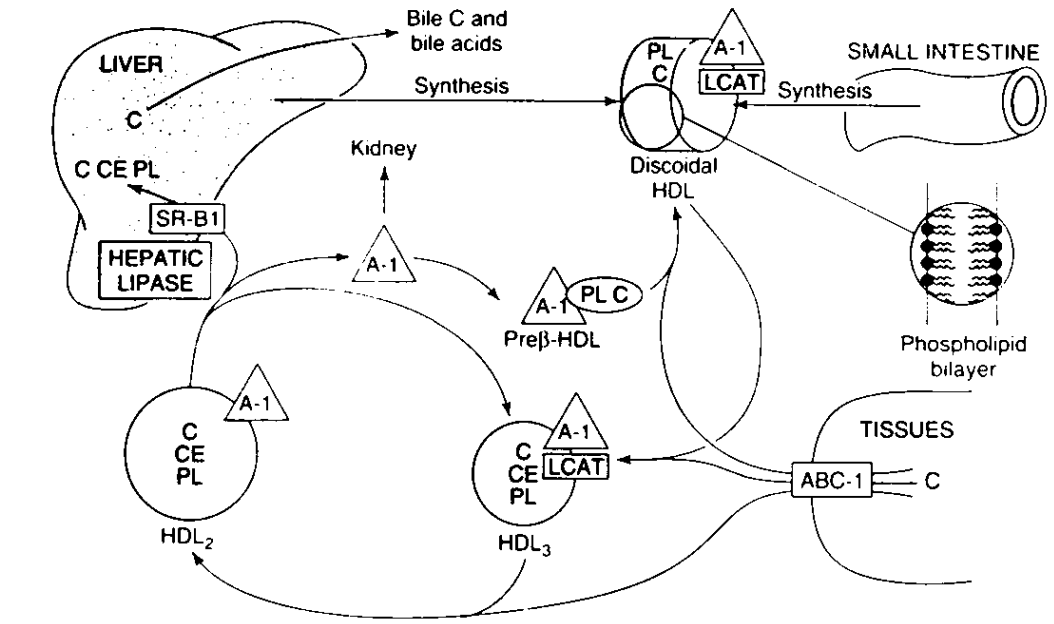


Fig. 4: Metabolism of HDL in reversed cholesterol transport



LIPOPROTEIN ABNORMALITIES IN TYPE 2 DIABETES

Quantitative Lipoprotein Abnormalities:

Table 2: Quantitative Lipoprotein Abnormalities in Type 2 Diabetes

↑ VLDL and total TG
↓ HDL
↔ LDL
↔/↑ Lp (a)
↑ IDL
↑ Postprandial remnants
↑ apoB
↓ apoA1

Raised serum VLDL and triglycerides and low levels of HDL cholesterol are the most frequent lipid abnormalities in type 2 diabetics.[11,31,32] Usually the elevation is moderate, 1.5-3 folds compared with non-diabetic subjects matched for sex, age and body mass index (BMI). Patients with severe hypertriglyceridemia and milky serums commonly have a concomitant genetic or secondary disorder of lipoprotein metabolism.[32] Higher production rates of triglycerides and VLDL particles are the most common metabolic abnormalities[30], clearance defects have been implicated in higher triglyceride levels with some authors reporting reduced postheparin lipoprotein lipase levels[33], while others have found no difference in this activity compared with diabetics without triglyceride elevations.[34] Plasma concentration of LDL cholesterol and LDL apo B are usually within normal limits.[35,36]

The concentration of HDL-C is on an average reduced by 10-20%.[37,38] The decrease in HDL-C occurs primarily in the HDL2 subfraction, the subfraction most

closely associated with protection against CAD.[39] High TG levels have been shown to be strongly associated with low HDL2 levels and with small HDL size in type 2 diabetics. Intermediate-density lipoprotein (IDL) levels have been shown to be increased in type 2 diabetes.[40] Even normolipidemic to mildly hyperlipidemic diabetic patients have increased IDL-C levels compared to controls.[40] Postprandial remnant lipoproteins are usually increased in type 2 diabetics and remnant levels following a meal correlate positively with fasting TG levels.[16,17] Most studies, but not all, have reported the concentration of Apo A-1 and HDL-C /apo A-1 ratio to be reduced.[30,41-43] Lipoprotein (a) [Lp(a)] concentrations are usually normal[44] and, in general, are strongly related to the mean Lp(a) levels of normoglycemic family members.[45] Lp(a) levels rise above baseline levels when diabetic patients develop renal insufficiency and/or proteinuria. Glycaemic control has no influence over Lp(a) levels[46] unlike other lipid parameters such as total cholesterol (TC), triglycerides (TG), LDL-C, HDL-C and apo B.

QUALITATIVE LIPOPROTEIN ABNORMALITIES

A number of qualitative changes in lipoproteins have been described in type 2 diabetics[11,32] that cannot be detected from routine measurements of lipoprotein concentrations and even mildly hyperlipidemic diabetics have been shown to have potentially important compositional abnormalities[46] (Table 3).

Table 3: Qualitative Lipoprotein Abnormalities in Type 2 Diabetes

VLDL	↑ TG content ↑ TG/apoB ratio ↑ FC and CE content
HDL	↑ TG content ↓ FC and CE content ↑ Glycosylation
LDL	↑ TG content ↑ Proportion of small, dense LDL ↑ Glycosylation ↑ Oxidation

VLDL has been shown to have an increase in TG and apo-B content and an increase in TG/apo-B ratio.[47] VLDL has been shown in some, but not all, studies to have an increased content of both free cholesterol (FC) and cholesteryl-ester (CE)[48,49]. Poorly controlled type 2 diabetics secrete mainly large VLDL particles (Sf 100-400)[50] and cholesterol-apo B ratio is increased in VLDL+IDL fraction (Sf 12-400)[51]. Apo E and apo C enrichment of VLDL also occurs.[52,53]

LDL has also been shown to be enriched in free cholesterol and triglycerides[30,31] although this has not been seen in all studies.[48] Fielding et al. isolated VLDL and LDL from diabetic plasma, and showed that both fractions had an increased content of FC along with an increased ratio of FC/ phospholipids.[48] Also, there is an increased proportion of small, dense LDL particles in type 2 diabetics.[46] Such small, dense LDL (phenotype B) are clearly associated with increased CAD risk in the general population.[54] The association of hypertriglyceridemia with small, dense

LDL subclass is well known in both general and type 2 diabetic populations.[54] McNamara et al. have shown that the amount of circulating TG's appears to be the single most important factor affecting LDL size.[55] Another important change found in the LDL fraction is the glycosylation of lysine residues of apoB to form glycosylated LDL.[56]

HDL typically shows a reduced content of both FC and CE.[57] Reduced HDL usually reflects preferential decrease in HDL2 sub-fraction.[57,58] HDL is also triglyceride enriched.[57,58] Apo A I concentration is found to be decreased.[58]

HYPERTRIGLYCERIDEMIA AS A RISK FACTOR FOR ATHEROSCLEROSIS

Many, epidemiological and clinical studies have demonstrated a relationship between plasma triglyceride levels and risk of coronary artery disease, and amplification of the risk with combined elevations of triglycerides and LDL-C.[59-61] Statistical adjustment for the relation between triglyceride and HDL generally reduces the strength of association between triglycerides and coronary artery disease, casting doubt on the importance of triglycerides as an independent coronary risk factor. However, statistical analyses have a limited ability to separate effects due to two highly co-linear variables.[62] Several issues are relevant. Firstly, adjustment of triglycerides for total cholesterol is inappropriate, because in individuals with high triglycerides, a significant fraction of the total cholesterol is VLDL cholesterol, and thus in effect, one is adjusting triglycerides for triglycerides. Secondly, triglycerides show a much larger intra-individual variation than other lipid/lipoprotein fractions, diluting its association in statistical models. Thirdly, triglycerides appear to be a risk

factor in specific population subgroups, such as women, diabetics, or those with low cholesterol levels. Lastly, high triglyceride levels often exist in a milieu of low HDL cholesterol, hypertension, insulin resistance and other abnormalities of the "syndrome-X" which is known to be associated with increased cardiovascular risk.

A recent meta analysis incorporating data from eight population based prospective studies in over 28,000 patients has indicated that for every 89 mg/dL increase in plasma triglycerides, there is approximately a 32% increase in coronary artery disease risk for men & a 76% increase in women.[59] Using data from studies where HDL-C was also measured, adjusting for effects of this variable resulted in a reduced estimate of risk, 14% in men and 37% in women, but the increase remained significant for both men and women. Best evidence for independent contribution from triglycerides to coronary risk was 6 year data from the PROCAM study, an observational follow-up of 4559 middle aged men, which showed the highest cardiovascular risk in patients with both an LDL: HDL ratio greater than 5 and a serum triglyceride concentration greater than 204 mg/dL.[60] Eight-year data from the PROCAM study has now shown a significant and independent association between serum triglyceride concentrations and the incidence of major coronary events. The Physician's Health Study has indicated that a random fasting plasma triglyceride level in the upper tertile (mean 229 mg/dl) was associated with more than 3 folds higher risk of coronary artery disease in subjects with total/HDL cholesterol ratios in the upper tertile (mean ratio 5.7) than in individuals with either lower triglyceride or total / HDL cholesterol ratio.[63] This is consistent with analysis from the Helsinki heart trial in which the highest risk subgroup, comprising approximately 10% of subjects in the trial had a combination of triglyceride > 204 mg /dl and LDL/HDL ratio >5.[64] This subgroup accounted for more than 70% of the reduction in incidence of clinical events

associated with gemfibrozil treatment. The pathological consequences of combined triglyceride and LDL cholesterol elevations are manifest by the relatively high prevalence of premature coronary artery disease in patients with familial combined hyperlipidemia and related disorders.[61]

HYPERTRIGLYCERIDEMIA AS A RISK FACTOR FOR ATHEROSCLEROSIS AMONG TYPE 2 DIABETICS

Several epidemiological studies have shown that hypertriglyceridemia is predictive of mortality from CAD in those with type 2 diabetes. Hypertriglyceridemia, in the metabolic setting of both impaired glucose tolerance (IGT) & type 2 diabetes, appeared as a major risk factor for CAD death in the Paris Prospective Study.[10] This study was a long term investigation of risk factors for CAD in over 7000 middle-aged men. A subgroup of 973 subjects had type 2 diabetes or IGT on OGTT as defined by WHO criteria. Within this subgroup, the plasma TG level was the most powerful predictor of subsequent CAD death. In the WHO multinational study, the single risk factor that correlated most closely with the occurrence of CAD in diabetics was serum TG concentration.[13] In a Finnish 7-year prospective study of 313 type 2 diabetics, high TG levels (>203 mg/dl) were associated with a 2-fold increase in risk for CAD events. In type 2 diabetics, elevated TG levels may be a better predictor of CAD than elevated LDL cholesterol levels.[12]

TRIGLYCERIDE RICH LIPOPROTEINS AND ATHEROSCLEROSIS

Studies involving measurements of specific triglyceride rich lipoprotein fractions have provided support for the hypothesis that particular types of triglyceride rich particles may be directly atherogenic.

Gofman, Lindgren and colleagues have shown that intermediate density lipoprotein (IDL) of Sf 10-20 are strong determinants of coronary artery disease risk compared to low-density lipoprotein Sf<10.[65] Current standards of LDL cholesterol measurement are based on Sf 0-20 lipoproteins and therefore, include cholesterol content of both IDL and LDL. Subsequently, evidence in support of a relation between Sf 12-60 lipoprotein (LDL+VLDL) and coronary heart disease has been provided.[66] In an angiographic trial, levels of IDL + remnant VLDL cholesterol, as well as levels of HDL cholesterol, were strong predictors of both coronary angiographic progression and clinical events during a four year observation period.[67] However neither plasma triglycerides nor LDL-C, LDL triglyceride or apo B was related to changes in stenosis. The mass of small VLDL (Sf 20-60) was the strongest independent predictor of progression of angiographically assessed coronary artery disease in the MARS (Monitored Atherosclerosis Regression Study) trial which employed lovastatin therapy for patients with coronary artery disease.[68] These studies suggest that triglyceride rich lipoprotein particles, particularly small VLDL and IDL, are involved in atherosclerosis progression more than LDL. Recent analysis of data from the MARS trial has shown that carotid artery intimal-medial thickness was strongly and independently related to levels of IDL mass measured by analytical ultracentrifugation[69] raising the possibility that IDL may be of particular importance in the early stages of atherogenesis detected as carotid intimal-medial thickness changes while both IDL and VLDL may be involved in the progression of more intrusive lesions that are also related to clinical events.

POSTPRANDIAL LIPID ABNORMALITIES AS A RISK FACTOR FOR CAD

Plasma lipids and lipoproteins are generally measured in the fasting state, and treatment strategies for prevention of cardiovascular disease are based on such measurements despite the fact that most of our lives are spent between the consumption of regular meals. A number of reports point to an association between impaired metabolism of postprandial triglyceride-rich lipoproteins (TRL's) and the presence or development of coronary artery disease (CAD).[15,21,22]

A mechanistic hypothesis linking the postprandial generation of TRL remnants to the development of atherosclerosis was formulated more than 20 years ago by Zilversmit.[21] The reasons for the relative lack of clinical evidence for an involvement of postprandial lipid and lipoprotein metabolism in the development of atherosclerosis is probably a consequence of biological, statistical and methodological issues. First, the perturbations of lipoprotein metabolism in the postprandial state are complex, with changes in both composition and concentration of potentially atherogenic lipoproteins. Presently, there is no consensus of what, or how to measure these lipoprotein species in this respect. Secondly, there is no consensus of how a standardized postprandial state should be elicited (dose or contents of an experimental meal). Thirdly, it is probable that the close statistical association between fasting plasma triglycerides and the accumulation of TRL's in the postprandial state will prevent us from determining the independent role of postprandial lipid abnormalities and atherosclerosis i.e., conventional statistical methods seem to be inappropriate or misleading in the study of casual relationship between the risk factor and disease.

In 1979, Zilversmit proposed that the postprandial accumulation of triglyceride rich lipoproteins (TRL's) resulted from a reduction in the rate of

clearance of the TG rich dietary remnant particles at the endothelial surface & promoted the development of atherosclerosis. Remnants of TRL's are certainly atherogenic in fat fed experimental animals & in humans with type III hyperlipoproteinemia.[20.25] TRL's derived from hypertriglyceridemic humans are toxic to endothelial cells & are taken up by macrophages resulting in foam cell formation. Moreover, case control studies have found an elevated level of postprandial TRL's in those with angiographically verified CAD as compared to normal controls.[15,21,22]

Barritt found significantly greater lipaemia in blood taken 7 hours after the ingestion of a high-fat meal from men with coronary artery disease than from controls.[70] In a case control study of 82 subjects with CAD, Simons et al measured a variety of plasma lipids and lipoproteins as well as other risk factors in both fasting subjects and the same subjects 4 hours after a triglyceride / cholesterol rich meal.[71] The presence of CAD was significantly related to postprandial plasma concentrations of cholesterol and triglycerides, the total cholesterol / HDL-cholesterol ratio, and the apo B-48 / apo B-100 ratio in the Sf<60 fraction. There was a 2.2 fold greater risk of CAD in individuals in the top quartile of the apo B-48/apo B-100 distribution, obtained 4 hours after the ingestion of the test meal than in those in the bottom quartile (after adjustment for other risk factors). Krauss et al. reported that coronary artery disease cases had higher TG and retinol palmitate concentrations 10 hours after the ingestion of a high-fat meal than did controls.[21]

In a case control study of about 100 individuals, Patsch evaluated the role of plasma triglycerides in CAD.[15] Following a standard fat load, triglycerides levels at 6-8 hours have been shown to distinguish patients with and without coronary artery disease, independent of fasting triglyceride levels and other lipoprotein

measurements, although low HDL was also a predictor of risk in these subjects. Multivariate analyses showed that the magnitude of postprandial hypertriglyceridemia was an independent predictor of the presence or absence of coronary artery stenosis. The authors have suggested the possibility of a "triglyceride intolerance hypothesis" in which CAD is linked to an impaired triglyceride transport. Because the triglyceride metabolic capacity determines the concentration of HDL-cholesterol, they postulated that the generally accepted relation between low HDL and CAD, may be a marker for the causal relation between plasma triglycerides and CAD. Furthermore, in persons with prolonged increases of plasma triglycerides, either fasting or postprandial, the process of lipid exchange would enrich the triglyceride rich particles in cholesteryl ester and thereby make these particles more atherogenic.

