A descriptive study of the type 2 diabetic population with hypertriglyceridemia of more than 2.5mmol/L at presentation with subsequent analysis of their baseline and follow up variables

BARBARA VERMOOTEN
VRMBAR002

Thesis presented in partial fulfilment of the requirements for the degree of
Master of Medicine (MMed) in the Faculty of Health Sciences
at the University of Cape Town.

Supervisor: Prof Dirk Blom

September 2022

Plagiarism Declaration

This thesis/dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor.

Name: Barbara Vermooten

Student number: VRMBAR002

Signature: Signed by candidate

Date: 2022/09/22

Abstract

Background

Type 2 diabetes mellitus and the dyslipidaemia that often accompanies it are major risk factors for atherosclerotic cardiovascular disease. Elevated triglycerides mark the accumulation of atherogenic remnant lipoproteins.
Because there is little South African data on hypertriglyceridaemia in diabetes we retrospectively reviewed baseline and follow-up data of patients attending a specialized lipid clinic.

Methods

We reviewed the medical records of 100 diabetic patients with hypertriglyceridemia of >2.5mmol/L who attended the Groote Schuur Hospital Lipid clinic for at least two years. We documented four six-monthly follow-up visits and also documented the visit with the lowest recorded triglyceride levels if it was outside the two-year follow-up.

Results

The study population was predominantly (63%) female with a mean (SD) age at presentation of 50.87 (10.44) years. Obesity (BMI >30 kg/m²) was highly prevalent (66.3%) and diabetes was generally poorly controlled (76.16% patients had a HbA1C >7%).

Baseline triglycerides ranged from 2.6mmol/L to 63.3mmol/L with a median and mean (SD) of 4.64 mmol/L and 10.47 (12.57) mmol/L, respectively. Baseline agarose electrophoresis patterns were: 0% type I, 0% type IIA, 51.4% type IIB, 10.3% type III, 18.7% type IV and 19.6% type V. LDL particle size determined by acrylamide gradient gel electrophoresis was intermediate or small in 61 of 64 (95%%) of patients with visible LDL.

At baseline calculated mean (SD) remnant cholesterol (48 patients) was 1.55 (0.24) mmol/L, ranging from 1.1mmol/L to 2mmol/L. Triglycerides and calculated remnant cholesterol were strongly correlated (r^2=0.9395, p=0.000). There was no correlation between baseline TG and HDL-C, baseline BMI and baseline waist circumference, but there was a positive correlation between triglycerides and alcohol intake, (r^2=0.224, P=0.012). There was no correlation between baseline triglycerides and HbA1C (p=0.8423) or fasting glucose (p=0.0857).

The change in total triglycerides from initial presentation to the follow-up visit with the lowest documented value was a mean(SD) decrease of 2.91 (4.98)mmol/L, ranging from either no change to a decrease of 32.68mmol/L. The mean (SD) reduction in HbA1C was 0.94 (1.64)% ranging from an increase of 1.7% to a decrease of 7.8%.

Fibrates were initiated in 43% of patients. Patients prescribed fibrates had higher mean (SD) baseline TG levels of 18.56(15.48)mmol/L, compared to levels of 4.11(1.85)mmol/L in patients who were not prescribed a fibrate.

At baseline mean (SD) TC values were 8.33 (3.18). The mean (SD) LDLC at baseline was 4.26 (1.45) mmol/L ranging from 0.5 to 7.6 mmol/L. Only 4.1% of all patients with a calculable LDL-C achieved values below 1.8mmol/L during follow-up .

Conclusions

In this study diabetic patients with elevated triglycerides who attended a specialist lipid clinic were frequently obese and often had poorly controlled diabetes. Although dyslipidaemia and glycaemia improved following intensification of therapy most patients did not reach their treatment goals. Our study highlights the heterogeneity of hypertriglyceridaemia, the difficulties of achieving good metabolic control, and the need for ongoing follow-up as severe hypertriglyceridaemia relapses readily.

Acknowledgements

The author wishes to dedicate this thesis to her parents Henk and Cornelia Vermooten for their continued support and encouragement.

In addition, the author wishes to acknowledge the following people for their various contributions towards the completion of this work:

- Professor Dirk Blom for his professional guidance and support, and
- Thinus Appel, her beloved, for his love, patience and input to this document.