Groot et al in a comparative study of normolipidemic men, with and without CAD, showed that the delayed clearance of chylomicron particles may account for an increased risk of CAD.[22]

In an angiographic study on survivors of a myocardial infarction before age 45, Karpe et al were unable to relate HDL cholesterol to the progression of coronary atherosclerosis, however, the number of small chylomicron remnants (Sf 20-60 apo B-48) during the postprandial phase appeared to explain the observed disease progression.[72] They examined plasma HDL concentration, particle size and composition and their relations to postprandial TRL's in 32 post-infarction patients and 10 healthy controls after intake of a standardized oral fat load of a mixed meal type. All patients had undergone coronary angiographies in connection with myocardial infarction and around 5 years later. The plasma HDL-C increased significantly postprandially, especially in hypertriglyceridemic subjects, with a

concomitant increase in HDL triglyceride. None of the HDL parameters measured either in the fasting state or postprandially (HDL-C, HDL-triglyceride, HDL sub-classes, apo AI, apoAI: AII) were related to the development of coronary atherosclerosis whereas the postprandial plasma levels of small chylomicron remnants, which showed a weak negative correlation with HDL, related positively to the progression of coronary atherosclerosis suggesting that the link between postprandial plasma levels of small chylomicron remnants and the progression of CAD was not accounted for by metabolic inter-relation with the HDL system, but by a direct atherogenic action of small chylomicron remnants. The patients in this study were, however, highly selected. First, females and patients with severe hyperlipoproteinemia were not investigated. Secondly, a certain number of patients died in the course of follow-up. It is also evident from this study that apoB-48 containing lipoproteins only constitute a very small fraction of the total TRL population in plasma.

Uiterwaal et al. showed an exaggerated triglyceridemic response (only at late time-points after fat intake) to a fatty meal in sons of patients with documented presence of CAD compared to sons of healthy men, though there was no difference in fasting plasma triglycerides. This study links the triglyceridemic outcome of a fat challenge with the heritable risk of CAD.[73]

All these studies involving an evaluation of postprandial metabolism and the risk of CAD are either retrospective or of association type. There is no prospective study, in which postprandial lipid metabolism has been related to future events of CAD or progression of atherosclerosis.

Also, all these studies relied on coronary angiography to define case status. Coronary angiography provides, at best, only an indirect measure of atherosclerosis because estimating lumen diameter does not really estimate wall thickness. While coronary angiography is critical for predicting clinical outcome, ultrasound measurements of wall thickness are more informative for quantifying the relation of risk factors to the presence and/or progression of atherosclerosis. In addition, because ultrasound is a noninvasive procedure, it may be used in an asymptomatic population, which would allow generalization of the results.

The intimal-medial thickness of the extracranial carotid artery as measured by high resolution B-mode USG is an excellent non-invasive and quantitative measure of generalized atherosclerosis & also a surrogate marker for CAD.[74-77] Increase in carotid IMT is associated with an increased risk of CAD and CVD in prospective studies.[76] It has been extensively used to examine the stage of atherosclerosis and to evaluate the regression of atherosclerotic lesions in intervention therapies. B-mode ultrasound is capable of imaging wall thickness and thus is able to define atherosclerosis with greater sensitivity than imaging methods that depend on narrowing of the arterial lumen e.g. angiography, Doppler ultrasound. Moreover, there is a good agreement between histological examination & carotid IMT as measured by B-mode USG.[77]

A number of factors have been identified as directly implicated in thickening of the intima-media, including elevated blood pressure, elevated fasting total plasma cholesterol & smoking. V. Mohan et al have studied the carotid IMT in the Chennai urban cohort.[78] They found that IMT showed a correlation with age, total cholesterol, LDL cholesterol, waist-hip ratio & systolic BP in non-diabetics whereas age &

duration of diabetes were the two most important predictors of IMT in diabetics. The mean IMT of diabetics (0.95±0.31mm) was significantly higher than those of the non-diabetic subjects (0.74±0.14mm) ($p<0.001$). They also determined the prevalence of carotid atherosclerosis using a cut off value of 1.1mm as described by Kawamori et al.[79] Using this cut off, they reported a 20 times higher prevalence of atherosclerosis among diabetics (20%) as compared to non-diabetic subjects (1%) ($p<0.001$).

ARE TRL REMNANTS ATHEROGENIC?

Triglyceride enriched apo B containing lipoproteins have been isolated from human atherosclerotic lesions, supporting the potential for direct involvement of triglyceride rich lipoproteins in atherogenesis.[80] Large VLDL particles from hypertriglyceridemic subjects have been shown to undergo endocytosis through specific receptors in macrophages, resulting in formation of cholesterol rich foam cells.[81] Lipolysis products of triglyceride rich lipoproteins have shown to be cytotoxic for endothelial cells and macrophages.[82] Liposomal structures with properties similar to lipoproteins remnants have been detected in arterial lesions.[83]

In order to assign a lipoprotein species an atherogenic potential, certain steps in the sequence of events leading to foam cell formation have to be fulfilled. The first of these hypothetical steps is the actual concentration of the lipoprotein species in the plasma compartment. Secondly, the size of the lipoprotein species should be small enough to penetrate the endothelium effectively. Thirdly, a subintimal localization of the lipoprotein should lead to proteoglycan binding and lipoprotein modification to allow recognition by macrophage scavenger receptors or other receptors for removal of TRL remnants. All these events have been clearly demonstrated for LDL especially small dense LDL. Considerably less is known about VLDL remnants and

chylomicron remnants and data are partly conflicting. Starting with the lipoprotein particle concentration, the absolute TRL apo B concentration, as well as the postprandial elevation, is small compared with the LDL apo B level.[84] Furthermore, the relative amount of chylomicron and chylomicron remnant particles is very small compared with equally sized apo B-100 TRL's.[85] Two human studies show that a major proportion of chylomicron remnants are removed from plasma long before they attain a size at which they may penetrate the arterial wall, i.e. at a size range of Sf 12-60.[86,87] The amount of cholesterol carried by apo B-48 remnants, both in fasting and postprandial plasma, is very small compared with all the other potentially atherogenic lipoprotein species in human plasma.[88]

The lipoprotein particle size is a major determinant of the ability of the particle to penetrate to a subintimal location from the plasma. Shaikh and coworkers studied this phenomenon in human arterial specimens by injecting labeled TRLs before vascular surgery with subsequent analysis of the lipoprotein deposition in arterial specimens.[89] The arterial localization of Sf 60-400 TRLs, i.e. 35-75 nm in diameter, was very small compared with Sf 12-60 TRLs (approximately 27-35 nm in diameter). Furthermore, the Sf 12-60 lipoproteins entering the arterial wall could not return to plasma, in contrast to both LDL and HDL. Hence, small TRL may be more atherogenic than the larger ones.

Rapp et al. used immunoaffinity chromatography to isolate apo B-containing lipoproteins from human arterial specimens removed during surgery.[90] They found that about one-third of the lipoprotein associated cholesterol was in the IDL/VLDL fraction. Furthermore, the characteristic of this lipoprotein fraction compared with its plasma equivalent was an excess of lipoprotein-carried apo E. Despite the use of

sensitive immunoblotting, apo B-48 was not found in the arterial IDL/VLDL fraction, arguing that the TRL cholesterol deposition in the arterial wall is due to apo B-100 lipoproteins and that the deposited material has remnant characteristics.

Binding of apo B-containing lipoprotein to subintimal matrix, above all proteoglycans, is a key mechanism for retention of lipoprotein-carried cholesterol in the vascular wall. Apo B displays several proteoglycan-binding sites which may be important in conferring atherogenicity to TRLs. Genetic engineering with a single point mutation in the apo B-100 peptide chain causes a protein with substantially reduced affinity for proteoglycans, though this site is not present in apo B-48 molecule.[90]

OTHER VASCULAR EFFECTS OF TRL'S

Metabolism of TRL's is recognized as a regulatory component of two important hemostatic proteins, i.e. plasminogen activator inhibitor-1 (PAI-1) and coagulation factor VII (FVII).[91,92] The high TRLs associated with alimentary lipemia lead to activation of FVII and increased levels of PAI-1.[92] Though it does not lead to any thrombus formation in itself, the procoagulant state augments the potential for thrombus formation in the event of plaque rupture.

The fatty acids locally accumulated by lipolysis of TRL's, have been shown to enhance trans-endothelial flux of LDL in in-vitro studies on cultured endothelial monolayers.[93] In addition, the lipolytic remnant lipoproteins may have the same potential.[94] Lundman et al showed that infusion of a chylomicron-like triglyceride emulsion reduces NO-dependent vascular reactivity in humans.[95] Similar effects have been observed after ingestion of a fatty meal.[96]

STUDIES ON POSTPRANDIAL LIPEMIA IN TYPE 2 DIABETICS

The magnitude of the postprandial lipemia is known to be dependent on the fasting plasma triglyceride concentration. Since type 2 diabetics generally present with mild hypertriglyceridemia, a disturbed postprandial triglyceride metabolism is expected in them.

Lewis et al[97] performed a retinyl palmitate fat loading test in untreated type2 diabetics with normotriglyceridemia (TG 102.7 ±7.9 mg%),untreated type2 diabetics with moderate hypertriglyceridemia (TG 232 ±31.9 mg%) and age and weight matched normotriglyceridemic controls (TG 112.4 ±8 mg%).[15] Only type2 diabetics with hypertriglyceridemia demonstrated a greater and prolonged increase in total plasma triglycerides ($r=0.88$, $p=0.0001$). The postprandial triglyceride increment in normotriglyceridemic type2 diabetics and controls was not different. Also the chylomicrons and chylomicron remnants showed a greater rise and prolonged residence time ($p< 0.011$) in hypertriglyceridemic type2 diabetics (13.7 hours), whereas no differences could be noted between normotriglyceridemic type2 diabetics (8.5 hrs) and controls (7.3 hrs). A highly significant positive correlation was noted between the fasting triglyceride concentration and the retinyl palmitate increment in total plasma ($r=0.85$, $p=0.0001$), chylomicrons and remnants ($r=0.34$, $p=o.0001$) in the postprandial state. Triglycerides did not correlate with fasting or postprandial insulin or glucose levels.

In a different study Chen et al[16] compared the postprandial triglyceride metabolism in normotriglyceridemic type2 diabetics and age, weight and triglyceride matched controls. In accordance with the study of Lewis et al[15], a normal postprandial triglyceride and retinyl palmitate increment was observed in the

chylomicron fraction in normotriglyceridemic type2 diabetics. However, the postprandial rise in triglycerides and retinyl palmitate in the chylomicron remnant fraction was significantly higher in the normotriglyceridemic type2 diabetics .

Syvanne et al[98] have reported an enhanced postprandial lipemia in normotriglyceridemic type2 diabetics in comparison with healthy, sex, age, and BMI matched controls. Differences were found in the chylomicron, chylomicron remnant and VLDL1 fraction (Sf > 60). It has been suggested that the discrepancy between the three studies may result from differences in the lipoprotein fractionating, favoring the more accurate fractionating methods in the latter two studies.[16,98]

Earlier studies from our institution clearly demonstrate the presence of postprandial hypertriglyceridemia among diabetic subjects, irrespective of fasting triglyceride levels.[18,19] This was independent of glycaemic control & insulin sensitivity but was related to the interaction of diabetic state & obesity. This was observed both in older type 2 diabetics[18] as well as young ketosis resistant subjects.[19]

PATHOPHYSIOLOGY OF POSTPRANDIAL HYPERLIPIDEMIA IN TYPE 2 DIABETES

Studies of whether the increased triglyceride rich lipoprotein-triglyceride concentration is due to exogenous or endogenous triglycerides have sought to define the nature of the lipoprotein particles that underlie postprandial hyperlipidemia. Both intestinally and hepatically derived TRLs contribute to the triglyceride rise after ingestion of a meal.[99] TRLs from the liver contribute significantly to the pool of triglyceride containing lipoproteins in the postprandial state.[99] This may result from an increased flow of substrates to the liver e.g. free fatty acids, glucose and lipoprotein remnants. Alternatively, a postprandial saturation of the common

chylomicron and VLDL removal pathway may contribute to triglyceride rise after ingestion of a meal.

Table 4: Enzymes involved in lipid metabolism and their function in type 2 diabetes

MOLECULE	NORMAL FUNCTION	EFECT ON FUNCTION IN TYPE2 DIABETES
ENZYMES		
Lipoprotein Lipase	1. Hydrolysis of TRL triglycerides and production of cholesterol rich remnants. 2. Binding of chylomicron remnants to the LDL receptor related protein. 3. Bridging of lipoproteins to proteoglycans. 4. Synthesis of LDL from VLDL.	Decreased
Hepatic Lipase	1. Catabolism of chylomicron remnants 2. Conversion of HDL_2 to HDL_3.	Same
RECEPTORS		
LDL receptor	Catabolism of IDL, LDL and chylomicron remnants.	No data available
LDL receptor related protein	Catabolism of chylomicron remnants and IDL	Decreased
Scavenger receptor	Direct removal of TRL and TRL remnants	No data available
APOLIPOPROTEINS		
Apo E	Binding of TRL remnants to the LDL receptor related protein and LDL receptor	Decreased (glycation)
Apo B 100	Binding of IDL to the LDL receptor	Decreased (glycation)
Apo C II	Co-factor in hydrolysis of TRL by lipoprotein lipase	Decreased (glycation)
Apo C III	1. Inhibitor of hydrolysis of TRL by lipoprotein lipase	No data available
	2. Inhibitor of hepatic remnant removal	No data available
OTHERS		
CETA (cholesteryl ester transfer activity)	Exchange of triglyceride and cholesteryl ester between apo B containing lipoproteins and HDL (production of cholesterol rich remnants and small dense LDL)	Increased
Free Fatty acids	1. Inhibition of hydrolysis of TRL by lipoprotein lipase 2. Inhibition of binding of LPL to TRL and endothelium bound proteoglycans.	Increased
Proteoglycans	1. Binding of LPL to vascular endothelium. 2. Uptake of lipoproteins.	No data available

Lipoprotein lipase activity is decreased in type 2 diabetics and this phenomenon may contribute to the delayed clearance of triglyceride rich lipoproteins as it is widely accepted that the amount of lipoprotein lipase available at the endothelium is the rate limiting factor in triglyceride hydrolysis. The underlying mechanism remains unresolved. Insulin resistance has been implicated in having a direct effect on the expression of lipoprotein lipase on the cell surface.[100] In non-obese type 2 diabetics, plasma free fatty acids have been found to be elevated consistently after the ingestion of a meal and in insulin glucose clamp experiments.[101,102] These higher plasma free fatty acid levels may contribute to peripheral insulin sensitivity. High free fatty acid concentrations have been shown to inhibit lipolysis and weaken the binding of lipoprotein lipase to TRLs and endothelium bound heparan sulfate.[103] Thus a disturbed fatty acid metabolism may play a key role in the impaired metabolism of TRL's in type 2 diabetics.

The remnant metabolism may also be influenced by a low lipoprotein lipase activity as lipoprotein lipase has been shown to increase the binding of lipoprotein remnants to LDL receptor related protein.[104] Descamps et al[105] have shown that insulin stimulates the uptake of apo E enriched VLDL in rat adipocytes, the effect resulting from an increased translocation of LDL receptor related protein to the cell surface. This may be evidence of effect of insulin resistance on remnant uptake. TRL remnants are also taken up by peripheral tissues including marrow, spleen, lungs and macrophages / monocytes.[106] In insulin resistant states, the peripheral tissues may be less sensitive to the stimulating effect of insulin on the lipoprotein lipase as well as LDL receptor related protein resulting in decreased elimination of remnants by peripheral tissues.

Rate of cholesteryl ester transfer is higher in type 2 diabetics compared to controls.[107] The cholesteryl ester transfer stimulating effect of diabetic VLDL may be due to changes in surface lipid or apolipoprotein content rather than core lipid content since the VLDL-TG concentrations were identical in both diabetics and controls. It has been suggested that the large TG rich VLDL particles prevalent in diabetes may be more potent TG donors and cholesteryl ester acceptors than normal VLDL. Lipid transfer is stimulated during alimentary lipemia, suggesting that the accelerated cholesteryl ester transfer may be more pronounced in the postprandial state as the magnitude of TRL response and the number of cholesteryl ester acceptor particles is increased in type 2 diabetes.[108] Exact clinical significance of enhanced cholesteryl ester transfer remains unknown in humans, but transgenic mice expressing high amounts of cholesteryl ester transfer protein develop more severe atherosclerosis than non-expressing ones.[109] Thus, the accelerated cholesteryl ester transfer in type 2 diabetics may contribute to increased CAD risk as the clearance of the cholesteryl ester accepting remnant particles is also delayed in them. Increased transfer may further reduce HDL-C levels and promote the formation of small dense LDL, thereby worsening the risk profile.

Increased glycation of apolipoproteins influences lipoprotein metabolism in type 2 diabetics.[110] An increased glycation has been reported in virtually all lipoprotein classes in diabetics, especially apo B 100. Glycation of LDL slows its catabolism. Catabolism of TRL's can also be influenced by glycation. Glycated VLDL particles show a reduced degradation in fibroblasts in vitro.[111] Increased apo E glycation may be responsible for the observed catabolic impairment of glycated VLDL. Glycational modification of apo C II may result in delayed triglyceride clearance.[112]

POSTPRANDIAL LIPID ABNORMALITIES AS A RISK FACTOR FOR ATHEROSCLEROSIS IN TYPE 2 DIABETICS

Since postprandial lipid abnormalities particularly postprandial hypertriglyceridemia, have been linked with asymptomatic and symptomatic macrovascular disease in both normo-and hypertriglyceridemic subjects and such abnormalities have been reported in type 2 diabetics, the increased risk of atherosclerosis among them, might therefore be related to the higher postprandial lipemia in them. Very few studies exist in literature which have investigated the relationship between postprandial triglyceridemia & atherosclerosis in type 2 diabetics.

Syvanne et al investigated whether abnormalities in alimentary lipemia explained the increased risk of CAD in subjects with type 2 diabetes.[99] They performed an oral vitamin A fat-load test in four groups of men (each n = 15): DM+CAD+, DM-CAD+, DM+CAD-, DM-CAD-. The postprandial plasma TG responses were significantly larger in both diabetic groups than in the healthy control group. The most marked differences were observed in the Sf 60-400 lipoproteins, whether measured as TG or RP responses. However, there were no differences between the DM+CAD+ and DM+CAD- groups. The between-group differences in alimentary lipemia were only partially explained by fasting TG levels. They concluded that the levels of atherogenic postprandial remnants are increased in type 2 diabetes, however, abnormal postprandial lipid metabolism in them is not related to CAD.

Shinichi Teno et al, investigated the correlation between 4 hour postprandial TG response to a mixed meal & carotid IMT in 61 patients with type 2 diabetes with

fasting normo-and hypertriglyceridemia. They found that the [113] carotid IMT of the patients with fasting hypertriglyceridemia was greater than that of the patients with normal fasting triglyceride (fTG) levels (0.85±0.12 vs. 0.76±0.14 mm; p = 0.02) .In patients with normal fTG levels, a tendency was evident for the carotid IMT to be greater when the postprandial triglyceride (pTG) levels were >2.27 mmol/l. The NN and NH groups consisted of patients with normal fTG levels but with pTG levels <2.27 and >2.27 mmol/l, respectively. Patients with both hypertriglyceridemia and pTG levels >2.27mmol/l formed the HH group. Carotid IMT was significantly increased in the NH (0.86±0.13mm) and HH (0.85±0.12 mm) groups compared with the NN group (0.73±0.13mm; p<0.01). Although postprandial plasma glucose, pTG, and fasting LDL cholesterol levels were all independently correlated with carotid IMT, pTG levels had the strongest statistical influence (p = 0.002).

However, certain lacunae in this study need to be pointed out. Firstly, a 4 hours postprandial TG value might not be representative of actual magnitude of hypertriglyceridemia especially in type 2 diabetics. Lewis et al found that peak TG response in normo-& hypertriglyceridemic type 2 diabetics, occurred 5.4 hours & 7.4 hours respectively after the test meal.[17] Earlier studies from our institution have found that peak TG response occurred at 4.6 hours & 5.5 hours after the test meal in non-diabetics & diabetics respectivel.[19,20] It has been suggested that measurement of peak postprandial TG value & the cumulative TG response as measured by the area under the fat tolerance curve, is more meaningful that a single 4 hour postprandial value. Secondly, for fat challenge, a mixed meal with fats providing only 20% of total energy has been used which may provide an inadequate fat challenge. A fat dense meal would provide a more sensitive measure of postprandial lipaemia than a mixed

meal, which have tried to ascertain the relationship between post prandial lipaemia and endothelial function from the Indian subcontinent.

POST-PRANDIAL HYPERTRIGLYCERIDEMIA AND ENDOTHELIAL DYSFUNCTION

The endothelium, the cell layer lining the blood vessels, has an enormous range of important hemeostatic roles. Normal endothelial functions include control over thombosis and thrombolysis, platelet and leucocytes interactions with the vessel wall, and regulation of vascular tone and growth. In addition, normal endothelium also plays an important role in vascular growth, leukocyte adhesion, immunological regulation, metabolism of circulating amines lipoprotein metabolism, and integration and transduction of blood-born signals.[114]

The endothelium responds to physical and chemical stimuli by synthesis and/or release of a variety of vasoactive, thromboregulatory and signal molecules as well as growth factors. Substances released by the endothelium include nitric oxide (NO, the endothelium-derived relaxing factor or EDRF), prostacyclin, endothelins, endothelial cell growth factor(s), interleukins, adhesion molecules, and fibrinolytic factors.[115]

The endothelium controls underlying smooth muscle tone in response to certain pharmacological and physiological stimuli.[116] This involves a number of membrane receptors, complex intracellular pathways, synthesis and release of a variety of relaxing and constricting substances. The existence of an endothelium-derived relaxing factor was first suggested by Furchgott and Zawadzki in 1980[116], when these investigators observed that rabbit aortic rings relaxed to acetylcholine

only in the presence of an intact endothelium. The biological effects of EDRF are mediated by NO. NO is synthesized from L-arginine by an enzyme, NO synthase.[117] The generation of NO from L-arginine can also be specifically blocked by arginine analogues, such as N^G-monomethyl-L-arginine (L-NMMA). NO maintains a low arterial tone at rest in both the systemic and pulmonary circulations.[118] The NO release is stimulated by increased flow (leading to increased shear stress on the endothelium)[119] and by bradykinin, thrombin, acetylcholine, serotonin, and a variety of other circulating agents, which increase NO release via activation of specific endothelial cell membrane receptors. In smooth muscle cells, this causes a reduction of intracellular calcium concentration, leading to vasorelaxation.[120]

Endothelial Dysfunction

Endothelial damage can result in an imbalance between relaxing and contracting factors, between anti-and procoagulant mediators, or growth-inhibiting and -promoting factors. Such dysfunction can result from mechanical or biochemical injury to the endothelium, or from stimulation of endothelial cells leading to inappropriate or abnormal physiological functions. Clinically, endothelial dysfunction can manifest as vasospasm, thrombus formation, hypertension, and atherosclerosis.

In normocholesterolemic animals, it has been shown that physical damage to the endothelium cells lead to atherosclerotic lesion formation.[121] The consequences of endothelial dysfunction that promote the formation of atherosclerotic lesions, such as fatty streaks and fibrous plaques, include increased adherence of monocytes which is prompted with cellular adhesion molecules[122], enhanced permeability to monocyte/macrophages and lipoproteins, which then accumulate in the vessel wall, increased platelet adherence, and increased smooth muscle cell migration and

proliferation.[123] The interaction with blood leukocytes is mediated by adhesion molecules i.e. vascular cellular adhesion molecules (VCAM-1), intercellular adhesion molecules (ICAM-1), E-selectin, P-selectin, cytokines like interleukins, monocyte-chemotactic factors and granulocyte or monocyte-colony stimulating factors. Growth factors modulate the proliferation of smooth muscle cells. Very high circulating level of soluble vascular cell adhesion molecules of (sVCAM) have been found in diabetic patients with atherosclerosis.[124,125] Therefore, it has been suggested that increased level of cellular adhesion molecules may be an index of endothelial activation during the early stage of atherosclerosis.[126,127] Endothelial dysfunction may also be accompanied by decreased production and/or local bioavailability of NO. In the presence of increased oxidative stress, this may be caused by excessive production of superoxide anions, with consequent degradation of NO before it can reach its target tissues. As NO acts as a vasodilator and inhibits platelet adherence and aggregation, smooth muscle proliferation and endothelial cell-leucocyte interactions, decreased NO activity may contribute importantly to the initiation and progression of atherosclerotic lesions.[127] Furthermore, experimentally induced acute hypertension has been shown to disturb endothelial integrity.[128] Several cardiovascular risk factors, such as high low density lipoprotein (LDL) cholesterol, low high density lipoprotein (HDL) lipoprotein[130], active and passive cigarette smoking[129], and diabetes[130] have been consistently associated with disturbances in normal endothelial physiology, even among young subjects without signs or symptoms of overt atherosclerosis. The various mechanisms whereby these risk factors cause endothelial damage are largely unknown; however, a common denominator for all these conditions is increased oxidative stress, which has therefore been suggested as an important cause of endothelial dysfunction.

It has been recently reported that postprandial hypertriglyceridemia can cause endothelial dysfunction which is recognized as an early process of arteriosclerosis even in healthy subjects.[131] As a result of many epidemiological studies, it now seems the HTG is a risk factor of atherosclerosis.[131,132] It has been reported that the risk of coronary heart disease is related more closely to postprandial serum triglyceride level or to delayed chylomicron remnant clearance than to fasting serum triglyceride level. Postprandial HTG following a high fat meal can cause endothelial [133] dysfunction even in healthy subject.[134,135] The mechanism of postprandial HTG-induced endothelial dysfunction is still not clear, although it has been suggested that oxidative stress or direct injury to the vascular wall by triglyceride rich lipoprotein particles may cause endothelial dysfunction.[135]

This endothelial dysfunction is an early process of atherosclerosis and consequently a very sensitive parameter of the early stage of atherosclerosis and thereby useful in the early diagnosis of patients with high risk of coronary artery disease.[134,136,137]

TESTING OF ENDOTHELIAL DYSFUNCTION

Qualities of an ideal test for endothelial dysfunction

Ideally, testing for arterial endothelial dysfunction should involve methods that are safe, noninvasive, readily available, economical, reliable, and reproducible, that correlate with the extent of subclinical atherosclerosis, predict the subsequent risk, and respond to therapy.

Table 5: Markers and assays for endothelial function

Direct assessment of nitric oxide and its metabolites in plasma and urine samples

Functional methods to measure nitric oxide dependent vasomotion

- Invasive coronary testing
- Invasive forearm test: plethysmography method
- Noninvasive coronary testing - position emission tomography
- Noninvasive ultrasound method (flow-mediated dilatation)

Circulating markers of endothelial function

- Asymmetric dimethylarginine (endogenous inhibitor of nitric oxide synthase)
- Endothelin-1
- von Willebrand Factor
- Tissue type plasminogen inhibitor

Adhesion molecules

- ICAM-1 (intercellular adhesion molecule-1)
- VCAM-1 (vascular cell adhesion molecule-1)
- E-selectin
- P-selectin

Studies on endothelial dysfunction

Vogel et al[138], demonstrated that endothelial function is impaired in a sample of middle aged men and women after a high fat meal and the transient endothelial dysfunction showed a significant correlation with PPTG. Doi et al,[139] observed that remnant lipoproteins contain a substantial amount of phospholipid hydroperoxides, capable of impairing flow mediated vasodilation. Kugiyama et al[140] showed that triglyceride rich remnant lipoproteins have, an independent association with endothelial dysfunction in epicardial coronary arteries in patients without angiographycally proven coronary artery disease. Marchesi et al[141], found that even in

young healthy subjects with few of cardiovascular risk factors, high fat diets may lead to atherosclerosis through mechanism at least in past independent of changes in cholesterol level, by impairing endothelial dependent vasoreactivity.

Anderson et al[28] found that in type 1 diabetes mellitus subjects when fasting endothelial dysfunction is present, postprandial lipemia appears to result in further decrements in endothelial function, which may related to TG, HDL, VLDL, LDL and a greater increase in oxidative stress. Vakkilainen et al[141] found that triglyceride level do not correlated with endothelial dysfunction whereas LDL size correlate with endothelial dysfunction Lewis et al[142] compared obese HTG subjects with lean normotriglyceridemic subjects. They found that endothelial function were impaired in the obese HTG group. Chowienczyk et al[143] and Schnell et al[144] found no correlation between fasting triglyceridmia & endothelial dysfunction.

Plotnik et al reported that pretreatment of subjects with antioxidants vit C and E block the postprandial endothelial dysfunction. Ceriello et al[145] found that both postprandial hyperglycemia and hypertryglyceridemia produce endothelial dysfunction in both diabetic and normal subjects. Postprandial hyperglycemia and hypertriglyceridemia have an independent and cumulative effect in determining endothelial dysfunction. Jang-HoBae et al[135] suggest that acute HTG causes endothelial dysfunction via enhanced oxidant stress. Simuna marchesi et al[131] found that a transient postprandial impairment of brachial artery FMD is evident in young healthy men after a high-fat meal, and it closely associated with triglyceride levels. Kiyotaka Kugiyama et al[140] found that remnant lipoprotein levels have a significant and independent correlation with impaired endothelial function in large and resistance

coronary arteries in humans. The decrease in coronary NO activity associated with the increase in the remnant levels may contribute in part to this correlation.