Abbreviations

IDF	International Diabetes Federation
MI	Myocardial Infarction
LDL-C	Low-density lipoprotein
HDL-C	High-density lipoprotein
VLDL	Very low-density lipoprotein
TG	Triglyceride
CVD	Cardiovascular disease
IHD	Ischemic Heart Disease
LPL	Lipoprotein Lipase
HL	Hepatic Lipase
CETP	Cholesteryl ester transfer protein
Apo	Apolipoprotein
HbA1C	Glycosylated hemoglobin
PCSK9	Proprotein convertase subtilisin-kexin type 9
hsCRP	high-sensitivity C-reactive protein
TGRL	Triglyceride-rich lipoprotein lipolysis
Lp(a)	Lipoprotein(a)
NCEP	National Cholesterol Education Program Evaluation Project
CTT	Cholesterol Treatment Trialists
BMI	Body Mass Index
mmol/L	Millimole per liter
mg	Milligram
TSH	Thyroid stimulating Hormone

Table of Contents

List of figures

List of tables

Chapter 1: Introduction and Literature Review

General background

1.1.1 South African and Western Cape population ethnicities

In 2020 South Africa had an estimated mid-year population of 59.62 million people, with 51.1% being female and 48.9% being male. The population consists of different ethnic groups, namely: Black Africans (80.8%), Coloured (Mixed Ancestry) (8.8%), Indian/Asian (2.6%) and White (7.8%).

The Western Cape population has been increasing consistently with a high migration inflow documented in the period 2006 – 2021 for which statistics are available. The estimated population of the Western Cape is 7 005 741 people of which 49.3% is male and 50.7% is female.[1]
Multiple ethnicities are found in the Western Cape. The majority of the population is of mixed ancestry and there is a high prevalence of metabolic syndrome and diabetes.[1]

1.1.2 Diabetes prevalence and burden of disease

Globally diabetes is a major cause of mortality. The Global Burden of Disease Study 2017 highlights the ever-increasing burden of non-communicable diseases compared to 1990. The global incidence of diabetes in 2017 was 22.9 million, the prevalence was 476 million and deaths attributed to diabetes were 1.37 million, respectively. It is projected that these figures may increase to an incidence of 26.6 million, a prevalence of 570.9 million and 1.59 million deaths, respectively, by 2025.[2]

The International Diabetes Federation (IDF) predicts a 140.0% increase in adults with diabetes by 2040 in Africa, and a concerning 126.4% increase in adults with impaired glucose tolerance in Sub-Saharan Africa. The IDF estimated the prevalence of diabetes in South Africa at 2.286 million adults (20-79 years of age) in 2015, 61.1% of these were undiagnosed with a population prevalence of 7%. [3] Most diabetics treated at primary care facilities in the Western Cape do not have good glycemic control for multiple patient, physician and health system related reasons.[4]

1.1.3 Cardiovascular disease risk, mortality in diabetes, and the clinical relevance thereof

Cardiovascular disease in diabetes is very common and macrovascular disease accounts for most of the mortality in diabetes.[5-7] The INTERHEART AFRICA Study confirmed diabetes as a major risk factor for myocardial infarction.[8] Dyslipidemia is an important component of the increased risk in diabetics but of course is not the sole factor.[9, 10]

The hazard ratios for all-cause death and vascular events in persons living with diabetes were reported to be 1.90 (95% CI, 1.19-1.31) and 2.32 (95% CI 2.11-2.56), respectively[11]. A 50-year-old person with diabetes dies approximately 6 years earlier than a person without diabetes.[11]

SATS SA: Statistical release P0302: Mid-year Population Estimates. Stats SA 2020,Pretoria. [Cited 2020 JULY 27]. Available from: http://www.statssa.gov.za/publications/P0302/P03022020.pdf

To emphasize the importance of aggressive risk factor management in diabetics Steven et al compared the coronary heart disease mortality of 1373 nondiabetic patients with a previous myocardial infarction (MI) to that of 1053 diabetic patients without a previous MI over a seven-year period. This observational study concluded that the hazard ratio for mortality from ischemic heart disease in patients with diabetes without prior MI compared to patients without diabetes with previous MI was 1.4 (95%CI 0.7-2.6) adjusted for age and sex. The hazard ratio remained close to 1 at 1.2 (95%CI 0.6-2.4) after further adjusting for age, gender, smoking, high blood pressure, LDL-C, HDL-C and TG, thus suggesting that some diabetics without prior MI have a risk of fatal ischemic heart disease that is as high as that seen in non-diabetic patients with established CVD.[12]

1.1.4 Diabetic dyslipidemia, insulin resistance and the metabolic milieu

Lipoprotein abnormalities are already evident in patients with abnormal glycaemia who do not have diabetes yet.[6] Diabetic dyslipidemia is also known as the atherogenic lipid phenotype and is characterized by elevated fasting and postprandial triglycerides, low HDL cholesterol, normal/elevated LDL cholesterol, the presence of small dense LDL particles and elevated remnant lipoproteins.[13-16] Poorly controlled diabetes, alcohol misuse and certain drugs may lead to severe hypertriglyceridemia and acute pancreatitis in genetically predisposed individuals.[13]