Lundman et al[146] found that transient triglyceridemia, produced by infusion of trigleceride infusion, decreased vascular reactivity in brachial artery in young, healthy men without risk factor for coronary heart disease. Forstermann et al[147] showed that atherosclerotic lesions lead to a selective attenuation of endothelial dependent vasodilation in human coronary arteries. Sorensen et al showed that endothelial dependent dilation is present in children with familial hypercholesterolemia as young as 7 year of age and the degree of impairment is related to the lipoprotein(a) level. Steinberg et al[148] showed that an elevated circulating FFA levels cause endothelial dysfunction and impaired endothelial function in the insulin resistant human may be secondary to the elevated FFA concentration.

There are very few studies comparing the post prandial lipaemia in patients with type 2 diabetes mellitus with and without macrovascular disease and healthy subjects.

With this background the present study was planned, to assess the post prandial lipaemia and the post prandial triglyceride levels and their clearance in patients with type 2 diabetes mellitus,with and without macrovascular disease and healthy subjects.

METHODOLOGY

SUBJECTS:

Assessment of post parandial lipaemia after oral fat load in fasting as well in post prandial state were made in 40 type 2 diabetic subjects and 20 healthy controls to investigate any relationship between them.Twenty type 2 diabetic patients with macrovascular disease and twenty type 2 diabetic patients without macrovascular disease were recruited consecutively from the diabetic clinic and medical wards at RLJH and RC if they fulfilled the criteria for inclusion.

Inclusion Criteria:

1. Patients with type 2 diabetes mellitus aged >30 years. The diagnosis of diabetes was made on the basis of revised American Diabetic Association Criteria i.e. fasting plasma glucose ≥126mg/dl & 2 hour post-prandial plasma glucose ≥ 200mg/dl.[149]
2. Duration of diagnosed diabetes ≥1 year.
3. clinical Evidence of MVD such as signs and symptoms like chest pain and shortness of breath in subjects of coronary artery disease (CAD), weakness or paralysis of one or more limbs, focal neurological deficit such as slurred speech, aphasia in subjects of cerebro vascular disease (CVD),claudication or history of Sores, wounds, or ulcers that heal slowly in subjects of peripheral vascular disease (PVD) in group I subjects.

Exclusion Criteria:

1. Fasting serum triglycerides >250 mg/dl
2. Inherited disorders of lipids/lipoprotein metabolism &/or family history of such disorders.

3. Deranged liver function tests.
4. Patients on drugs affecting lipid metabolism like β-blockers, diuretics, lipid lowering drugs & alcohol.
5. Patients taking vitamin supplements or antioxidants for last two weeks.
6. Patients who were on insulin during last 4 months.
7. Clinical and/or biochemical evidence of hypothyroidism or Cushing's syndrome.
8. Patients with Congestive heart failure (CHF) and hypercholesterolemia (fasting total serum cholesterol $\geq$ 250 mg/dl).
9. Women on hormone replacement therapy (HRT).
10. Acute infection anywhere in body or gangrene.

Diabetic subjects on oral hypoglycemic agents, were asked to omit the drug on the morning of the test.

Twenty non diabetic individuals who were age, sex and body mass index (BMI) matched, who were non smokers, non-alcoholic and do not have overt clinical evidence of coronary artery disease (CAD), cerebrovascular disease (CVD) or peripheral vascular disease (PVD) were recruited as controls.

Thus there were three study groups:

Group – I (n=20)**:** The subjects with Type 2 Diabetes Mellitus with macrovascular disease (CAD, CVD, PVD).

- CAD was defined on the basis of (1) ECG evidence of CAD or (2) TMT evidence for reversible ischemia.
- CVD was defined on the basis of history of diagnosed stroke.
- PVD was defined on the basis of history and clinical examination and / or ankle / brachial index less than 0.9.

Group – II (n=20)**:** The subjects with type 2 diabetes Mellitus without macrovascular disease These were type 2 diabetic patients with no h/o or clinical evidence of overt macrovascular disease.

Group – III (n=20)**:** Non diabetic individuals without macrovascular disease).

STUDY DESIGN

An informed consent was obtained from each subject prior to entering the study. A detailed history and physical examination was carried out for every subject who entered in the study as per a pre-designed proforma. This included h/o duration of diabetes, family h/o premature atherosclerosis, h/o macrovascular disease in the form of CAD, CVD or PVD, dyslipidemia, history regarding complications of diabetes as well as history and details of treatment received.

Examination included thorough physical examination, assessment of vital parameters, anthropometry (ht, wt, waist/hip ratio) and systemic examination.

Screening investigations included Hb concentration, glycosylated hemoglobin, fasting and post-prandial blood glucose, blood urea, serum creatinine, liver function tests, fasting lipid profile, chest x-ray, routine urine analysis, electrocardiography (ECG) & fundus examination by ophthalmologist.

All subjects were admitted to the medical ward a day prior to the study. After 14 hours fasting, during which water intake was allowed, blood was collected for various biochemical parameters (0 hr). The subjects were then given a standardized fatty meal containing 729 kcal/m^2 body surface area (BSA) and 5.3 gm protein, 24.75 gm. carbohydrates, 240 mg. cholesterol and 65.2 gm. Fat (ref). This was in the form

of whipped cream and fruits. It was given over 10-15 minutes. Blood samples were drawn at 2,4,6 and 8 hrs after the oral fat challenge.

Serum was separated in all the samples by centrifuging it immediately after collection and stored at –20° Celsius for various estimations to be done later.

ESTIMATION OF BIOCHEMICAL PARAMETERS

Blood glucose and serum lipids were estimated in the 0,2,4,6&8 hr samples.

Plasma glucose

Plasma glucose was estimated by glucose oxidase/peroxidase method (Barham and Trinder, 1972)[150].

Principle:

The enzyme glucose oxidase (GOD) catalyses oxidation of glucose to gluconic acid and hydrogen peroxide (H_2O_2).

$$\text{Glucose} + 2H_2O + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + 2H_2O_2$$

On addition of enzyme peroxidase (POD) the H_2O_2 so produced oxidatively couples with 4-aminoantipyrine and phenol to produce a red quinoneimine complex, absorbance of which is measured at 510 nm.

$$H_2O_2 + \text{Phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} \text{Red quinoneimine complex} + H_2O$$

Lipid Profile

a) Total serum cholesterol

Total serum cholesterol was estimated by enzymatic method using cholesterol esterase (Allain et al. 1974)[151].

Principle:

1) Cholesterol esters are hydrolyzed by cholesterol esterase into free cholesterol and fatty acids.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

2) Cholesterol oxidase (CHO) catalyzes oxidation of free cholesterol to cholest–4-en-3one and hydrogen peroxide.

$$\text{Cholesterol} + O2 \xrightarrow{\text{CHO}} \text{Cholest-4-en-3one} + H_2O_2$$

3) In the presence of peroxidase (POD), hydrogen peroxide oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye, has maximum absorbance at 510 nm. The intensity of red color is proportional to the amount of cholesterol in specimen.

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\textbf{POD}} \text{Red quinoneimine dye} + 2H_2O$$

HDL-Cholesterol

HDL cholesterol was estimated by method of Burstein et al (1970)[152], using kits from Accurex Biomedical Private Limited, Mumbai.

Principle:

The lipoproteins containing apoliprotein-B such as chylomicrons, low density lipoprotein (LDL) and very density lipoprotein (VLDL) are precipitated by phosphotungstate in the presence of Mg^{2+}. After centrifugation, high density lipoprotein (HDL) remains in supernatant, cholesterol content of which is estimated by the enzymatic method as described above.

Serum triglycerides

Serum triglycerides were estimated by an enzymatic method (Werener and Gabriesulsen, 1981)[153].

Principle:

(1) Triglycerides are hydrolyzed by lipoprotein lipase (LPL) into glycerol and fatty acids.

$$\text{Triglycerides} + 3H_2O \xrightarrow{\textbf{LPL}} \text{Glycerol} + 3\ \text{Fatty Acids}$$

(2) Catalyzed by glycerol kinase (GK), glycerol is phosphorylated to glycerol-3phophate.

$$\text{Glycerol} + \text{ATP} \xrightarrow{\textbf{GK}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

(3) Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate and hydrogen peroxide, by glycerol phosphate oxidase (GPO):

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\textbf{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

(4) In the presence of peroxidase (POD), hydrogen peroxide causes oxidation of phenolic chromogen to a red coloured compound.

$$\text{H2O2} + \text{Phenolic chromogen} \xrightarrow{\textbf{POD}} \text{Red coloured compound}$$

Absorbance of this red coloured compound is measured at 500 nm.

Serum VLDL and LDL

- Serum VLDL was estimated by using VLDL = TG/5 based on the average ratio of TG to cholesterol in VLDL
- Serum LDL was determined from the Friedwald's and Fredrickson's formula (1972)[154], which is :

  LDL = Total Cholesterol – (HDL + VLDL).

Glycosylated Hemoglobin

Glycosylated hemoglobin was determined by ion-exchange chromatography as described by Goldstein et al. (1986)[155], using kits from ERBA test.

Principle:

Ion exchange chromatography separates hemoglobin variants on the basis of charge. The weak binding cation exchange resin (negatively charged), packed in a disposable minicolumn, has an affinity for hemoglobin, which is positively charged. The patients's sample is hemolyzed, and an aliquot of hemolysate is applied to column and mixed continuously for 5 minutes. During mixing non-glycosylated hemoglobin binds to the resin and glycosylated hemoglobin remains in supernatant which is then separated from the resin and absorbance is measured at 415 nm. Total hemoglobin is measured separately by adding an aliquot of hemolysate to deionised water and absorbance measured at 415 nm. The ratio of the two absorbances gives the percentage glycohaemoglobin.

Reagents:

1) Cation exchange resin (pH –6.9)
2) Lysing reagent
3) Glycohemoglobin Calibrator (10%) or Glycohemoglobin standard (10%).

Procedure:

1) Blood sample was collected in EDTA vial and hemolysate was prepared by adding 500µl of lysing reagent to 1000ml of test sample. Hemolysate of glycohemoglobin calibrator was also prepared in the same way. Both the tubes were mixed well and allowed to stand for 5 minutes till complete lysis.
2) 100 µl of hemolysate was added to appropriately marked ion exchange tubes.
3) The filter separator was then positioned approximately 2 cm above the liquid level in the tube and tubes were mixed on a shaker for 5 minutes.
4) Separator was pushed until the resin was firmly packed and supernatant containing glycohemoglobin was separated.
5) For glycohemoglobin, absorbance of supernatant was taken at 415 nm against deionised water blank.
6) To estimate the total haemoglobin, 20 µl each of calibrator and sample hemolysate were added to 5.0 ml of deionised water, mixed well and absorbance was taken at 415 nm.

Calculations:

Ratio of glycohemoglobin absorbance to the total hemoglobin absorbance was calculated using the following equations:

R_C = Absorbance of calibrator (Glyco) / Absorbance of calibrator (Total)

R_U = Absorbance of unknown (Glyco) / Absorbance of unknown (Total)

$$\% \text{ glycohemoglobin of known} = \frac{R_U}{R_C} \times \text{value of calibrator.}$$

Plasma glucose will be analyzed by glucose oxidase peroxidase method. TGs will be estimated by Lipase method. Total cholesterol will be determined by cholesterol esterase/oxidase method. HDL-C will be quantitated in the supernatant after precipitation of other lipoproteins with phosphotungstate/magnesium. LDL will be calculated using the friedwald formula. VLDL cholesterol will be determined by the formulaTG/5. Fibrinogen level will be determined by Ellis and Stransky method. Insulin will be measured by radio immuno assay kit. sVCAM will be measured by a solid phase sandwich ELISA kit.

STATISTICAL ANALYSIS

The data were expressed as mean ± SD for all the study groups. The significance of difference was determined using ANOVA followed by Tukey test correlation between different parameter were determined by Pearson correlation coefficient.

OBSERVATIONS AND RESULTS

The findings of the study are presented in this section. The following were the observations made in patients with type 2 diabetes mellitus with macrovascular disease (categorized as Group 1), patients with type 2 diabetes without macrovasular disease (categorized as Group 2) and healthy subjects (categorized as Control group).

Table 6: Age and Physical Findings of the Subjects in Three Study Groups

		N	Mean	Std. Deviation
AGE	Group 1	20	53.80	10.670
	Group 2	20	54.10	9.078
	Control	20	46.25	8.522
HEIGHT	Group 1	20	166.95	7.877
	Group 2	20	169.20	7.851
	Control	20	164.95	6.057
WEIGHT	Group 1	20	68.55	7.345
	Group 2	20	62.95	7.729
	Control	20	58.05	7.171
BMI	Group 1	20	23.55	2.164
	Group 2	20	22.90	3.354
	Control	20	23.35	2.185
WC	Group 1	20	89.80	6.363
	Group 2	20	83.45	4.628
	Control	20	81.65	4.499
HC	Group 1	20	95.55	5.862
	Group 2	20	90.40	4.946
	Control	20	94.70	4.473

The above table shows the age and physical parameters of the subjects in the three study groups. It elucidates that the mean age of group 1 was 53.8 years, of group 2 was 54.1 years and of the control group was 46.2 years. The mean BMI was 23.5 in group 1, 22.9 in group 2 and 23.3 in control group. The WC mean was observed to be 89.8 in group 1, 83.4 in group 2 and 81.6 in control group. The HC mean was 95.5 in group 1, 90.4 in group 2 and 94.7 in control group. As seen in Table 1, all the three study groups are age, sex and BMI matched. Waist was significantly higher in diabetic subjects as compared with control however, no significant difference was found between group 2 and group 3 Waist hip ratio was significantly higher in diabetic groups (group 1 and 2) as compared to controls (group 3). Diabetic subjects with macrovascular disease were having significantly higher WHR than diabetic subjects without macrovascular disease.

Family history of CAD was present in 3/13 (23.0%) in subjects with macrovascular disease and 2/13 (15.38%) in diabetic subjects without macrovascular disease.

Table 7: Gender Representation of the Subjects in the Three Groups

SEX * Group Cross tabulation					
Count					
		Group			Total
		Group 1	Group 2	Control	
SEX	F	5	8	11	24
	M	15	12	9	36
Total		20	20	20	60

The above table illustrates the gender distribution of the subjects in the three groups. The table shows that the total numbers of females were 24 and males were 36. In group 1 and 2, majority of the subjects were male and in control group, majority of them were female.

Figure 5: Gender Representation of the Subjects in the Three Groups

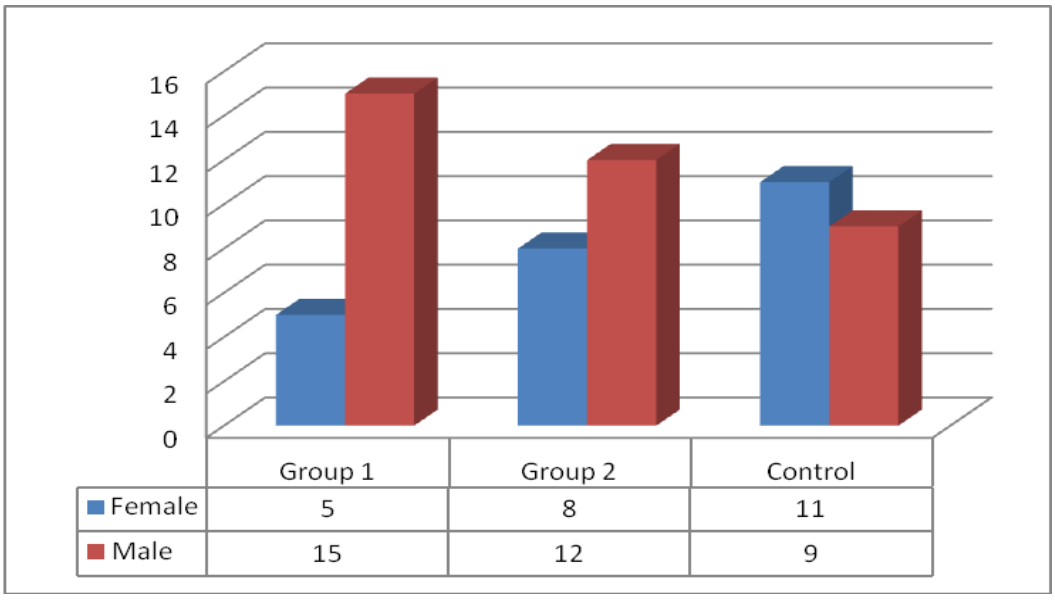


Table 8: Family History Representation of the Subjects in Group 1 and 2

FAMILY HISTORY OF DM * Group				
Count				
		Group		Total
		Group 1	Group 2	
FAMILY HISTORY OF DM	NO	11	15	26
	YES	9	5	14
Total		20	20	40

The above table represents the family history of Diabetes Mellitus (DM) in group 1 and 2. It shows that in total, 14 subjects had a family history of DM out of 40. In group 1, 9 subjects and in group 2, 5 subjects were noted to have a family history of DM.

Figure 6: Family History Representation of the Subjects in Group 1 and 2

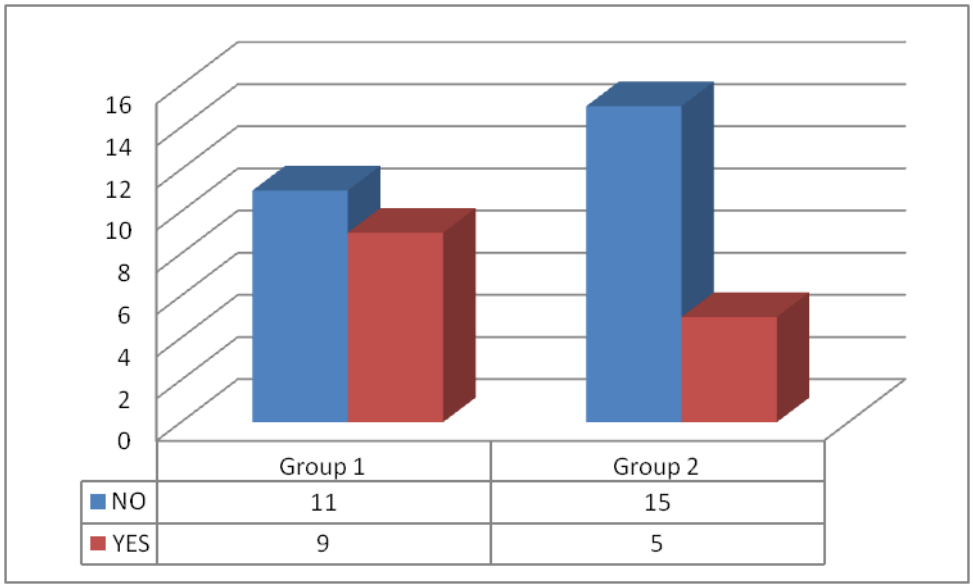
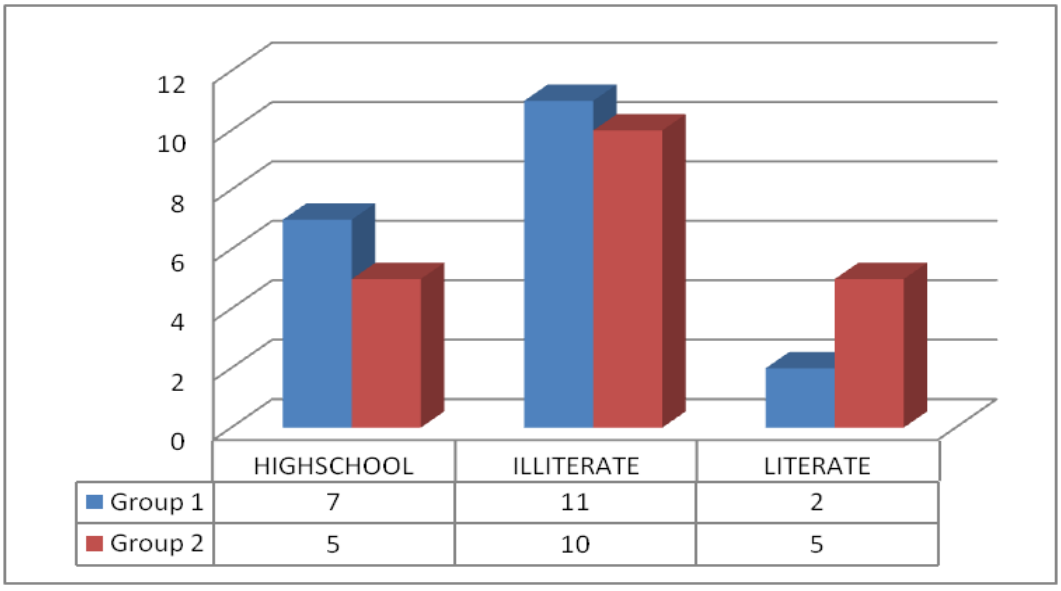


Table 9: Literacy Level of the Subjects in Group 1 and 2

LITERACY * Group				
Count				
		Group		Total
		Group 1	Group 2	
LITERACY	HIGHSCHOOL	7	5	12
	ILLITERATE	11	10	21
	LITERATE	2	5	7
Total		20	20	40

Figure 7: Literacy Level of the Subjects in Group 1 and 2

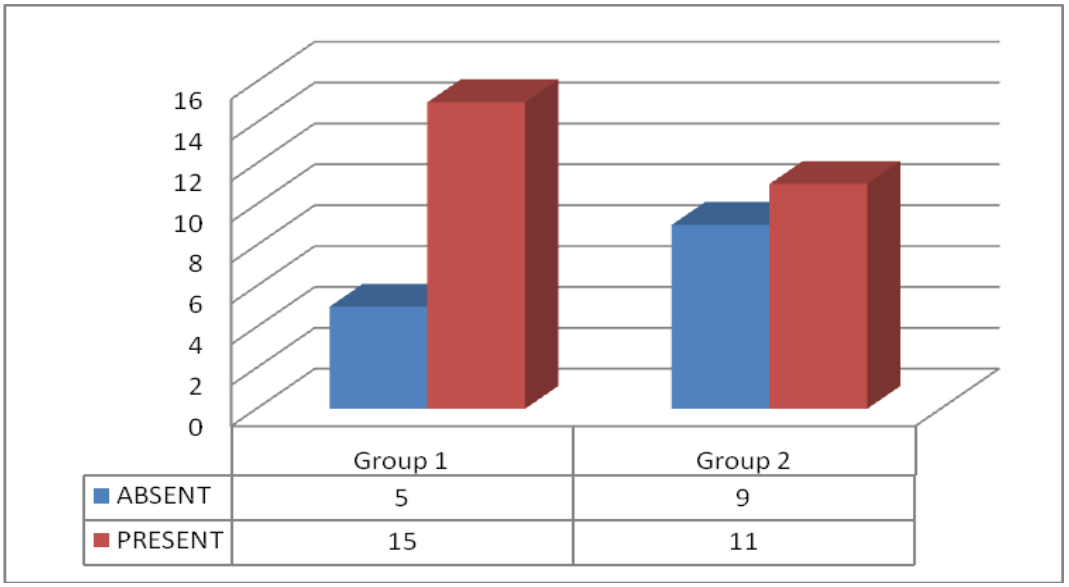


The above figure shows that majority of the participants i.e., 21 out of 40 were illiterate, followed by 12 who studied high school and 7 subjects who had some education.

Table 10: Dyslipidemia profile of the Subjects in Group 1 and 2

DYSLIPIDEMIA * Group				
Count				
		Group		Total
		Group 1	Group 2	
DYSLIPIDEMIA	ABSENT	5	9	14
	PRESENT	15	11	26
Total		20	20	40

Figure 8: Dyslipidemia profile of the Subjects

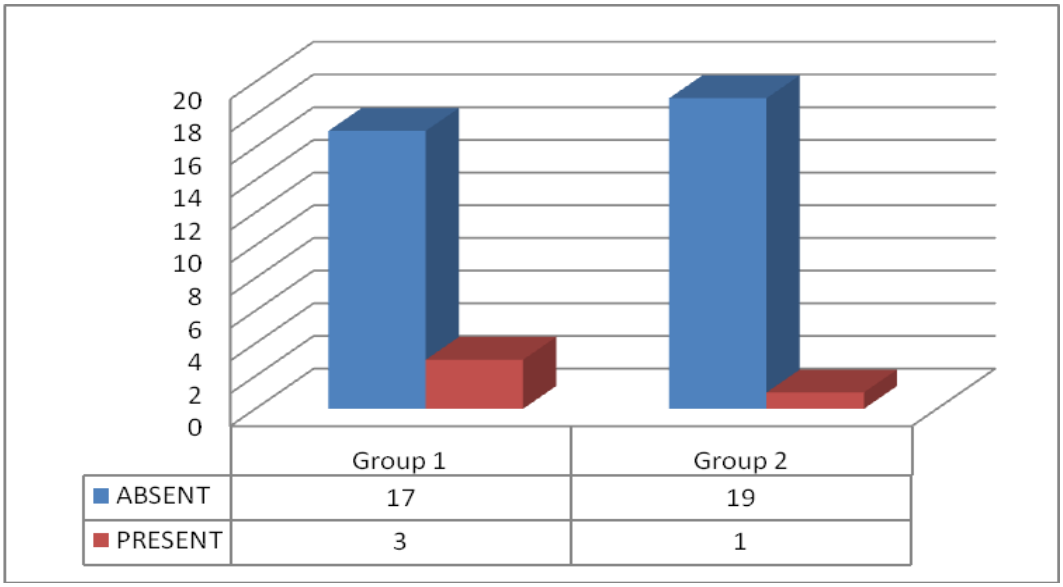


The above figure shows the total subjects with and without Dyslipidemia. It can be interpreted that a total of 26 subjects (65%) had Dyslipidemia and 14 subjects (35%) did not have it.

Table 11: Nephropathy profile of the Subjects in Group 1 and 2

NEPHROPATHY * Group				
Count				
		Group		Total
		Group 1	Group 2	
NEPHROPATHY	ABSENT	17	19	36
	PRESENT	3	1	4
Total		20	20	40

Figure 9: Nephropathy profile of the Subjects

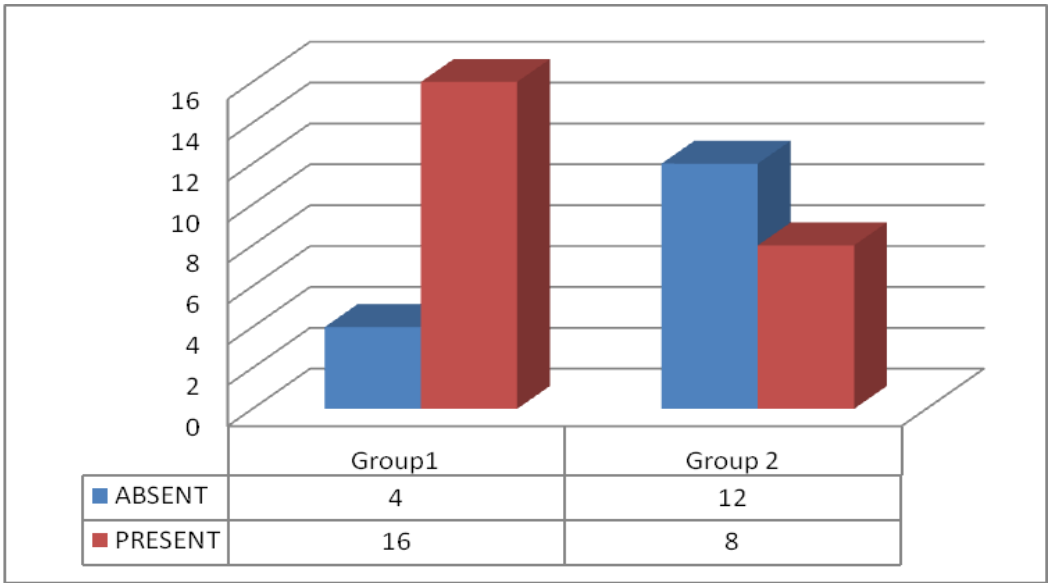


The above figure represents the total subjects with and without Nephropathy. It can be interpreted that a total of 4 subjects (10%) had Nephropathy and 36 subjects (90%) did not have the disease. Which implies that majority did not have the disease.

Table 12: Retinopathy profile of the Subjects in Group 1 and 2

RETINOPATHY * Group				
Count				
		Group		Total
		Group 1	Group 2	
RETINOPATHY	ABSENT	4	12	16
	PRESENT	16	8	24
Total		20	20	40

Figure 10: Retinopathy profile of the Subjects



The above figure demonstrates the total subjects with and without Retinopathy. It can be interpreted that a total of 24 subjects (60%) had Retinopathy and 16 subjects (40%) did not have the disease.

Table 13: Age of Onset of DM, its Duration and Ankle Brachial Index (ABI) of the Subjects of Group 1 and 2

	Group	N	Mean	Std. Deviation
AGE AT ONSET OF DM	Group 1	20	47.75	8.039
	Group 2	20	48.45	6.886
DURATION OF DM	Group 1	20	5.95	3.120
	Group 2	20	5.65	4.095
ABI	Group 1	20	1.000500	.1213293
	Group 2	20	.986500	.0423364

The above table shows the mean age of onset of DM in group 1 was 47.7 and group 2 was 48.4 years respectively. The mean duration of DM was 5.9 years and 5.65 years in group 1 and 2 respectively. The table also demonstrates the mean Ankle Brachial Index as 1 in group 1 and 0.98 in group 2.

Table 14: Comparison of 5 Occasions of Glucose levels among the Subjects in Three Groups

	Group					
	Group 1		Group 2		Control	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
GLUCOSE0	247.15	81.74	161.95	45.11	101.20	10.81
GLUCOSE2	357.80	102.05	238.90	57.43	157.55	17.59
GLUCOSE4	378.85	82.65	300.75	52.63	201.15	23.03
GLUCOSE6	335.35	71.92	298.95	53.26	182.35	19.42
GLUCOSE8	286.35	59.28	240.05	42.18	152.90	18.37

The table above elucidates the glucose level comparison of the subjects of the three groups in five occasions. The five occasions of glucose reading considered was at 0, 2, 4, 6 and 8 hours. It can be inferred that the mean glucose levels of group 1 ranged from 247 to 378 while that of group 2 ranged from 162 to 300 and that of control group ranged from 101 to a maximum of 201.