In diabetes or insulin resistant states atherogenic dyslipidemia is often defined as triglyceride levels >2.2mmol/L and HDL-C <1mmol/L.[17] The atherogenicity of diabetic dyslipidemia is mediated by multiple factors, including the presence of small dense LDL particles, elevated remnant cholesterol which is associated with a 2.8 fold increased cardiovascular risk, and dysfunctional HDL.[13] The Framingham Offspring study highlighted the significant elevation of remnant like particles in patients with diabetes compared to those without diabetes.[18]

Insulin resistance is characterized by increased secretion of triglyceride rich VLDL and inefficient and incomplete clearance of these lipoproteins results in higher concentrations of remnant lipoproteins. Normally insulin inhibits Hormone Sensitive Lipase (HSL) [19] and also inhibits transcription of microsomal triglyceride transfer protein. Thus, in insulin resistant states more triglycerides are transferred to nascent apo B, with excess secretion of triglyceride-rich VLDL1, which following lipolysis by Lipoprotein lipase (LPL) and Hepatic lipase (HL) and the actions of CETP ultimately yield small dense LDL.[13, 20]

Increased circulating triglyceride-rich VLDL leads to low HDL-C and small dense LDL particles via cholesteryl ester transfer protein (CETP) mediated exchanges.[6, 19, 20] Hypertriglyceridemia stimulates CETP activity leading to increased TG content of HDL-C and LDL.[13, 17] CETP facilitates predominant exchange of cholesterol esters for triglyceride from HDL to VLDL particles, ultimately leading to triglyceride-rich LDL which then becomes a substrate for LPL and hepatic lipase, ultimately leading to the formation of small dense LDL particles.[6, 13, 17] Small triglyceride enriched HDL is catabolized more rapidly, leading to the low HDL-C and predominance of small HDL particles seen in diabetes.[6, 20] Apolipoprotein A-V on Triglyceride rich lipoproteins activates LPL (Lipoprotein lipase), and apo A-V polymorphisms can contribute to hypertriglyceridemia.[21]

In insulin resistance Apo A1 production is not upregulated appropriately, and high tumor necrosis factor alpha(TNF alpha) levels seen in chronic inflammatory states also suppress HDL formation and Apo A1 production.[15] The decreased concentration of HDL-C in diabetes not

only act as a marker for its impaired functionality, but is also a marker of the impaired metabolism of triglyceride-rich apo B lipoprotein[13] - in fact HDL-C cholesterol has been called the 'HbA1c of triglyceride rich lipoprotein metabolism'.

Impaired LPL activity results in decreased VLDL delipidation and post prandial chylomicronemia with the subsequent elevated triglyceride concentrations serving as a marker of elevated remnant cholesterol levels.[14] Elevated free fatty acids causes LPL to detach from endothelial surfaces thus impairing its activity.[13] Saturation of LPL at VLDL triglyceride concentration above approximately 5-6mmol/L impairs chylomicron hydrolysis.[20]

Apo CIII expression is increased by hyperglycemia and deceased by insulin. Apo CIII inhibits LPL and additionally interferes with the hepatic uptake of apo B-containing lipoproteins by interfering with their binding to hepatic lipoprotein receptors.[13] Clearance of remnants by the liver LDL receptor and remnant receptor is negatively regulated by PCSK9 (Proprotein convertase subtilisin-kexin type 9) which prevents the recirculation of internalized LDL receptors by targeting them for lysosomal degradation.[14]

1.1.5 Remnant cholesterol and its role in CVD

Remnant cholesterol represents the cholesterol carried in the remnants of VLDL and chylomicrons.[22, 23] The larger size of these remnant particles allows them to carry 5 – 20 times the amount of cholesterol per particle compared to LDL.[24] Non-fasting triglyceride concentrations and remnant cholesterol concentrations are highly correlated ($r^2 = 0.96$) and triglyceride concentrations therefore not only acts as an indication of insulin resistance, but also of the atherosclerosis prone metabolic milieu.[6, 21, 22, 25] Triglycerides themselves likely do not cause atherosclerosis, but remnant particles are able to infiltrate the blood vessel walls, oxidize readily and are frequently ingested by macrophages in the same way LDL particles are.[6, 21, 25]

The role of remnant cholesterol in atherosclerotic cardiovascular disease has been evaluated in multiple studies.[18, 21, 22] These studies highlight the contribution of remnant cholesterol to cardiovascular risk with most studies identifying remnant cholesterol as a causal risk factor for IHD especially after LDL reduction was obtained [23]. In a short-term (12 month) study of 328 Chinese diabetics with new onset coronary artery disease remnant cholesterol was not an independent prognostic biomarker following multivariate analysis, however, remnant cholesterol was associated with increased inflammatory parameters such as: hsCRP (high-sensitivity C-reactive protein), fibrinogen and neutrophil count contributing to the inflammatory milieu that facilitates atherosclerosis. [24]

In a multidirectional Mendelian randomization study of 60608 Danish participants from the Copenhagen General Population Study, the Copenhagen City Heart Study, and the Copenhagen Ischemic Heart Disease Study a causal association was shown between elevated non-fasting remnant cholesterol and low-grade inflammation with an elevated risk of IHD. This inflammatory causality was not shown for LDL.