Table 15: Comparison of 5 Occasions of Triglyceride levels among the Subjects in Three Groups

	Group					
	Group 1		Group 2		Control	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
TRIGLY0	169.10	29.92	118.40	20.36	93.45	35.23
TRIGLY2	255.60	41.05	165.75	21.29	146.60	32.05
TRIGLY4	322.60	38.94	211.40	31.21	169.25	18.99
TRIGLY6	364.35	40.22	189.85	26.49	154.25	20.71
TRIGLY8	370.70	52.41	157.55	25.39	132.60	19.26

The table above demonstrates the triglyceride level comparison of the subjects of the three groups in five occasions at 0, 2, 4, 6 and 8 hour. It shows that the mean triglyceride levels of group 1 ranged from 169 to 370 while that of group 2 ranged from 118 to 211 and that of control group ranged from 93 to 169.

Table 16: Comparison of 5 Occasions of Cholesterol levels among the Subjects in Three Groups

	Group					
	Group 1		Group 2		Control	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
CHOLES0	218.50	22.91	186.25	21.39	161.40	14.84
CHOLES2	262.75	28.22	229.30	34.89	197.20	20.02
CHOLES4	292.70	14.48	270.15	33.78	254.10	26.17
CHOLES6	316.45	20.24	260.10	24.75	231.70	21.96
CHOLES8	331.80	26.16	217.20	24.95	195.60	19.64

The table above shows the cholesterol level comparison of the subjects of the three groups in five occasions. It can be seen that that the mean cholesterol levels of group 1 ranged from 218 to 332 while that of group 2 ranged from 186 to 270 and that of control group ranged from 161 to 254.

Table 17: Comparison of 5 Occasions of Very-Low-Density Lipoprotein (VLDL) Cholesterol levels among the Subjects in Three Groups

	Group					
	Group 1		Group 2		Control	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
VLDL0	33.80	5.98	23.75	3.89	18.65	7.13
VLDL2	51.10	8.10	33.20	4.24	29.20	6.43
VLDL4	64.40	7.73	42.25	6.24	33.75	3.91
VLDL6	72.75	8.06	37.95	5.32	30.95	4.08
VLDL8	74.20	10.45	31.60	5.11	26.50	3.89

The table above shows the VLDL cholesterol level comparison of the subjects of the three groups in five occasions. The mean VLDL levels of group 1 ranged from 34 to 74 while that of group 2 ranged from 24 to 42 and that of control group ranged from 19 to 34.

Table 18: Comparison of 5 Occasions of Low-Density Lipoprotein (LDL) Cholesterol levels among the Subjects in Three Groups

	Group					
	Group 1		Group 2		Control	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
LDL0	160.58	23.39	125.72	21.66	94.61	16.92
LDL2	190.93	30.71	163.90	36.17	123.38	20.51
LDL4	210.08	16.24	199.27	35.99	177.30	27.98
LDL6	227.08	20.27	196.68	25.82	154.35	22.77
LDL8	242.56	26.27	157.84	25.87	121.23	21.44

The table above illustrates the LDL cholesterol level comparison of the subjects of the three groups in five occasions. The mean LDL levels of group 1 ranged from 160 to 242 while that of group 2 ranged from 125 to 157 and that of control group ranged from 94 to 121.

Table 19: Levels of 5 Occasions of High-Density Lipoprotein (HDL) Cholesterol levels among the Subjects in Three Groups

	Group					
	Group 1		Group 2		Control	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
HDL0	24.10	4.08	36.85	5.10	48.10	6.22
HDL2	20.70	3.87	32.25	4.24	44.50	6.24
HDL4	18.10	3.81	28.60	3.80	42.95	6.27
HDL6	16.50	3.36	25.45	3.05	46.50	6.53
HDL8	15.10	2.81	27.85	3.27	47.85	6.06

The table above shows the HDL cholesterol levels of the subjects of the three groups in five occasions. The mean HDL levels of group 1 ranged from 15 to 24 while that of group 2 ranged from 25 to 37 and that of control group ranged from 43 to 48.

DISCUSSION

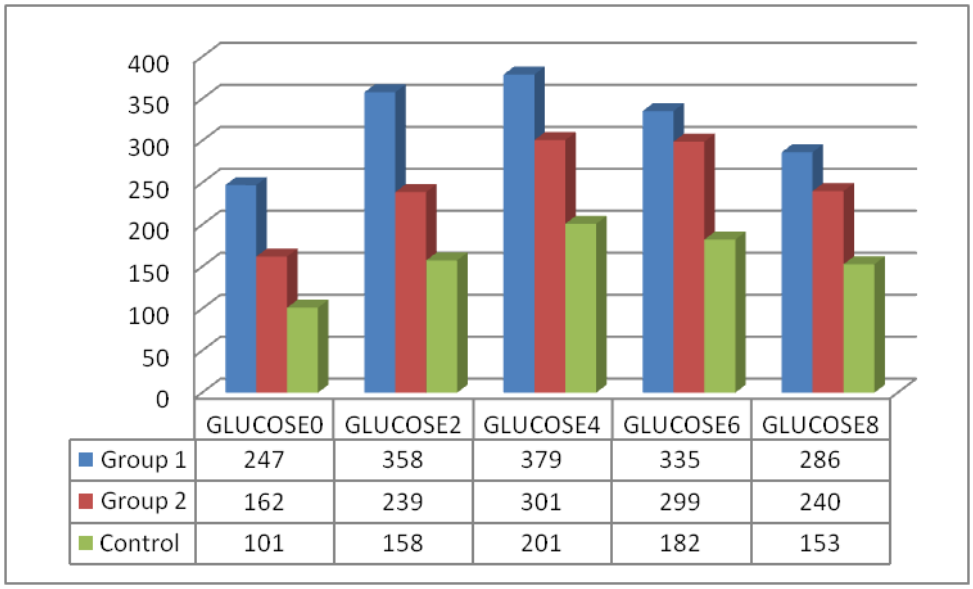
Diabetes Mellitus is associated with metabolic derangement of hyperglycemia, insulin resistance and dyslipidemia. Diabetes leads to several vascular and non-vascular complications and the macro vascular disease associated with diabetes is a major cause of morbidity and mortality. Dyslipidemia that accompanies type 2 DM may play an important role in the pathogenesis of diabetes associated atherosclerosis. TG rich lipoproteins (VLDL, IDL) are believed to cause an endothelial dysfunction an early event in atherosclerosis.

The present study was conducted in 20 type 2 diabetic patient with macrovascular disease (group 1), 20 type 2 diabetic patients without macrovascular disease (group 2) and 20 healthy controls (group 3) to determine whether postprandial lipid abnormalities particularly postprandial hypertriglyceridemia and the triglyceride clearance in the two different groups of diabetics and controls.

All the subjects in current study were age, sex and BMI matched. Measurement of waist as well as Waist: Hip ratio which indicate truncal obesity and serve as surrogate markers of insulin resistance, were higher in diabetic patient with and without macrovascular disease compared to controls.

This would suggest that despite having similar BMI, the diabetic subjects were, more insulin resistance than controls also the near normal BMI of both diabetics groups suggest that despite being non-obese they displayed abnormalities of body fat distributions which favour insulin resistance. Several early studies have found their truncal obesity among Indian diabetics both obese and non-obese.[156,157]

Figure 11: Comparison of 5 Occasions of Glucose levels among the Subjects in Three Groups



	GLUCOSE0	GLUCOSE2	GLUCOSE4	GLUCOSE6	GLUCOSE8
Group 1	247	358	379	335	286
Group 2	162	239	301	299	240
Control	101	158	201	182	153

Comparison between 5 occasions F=138.347, P<0.001

Comparison between 3 groups F=56.147, P<0.001

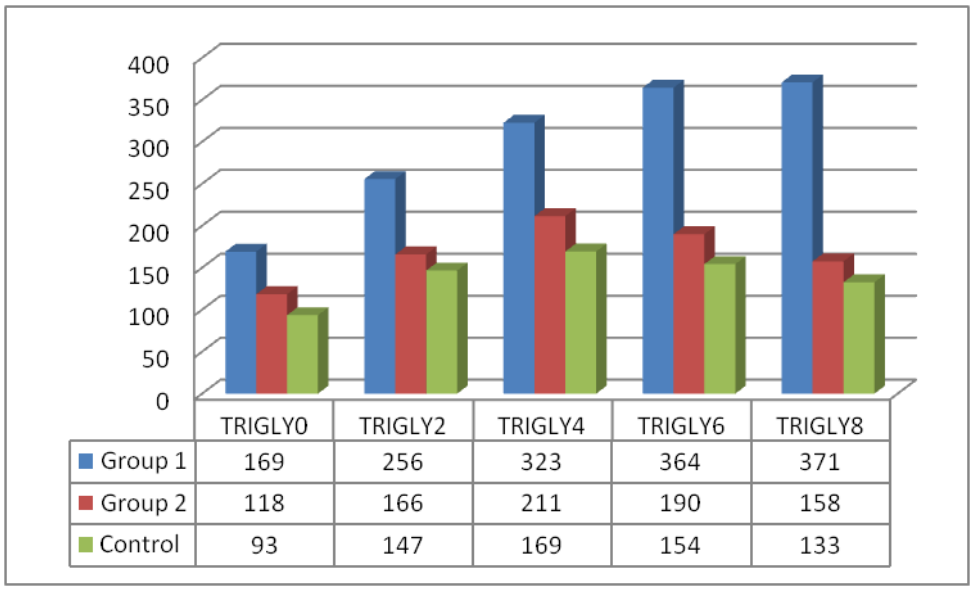
Group1 vs group2 p<0.001

Group1 vs control p<0.001

Group2 vs control p<0.001

The figure above elucidates the glucose level comparison of the subjects of the three groups in five occasions. The five occasions of glucose reading considered was at 0, 2, 4, 6 and 8 hours. Repeated measure ANOVA was applied to compare between 5 occasions and 3 groups. It showed that there was significant difference observed when the five occasions of glucose level reading were compared. Even when the three groups were compared, significant difference was observed. Group 1and 2 were found to have higher glucose levels than Controls and Group 1 was found to have higher glucose levels when compared to Group 2 All of which were found significant at 99% C.I. (Confidence interval).

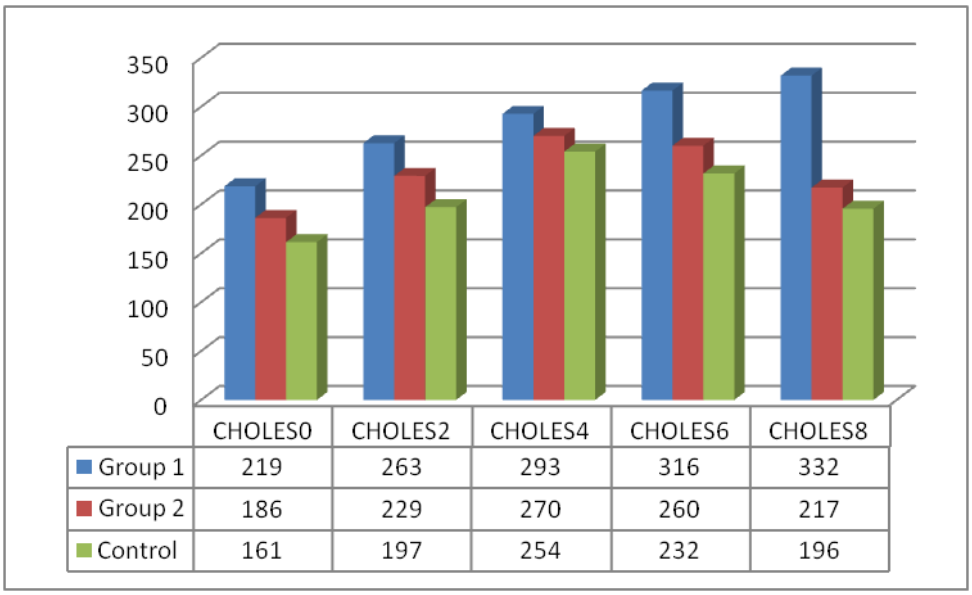
Figure 12: Comparison of 5 Occasions of Triglyceride levels among the Subjects in Three Groups



	TRIGLY0	TRIGLY2	TRIGLY4	TRIGLY6	TRIGLY8
Group 1	169	256	323	364	371
Group 2	118	166	211	190	158
Control	93	147	169	154	133

The figure above demonstrates the triglyceride level comparison of the subjects of the three groups in five occasions at 0, 2, 4, 6 and 8 hour. Repeated measure ANOVA when applied showed that there was significant difference observed when the five occasions of triglyceride level reading were compared and also when the three groups were compared. The significance was tested at 99% C.I. It was found that Group 1 subjects had a progressively elevated TG levels than Group 2 and Controls where the later were found to have a peak TG levels at 4 hrs followed by their decline.

Figure 13: Comparison of 5 Occasions of Cholesterol levels among the Subjects in Three Groups



	CHOLES0	CHOLES2	CHOLES4	CHOLES6	CHOLES8
Group 1	219	263	293	316	332
Group 2	186	229	270	260	217
Control	161	197	254	232	196

Comparison between 5 occasions F=324.094, P<0.001

Comparison between 3 groups F=73.20, P<0.001

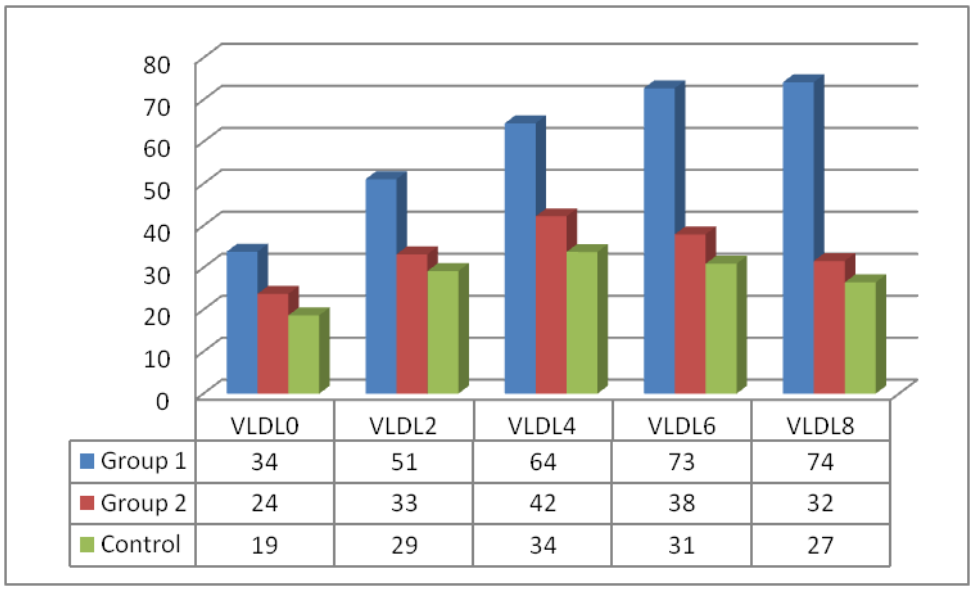
Group1 vs group2 p<0.001

Group1 vs control p<0.001

Group2 vs control p<0.01

The figure above shows the cholesterol level comparison of the subjects of the three groups in five occasions. Statistical test showed that there was significant difference observed when the five occasions of cholesterol level reading were compared and also when the three groups were compared. They were found significant at 99% C.I. It was found that Group 1 subjects had a progressively elevated cholesterol levels than Group 2 and Controls where the later were found to have a peak total cholesterol levels at 4 hrs followed by their decline.

Figure 14: Comparison of 5 Occasions of Very-Low-Density Lipoprotein (VLDL) Cholesterol levels among the Subjects in Three Groups



	VLDL0	VLDL2	VLDL4	VLDL6	VLDL8
Group 1	34	51	64	73	74
Group 2	24	33	42	38	32
Control	19	29	34	31	27

Comparison between 5 occasions F=279.077, P<0.001

Comparison between 3 groups F=216.649, P<0.001

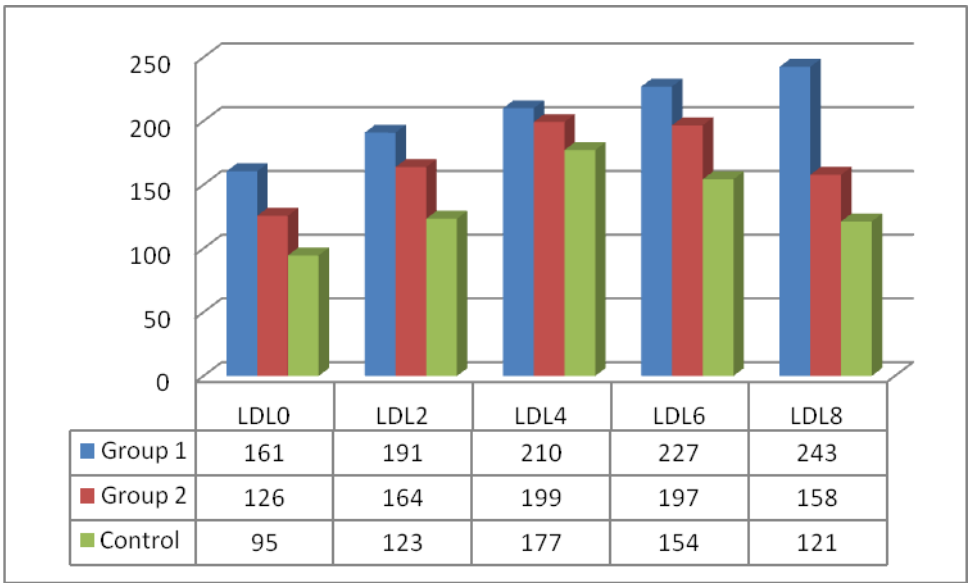
Group1 vs group2 p<0.001

Group1 vs control p<0.001

Group2 vs control p<0.01

The figure above shows the VLDL cholesterol level comparison of the subjects of the three groups in five occasions.. Statistical test showed that there was significant difference observed when the five occasions of VLDL level reading were compared. Even when the three groups were compared, significant difference was observed at 99% C.I. It was found that Group 1 subjects had a progressively elevated VLDL levels the last 2 readings being almost equal.whereas Group 2 and Controls were found to have a peak VLDL levels at 4 hrs followed by their decline.

Figure 15: Comparison of 5 Occasions of Low-Density Lipoprotein (LDL) Cholesterol levels among the Subjects in Three Groups



	LDL0	LDL2	LDL4	LDL6	LDL8
Group 1	161	191	210	227	243
Group 2	126	164	199	197	158
Control	95	123	177	154	121

Comparison between 5 occasions F=194.243, P<0.001

Comparison between 3 groups F=57.185, P<0.001

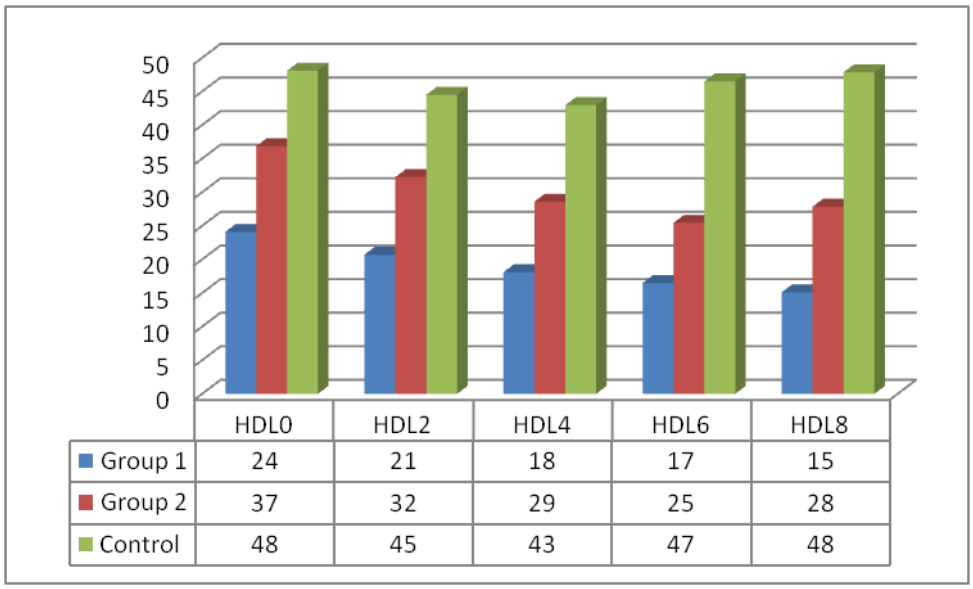
Group1 vs group2 p<0.001

Group1 vs control p<0.001

Group2 vs control p<0.001

The figure above illustrates the LDL cholesterol level comparison of the subjects of the three groups in five occasions. Statistical test showed that there was significant difference observed when the five occasions of LDL level reading were compared and when the three groups were compared at 99% C.I. It was found that Group 1 subjects had a progressively elevated LDL levels than Group 2 and Controls where the later were found to have a peak LDL levels at 4 hrs followed by their decline.

Figure 16: Comparison of 5 Occasions of High-Density Lipoprotein (HDL) Cholesterol levels among the Subjects in Three Groups



	HDL0	HDL2	HDL4	HDL6	HDL8
Group 1	24	21	18	17	15
Group 2	37	32	29	25	28
Control	48	45	43	47	48

Comparison between 5 occasions F=120.264, P<0.001

Comparison between 3 groups F=190.41, P<0.001

Group1 vs group2 p<0.001

Group1 vs control p<0.001

Group2 vs control p<0.001

The figure above shows the HDL cholesterol level comparison of the subjects of the three groups in five occasions.. Statistical test showed that there was significant difference observed when the five occasions of HDL level reading were compared and when the three groups were compared at 99% C.I. A constant reduction in HDL levels was found in Group 1 subjects whereas a decrease in HDL levels till the 6 hr followed by its increase was observed in Group 2 subjects however in controls the HDL levels were reduced till the 4 hr followed by their elevation.

Table 20 : Basal Glycemic parameters and insulin levels in three study groups

	Group I		Group II		Group III		p-value
	mean±SD	range	mean±SD	Range	mean±SD	Range	
Fasting plasma glucose (mg/dl)	157.2±45.3[b]	80-230	145.9±46.2c	108-244	84.8±9.8	70-102	b=0.000 c=0.001
Post-prandial plasma glucose (mg/dl)	250.2±41.2[b]	194-310	227.1±74.9[c]	119-354	113.8±9.0	104-103	b=0.000 c=0.001
Glycosylated hemoglobin (%)	8.6±1.9	6.4-13.6	8.5±1.6	6.8-12.4	6.1±0.98	4.2-7.2	b=0.001 c=0.001

Table 15 shows glycemic parameter in the three study groups. The fasting, postprandial plasma glucose levels were significantly higher in diabetic subjects (group I and group II) as compared to controls (group III). The Glycosylated Hemoglobin levels were significantly higher in diabetic subjects (group I and group II) as compared to controls (group III).

Table 21: Fasting lipid and glucose profile (0 hr) in three study groups

	Group I	Group II	Group III	p-value
Glucose (mg/dl)	247.15±81.74[b]	161.95±45.11c	101.20±10.81	b=0.003 c=0.006
TC (mg/dl)	218.50±22.91[a,b]	186.25±21.39	1161.40±14.84	a=0.012 b=0.008
TG (mg/dl)	169.10±29.92	118.40±20.36	93.45±35.23	
HDL (mg/dl)	24.10±4.08[b]	36.85±5.10[c]	48.10±6.22	b=0.000 c=0.008
VLDL (mg/dl)	33.80±5.98	23.75±3.89	18.65±7.13	
LDL (mg/dl)	123.0±40.8[a,b]	96.22±23.4	85.4±3.0	a=0.048 b=0.004

[a]Group II vs group I

[b]Group III vs group I

[c]Group II vs group III

Table 3 shows that fasting (0hr) plasma glucose was significantly higher in diabetic subjects as compared to controls. Fasting level of HDL was significantly lower in diabetic subjects as compared to controls. Fasting TC and LDL was significantly higher in diabetic subjecs with macrovascular disease as compared to controls and diabetic subjecs without macrovascular disease.

Table 22: Postprandial lipid and glucose response (AUC) in three study groups

	AUC (mg/dl.hr)			p-value
	Group I	Group II	Group III	
TC AUC (mg/dl.hr)	1672.15 ±392.54[b]	14360 ±179.8	1318.5 ±99.3	b=0.003
TG AUC (mg/dl.hr)	2114.1 ±1392.7[b]	1409.2 ±364.01	901.31 ±152.51	b=0.002
HDL-C AUC (mg/dl.hr)	284.36 ±27.7[b]	312.3 ±55.7[c]	396.0 ±36.2	b=0.000 c=0.000
VLDL-C AUC (mg/dl.hr)	422.8 ±278.5[b]	281.8 ±72.8	179.4 ±31.94	b=0.002
LDL AUC (mg/dl.hr)	966.5 ±333.7[b]	828.9 ±170.6	732.5 ±79.9	b=0.027
Glucose AUC (mg/dl.hr)	130.77 ±365.65[b]	1327.85 ±0.85[c]	758.38 ±77.52	b=0.000 c=0.000

[a]Group II vs group I

[b]Group III vs group I

[c]Group II vs group III

Table 4 shows AUC TC and AUC TG, AUC HDL (inverse), AUC VLDL and AUC LDL was significantly higher in diabetic subjets with macrovascular disease as compared to controls. Significant difference was also found for AUC HDL between diabetics subjects without macrovascular disease amnd healthy controls. The AUC glucose level was significantly higher in diabetic subjets with macrovascular disease as compared to controls.

Table 23: Postprandial lipid and glucose response (iAUC) in three study groups

	iAUC (mg/dl/hr)			p-value
	Group I	Group II	Group III	
TC (mg/dl/hr)	162.92±208.00	187.25±98.4	98.30±55.12	
TG (mg/dl/hr)	890±725[b]	569.8±282.2[c]	123.8±55.35	b=0.000 c=0.042
HDL-C (mg/dl/hr)	10.74±26.84	-37±12.39	18.15±19.5	
VLDL-C (mg/dl/hr)	178±0.145[b]	113.9±56.4[c]	23.67±11.80[c]	b=0.000 c=0.039
LDL (mg/dl/hr)	-24.72±148.5	52.25±121.8	42.07±63.6	
Glucose (mg/dl/hr)	178.07±104.0b	236.1±246.9[c]	72.69±58.37	C=0.031 B=0.021

[a]Group II vs group I

[b]Group III vs group I

[c]Group II vs group III

Table 5 shows that incremental iAUC TG and incremental iAUC VLDL was significantly higher in diabetic as compared to controls. The incremental of glucose was significantly higher in diabetic as compared to control.

Table 24: Peak Postprandial lipid and glucose response in three study groups

	Peak response (mg/dl)			p-value
	Group I	Group II	Group III	
TC (mg/dl)	237.0 ±66.9[b]	199.6 ±27.8	176.1 ±16.56	b=0.003
TG (mg/dl)	370.6 ±232.2[b]	236.0 ±69.2	127.4 ±23.30	b=0.000
HDL-C (mg/dl)	38.2 ±4.28[b]	40.4 ±6.97[c]	51.4 ±4.65	b=0.000 c=0.000
VLDL-C (mg/dl)	74.1 ±46.4[b]	47.2 ±13.84	25.4 ±4.68	b=0.000
LDL (mg/dl)	139.9 ±47.3[b]	122.7 ±30.5	104.8 ±17.8	b=0.034
Glucose (mg/dl)	203.62 ±69.66[b]	199.38 ±64.69[c]	106.38 ±9.30	b=0.000 c=0.000

[a]Group II vs group I

[b]Group III vs group I

[c]Group II vs group III

Table 6 shows the peak TC and peak TG, nadir HDL, peak VLDL, peak LDL was significantly higher in diabetics subjects with macrovacular disease as compared to diabetic subjects without macrovascular disease and healthy controls. Significant difference was also found for nadir HDL between diabetic subjects without macrovascular disease and healthy controls. The peak glucose level was significantly higher in diabetic subjects as compared to controls.

In the current study all glycemic parameter viz. fasting and postprandial plasma glucose concentration as well as GHB was higher in diabetic subjects as compared controls. But did not differ much significantly in diabetics subjects with and without macro vascular disease.

A significant fasting dyslipidemia as evidence by a significantly higher TC & LDL and significantly lower HDL cholesterol was seen in diabetic with macro vascular disease as compared to those without macrovascular disease and healthy controls. In addition serum triglyceride level was higher in diabetic with macrovascular disease as compared to other two groups with a constant elevation till the 8 hr indicating a delayed clearance.Several previous studies have reported significant fasting dyslipidemia among diabetics, which includes high serum triglyceride and the low HDL, high TC and LDL cholesterol[88]

The present study found significant postprandial lipid abnormalities particularly postprandial hyper tryglyceridemia in diabetics with and without macrovascular disease as compared to controls and a delayed trygliceride clearance in diabetics with macrovascular disease .

In response to an oral fat challenge, the PP AUC and PP peak levels of TC, TG, VLDL and LDL were significantly higher and the pp nadir for HDL was significantly lower in diabetics subjects with macrovascular disease when compared with healthy controls. However, when PP incremental curve, were compared for different lipid parameters, it became clear that only triglyceride and VLDL remain significantly higher. This suggests that hyper triglyceridemia is the dominant abnormality of lipid metabolism in the post prandial phase.

This is an agreement with earlier studies of post prandial metabolism in diabetic subjects.[73,84,97,133]

The present study also demonstrated a significant postprandial hyperglycemic load following a high fat and low carbohydrate meal challenge in diabetic subjects with and without macrovascular disease when compared to controls. The peak postprandial glucose levels as well as the overall glycemic burden for 8 hours following the meal (AUC glucose) were significantly higher among both diabetic groups. A significantly higher postprandial iAUC glucose in the diabetic groups with macrovascular disease suggests that a postprandial rise in blood glucose, independent of the fasting glucose level, significantly contributes to the over all glycemic burden in the postprandial phase in this group.

It seems possible that the postprandial rise in blood glucose and triglycerides following a high fat meal may alter the metabolic melieu in this phase which is pro-atherogenic.[158]

The finding of a high postprandial hyperglycemic and hyperlipideimic burden in diabetic subjects with a delayed Triglyceride clearance which is higher in the group with macrovascular disease suggests that these PP parameters provide a pro-atherogenic environment following a high fat meal. This may, through a series of pathways involving TRL's result in a heightened endothelial dysfunction postprandially and in the long term lead to artheriosclerosis and macrovascular disease in diabetes mellitus.

These findings support the results of earlier studies which have reported that high PP TG's and low PP HDL cause endothelial dysfunction in healthy as well as diabetic subjects.[28.159] The effects of PP Lipemia on endothelial dysfunction may be mediated by an enhanced PP oxidative stress[135]

It is possible that high fat meals in daily life particularly in diabetic patients will cause postprandial decline in endothelial function. This phenomenon may be entirely reversible to begin with but repeated insults may make this progressively less reversible resulting in a persistent dysfunction of the endothelium even in the preprandial state. Ultimately this leads to clinically manifest atherosclerotic vascular disease.

To the best of our knowledge this is one of the few studies, which is investigating postprandial lipemia and Triglyceride clearance in two subgroups of diabetic patients viz those with and without macro vascular disease.

The results of the present study therefore add further support to the role of post prandial lipid abnormalities and delayed Triglyceride clearance in the pathogenesis of diabetes related atheroscloritic vascular complications.

CONCLUSION

It may be concluded from the present study that there is a significant fasting and postprandial dyslipidemia in patients with diabetes mellitus particularly in those who manifest macrovascular complications. This dyslipidemia is much greater postprandially than in the fasting state.

It was also found that there is a delayed Triglyceride clearance in subjects with MVD after an oral fat challenge compared to the other two groups.

An enhanced PP glycemic and lipaemic burden with delayed PP TG clearance following a high fat meal may be responsible for the aforementioned endothelial dysfunctions. The results of the study would thus support the view that repeated challenges with high fat meals in diabetic patients may lead to atherosclerotic vascular disease by causing recurrent and persistent endothelial dysfucntion.

SUMMARY

The present study was undertaken to evaluate the postprandial dyslipidemia and postprandial triglyceride clearance in patients with type 2 diabetes mellitus.

Twenty type 2 diabetic subjects with macrovascular disease, twenty type 2 diabetic subjects without macrovascular disease and twenty healthy controls were included in this study.

Following results were obtained: -

1. All the groups were age, sex and BMI matched. Waist circumference and waist: hip ratio was significantly higher in diabetic patients compared to controls. Duration of diabetes was comparable in both diabetic groups (groups 1 and group 2).
2. Fasting and postprandial plasma glucose and glycosylated hemoglobin was higher in diabetic patients compared to controls. ($p<0001$) Glycemic control was comparable in diabetic subjects with macrovascular disease and diabetics without macrovascular disease.
3. Significant fasting dyslipidemia as indicated by a significantly higher TC, LDL and lower HDL cholesterol was found in diabetic subjects with macrovascular disease as compared to those without macrovascular disease and healthy controls. In addition fasting serum TG levels were higher in the diabetics with macrovascular disease as compared to other groups.
4. The current study found a significant PP lipid abnormality particularly PP hypertryglyceridemia (Tg-iAUC) and a delayed Triglyceride clearance after an oral fat challenge in diabetics with and without macrovascular disease as compared to controls. (In gp 1 vs. gp 3, $p<0.001$, in gp 2 vs.gp 3 $P<0.01$)

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ANNEXURES

ANNEXURE – I

PROFORMA

POST-PRANDIAL HYPERTRIGLYCERIDEMIA IN TYPE II DIABETES MELLITUS WITH OR WITHOUT MACRO-VASCULAR DISEASE

(A) PATIENT PARTICULARS **Date :**

Name :

Age/ Sex :

Address :

Phone :

Age at onset of Diabetes: Literacy :

Duration of Diabetes: Socio-economic Status:

(B) PRESENTING COMPLAINTS:

(C) HISTORY:-

1. Macro vascular disease
   (a) Coronary Artery Disease (CAD)
   (b) Cerebrovascular Disease (CVD)
   (c) Peripheral Vascular Disease (PVD)
2. Hypertension
3. Dyslipidemia
4. Hepatic or Renal Disease
5. Any endocrine disease (esp. hypothyroidism or Cushing`s syndrome)
6. Recent stress
7. History of Complications of Diabetes

(a) Acute

(b) Late	Nephropathy	Neuropathy
	Retinopathy	Macrovascular disease
❖ Drug History	Insulin	Diuretics
	OHA	B-Blockers
	Lipid Modifying Drugs	Anthihypertensives
	Steroids/OCP's	Vitamins or antioxidants
	Others	HRT
❖ Dietary History	Vegetarian	Non-vegetarian and frequency
	Saturated Fats	Unsaturated Fats
	Total oil consumtion	Tobacco chewing
	Smoking	Alcohol
❖ Family History	Diabetes Mellitus	Hypertension
	Lipid Metabolism Defects	CAD
	CVD	PVD

(D) EXAMINATION

• Height	• Weight	• BMI
• Waist circumference	• hip circumference	-Waist/Hip Ratio
• Pulse	- Peripheral Pulses	- Carotid Bruit
• BP	- Supine	- Standing
• Skin	- Pigmentation	- Acanthosis Nigricans
	- Texture	- Others
• Xanthomas	• Thyroid	• Foot Examination

SYSTEMIC EXAMINATION

- **Respiratory System**
- **Cardiovascular System**
- **Abdomen Examination**
- **CNS Examination**

(E) ROUTINE INVESTIGATIONS

HAEMOGRAM		LIVER FUNCTION TESTS	
HB (gm %)		T. PROTEIN	
TC		ALBUMIN	
DC(N,L,E)		BILIRUBIN	
ESR		SGOT	
PLATELET COUNT		SGPT	
		ALK PHOS	
BLOOD GLUCOSE		GGT	
FASTING		A/G RATIO	
POST PRANDIAL			
RENAL FUNCTION TESTS		**LIPID PROFILE**	
BLOOD UREA		CHOLESTROL	
S. CREATININE		VLDL	
SODIUM		TG	
POTASSIUM		HDL	
		LDL	
GLYCOSYLATED Hb			
		URINE ANALYSIS	
HBsAG		ALBUMIN	
HIV		SUGAR	
		MICROSCOPY	
CHEST X RAY			
ECG			
FUNDOSCOPY			

(F) SPECIAL INVESTIGATIONS

(i) ORAL FAT CHALLENGE TEST

Time(hrs)	**0**	**2**	**4**	**6**	**8**
Glucose					
Triglyceride					
Total Cholesterol					
VLDL					
LDL					
HDL					

(ii) ANKLE BRACHIAL INDEX

	RIGHT	**LEFT**
ANKLE SYSTOLIC PRESSURE		
ANKLE SYSTOLIC PRESSURE		

ABI = ANKLE SYSTOLIC PRESSURE =
BRACHIAL SYSTOLIC PRESSURE

(G) IMPRESSION

(H) CONCLUSION

(I) REMARKS OF THE GUIDE

SIGNATURE **SIGNATURE**

PROF. DR. K. PRABHAKAR **DR.SAMARSENPOPURI**

ANNEXURE - II

INFORMED CONSENT

I, _____ unreservedly in my full sense give my consent to take part in the study and consume the oral fat challenge given to me and give blood samples when ever required for investigation, the risks and benefits of which have been explained to me in my vernacular language.

Further I do not have any objections for the presentation of this study as a part of any publication.

Signature of witness

Signature of patient / guardian

SL NO	NAME	AGE	SEX	HOSPITAL NO	HEIGHT	HEIGHT2	WEIGHT	BMI	WC	HC	WHR	PULSE	BP	GLUCOSE					TRIGLYCERIDES					
														O HOURS	2 HOURS	4 HOURS	6 HOURS	8 HOURS	O HOURS	2 HOURS	4 HOURS	6 HOURS	8 HOURS	O HOURS
C1	Venkatamma	44	F	327597	160	25600	54	21.09375	77	96	0.802083333	66	120/80	97	150	198	164	143	45	97	176	113	102	155
C2	Chowdamma	55	F	327201	157	24649	56	22.7189744	80	93	0.860215054	68	120/80	103	166	207	199	154	62	91	156	145	120	146
C3	Dishak begum	40	F	327139	165	27225	62	22.7731864	79	96	0.822916667	70	120/80	116	154	177	191	143	77	126	121	119	107	165
C4	Nataraj	35	M	328369	176	30976	58	18.7241736	84	94	0.893617021	64	130/80	109	142	186	173	129	81	187	172	164	134	178
C5	Vasanthamma	48	F	328346	163	26569	60	22.5827092	85	97	0.87628866	72	120/80	84	133	197	201	163	59	169	150	134	109	160
C6	Shashikala	38	F	328721	166	27556	54	19.5964581	76	89	0.853932584	60	120/80	102	160	218	169	155	44	121	164	140	138	172
C7	Asif ali khan	32	M	328653	170	28900	66	22.8373702	84	93	0.903225806	68	128/84	93	155	190	178	143	78	176	157	160	121	159
C8	Byra reddy	60	M	328944	173	29929	70	23.3886866	89	102	0.87254902	70	130/80	99	178	191	234	170	90	142	187	153	129	133
C9	Narayanamma	60	F	328969	164	26896	50	18.5901249	77	91	0.846153846	62	120/80	105	166	194	169	144	85	125	165	153	107	174
C10	Prakashini	48	F	328728	157	24649	47	19.0677107	74	87	0.850574713	66	120/76	87	124	188	170	166	62	117	152	149	133	163
C11	Rama chandra	40	M	326508	169	28561	64	22.408179	84	95	0.884210526	74	130/90	100	149	233	161	143	41	89	146	140	127	153
C12	Mahadevayya	55	M	327018	165	27225	57	20.9366391	86	95	0.905263158	68	130/86	91	154	199	163	120	112	154	177	145	140	167
C13	Muneer	46	M	329249	175	30625	75	24.4897959	89	102	0.87254902	72	128/80	104	164	177	164	140	145	176	167	160	133	154
C14	DHanalakshmamma	50	F	329400	159	25281	55	21.7554685	84	99	0.848484848	70	130/80	81	155	170	188	169	124	154	178	150	126	136
C15	Eraiah	47	M	329476	164	26896	50	18.5901249	79	87	0.908045977	60	116/80	110	166	199	155	122	133	166	199	177	155	156
C16	Narayanamma	56	F	329505	157	24649	55	22.3132784	83	97	0.855670103	72	120/84	107	144	220	196	178	112	144	188	155	133	163
C17	Seenappa	55	M	329632	167	27889	64	22.9481157	86	101	0.851485149	62	120/80	120	160	240	180	150	106	172	165	180	143	178
C18	Susheelamma	40	F	329643	166	27556	50	18.1448686	75	90	0.833333333	68	120/80	92	159	188	201	184	149	194	188	176	167	193
C19	Ragavendra	38	M	329858	170	28900	60	20.7612457	79	93	0.849462366	66	126/80	116	161	189	198	177	138	156	195	173	166	176
C20	Sarojamma	38	F	330015	156	24336	54	22.1893491	83	97	0.855670103	64	120/80	108	211	262	193	165	126	176	182	199	162	147

TOTAL CHOLESTEROL				VLDL					LDL					HDL					HDL + VLDL				
2 HOURS	4 HOURS	6 HOURS	8 HOURS	O HOURS	2 HOURS	4 HOURS	6 HOURS	8 HOURS	O HOURS	2 HOURS	4 HOURS	6 HOURS	8 HOURS	O HOURS	2 HOURS	4 HOURS	6 HOURS	8 HOURS	O HOURS	2 HOURS	4 HOURS	6 HOURS	8 HOURS
178	234	196	165	9	19.4	35.2	22.6	20.4	92	109.6	151.8	123.4	93.6	54	49	47	50	51	63	68.4	82.2	72.6	71.4
189	214	178	155	12.4	18.2	31.2	29	24	85.6	125.8	136.8	100	81	48	45	46	49	50	60.4	63.2	77.2	78	74
189	246	221	188	15.4	25.2	24.2	23.8	21.4	92.6	109.8	169.8	144.2	111.6	57	54	52	53	55	72.4	79.2	76.2	76.8	76.4
212	267	233	190	16.2	37.4	34.4	32.8	26.8	112.8	130.6	192.6	157.2	116.2	49	44	40	43	47	65.2	81.4	74.4	75.8	73.8
179	254	221	210	11.8	33.8	30	26.8	21.8	96.2	95.2	176	144.2	137.2	52	50	48	50	51	63.8	83.8	78	76.8	72.8
201	259	237	181	8.8	24.2	32.8	28	27.6	107.2	123.8	176.2	154	96.4	56	53	50	55	57	64.8	77.2	82.8	83	84.6
185	199	215	178	15.6	35.2	31.4	32	24.2	95.4	104.8	124.6	137	105.8	48	45	43	46	48	63.6	80.2	74.4	78	72.2
178	227	218	193	18	28.4	37.4	30.6	25.8	65	100.6	143.6	138.4	113.2	50	49	46	49	54	68	77.4	83.4	79.6	79.8
203	254	243	227	17	25	33	30.6	21.4	112	134	173	153.4	155.6	45	44	48	59	50	62	69	81	89.6	71.4
198	276	250	213	12.4	23.4	30.4	29.8	26.6	99.6	127.6	199.6	171.2	135.4	51	47	46	49	51	63.4	70.4	76.4	78.8	77.6
187	240	218	183	8.2	17.8	29.2	28	25.4	91.8	119.2	160.8	136	101.6	53	50	50	54	56	61.2	67.8	79.2	82	81.4
215	263	231	180	22.4	30.8	35.4	29	28	105.6	150.2	196.6	166	112	39	34	31	36	40	61.4	64.8	66.4	65	68
201	277	260	194	29	35.2	33.4	32	26.6	78	121.8	202.6	180	119.4	47	44	41	48	48	76	79.2	74.4	80	74.6
178	214	210	188	24.8	30.8	35.6	30	25.2	56.2	99.2	133.4	134	115.8	55	48	45	46	47	79.8	78.8	80.6	76	72.2
196	269	245	210	26.6	33.2	39.8	35.4	31	83.4	120.8	184.2	162.6	131	46	42	45	47	48	72.6	75.2	84.8	82.4	79
188	259	258	221	22.4	28.8	37.6	31	26.6	107.6	129.2	186.4	190	161.4	33	30	35	37	33	55.4	58.8	72.6	68	59.6
214	278	250	211	21.2	34.4	33	36	28.6	111.8	138.6	205	171	137.4	45	41	40	43	45	66.2	75.4	73	79	73.6
266	289	254	224	29.8	38.8	37.6	35.2	33.4	115.2	185.2	216.4	181.8	149.6	48	42	35	37	41	77.8	80.8	72.6	72.2	74.4
199	265	256	211	27.6	31.2	39	34.6	33.2	111.4	133.8	196	185.4	138.8	37	34	30	36	39	64.6	65.2	69	70.6	72.2
188	298	240	190	25.2	35.2	36.4	39.8	32.4	72.8	107.8	220.6	157.2	111.6	49	45	41	43	46	74.2	80.2	77.4	82.8	78.4