In a further Mendelian randomization study Nordestgaard et al. showed that each 1 mmol/L increase in non-fasting remnant cholesterol caused a 2.8-fold risk for IHD independent of HDL-C reduction.[22, 24]

In yet another Mendelian randomization study, Nordestgaard et al evaluated the effects of a genetic variants in APOA-V leading to increased non-fasting triglycerides and calculated remnant cholesterol and confirmed the causal association for myocardial infarction with remnant cholesterol in hypertriglyceridemia. The observational hazard ratios for a doubling in non-fasting TG compared to a causal genetic odds ratio were 1.57 (1.32-2.68) and 1.94 (1.40-1.85) respectively. The calculated remnant cholesterol observational hazard ratio compared to causal genetic odds ratio were 1.67(1.38—2.02) and 2.23(1.48-3.35) respectively.[21]

Dysbetalipoproteinemia which results from mutations in apolipoprotein E, is characterized by the accumulation of cholesterol enriched remnants and serves as a natural genetic model to demonstrate the atherogenicity of remnant lipoproteins.[26]

In a review article regarding the pathogenicity of TGRL Goldberg presents two hypotheses:

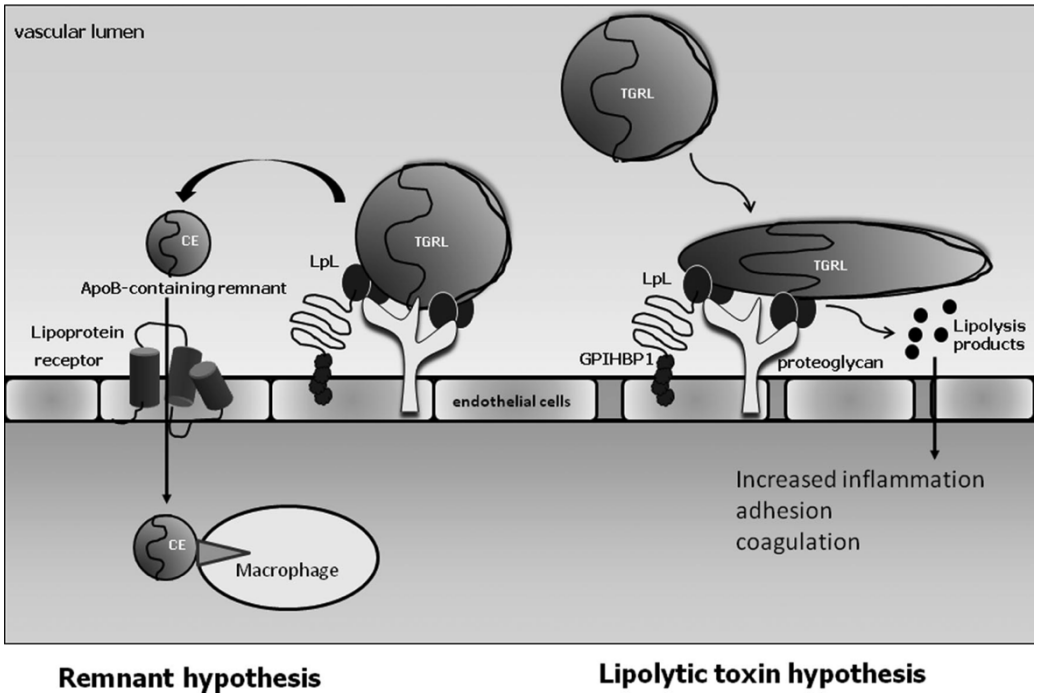


Figure 1 - Atherogenicity of triglyceride-rich lipoproteins. [20]

Remnant Hypothesis by Zilversmit: Remnant lipoproteins can be detected in human arterial walls. Triglyceride rich lipoproteins converted to remnants enter arterial walls in a process mediated by LPL, carrying both triglycerides and cholesterol and are ingested by macrophages leading to foam-cell formation.[20-22]

The Lipolytic Toxin hypothesis: VLDL lipolysis induces inflammation. With lipolysis of TGRL inflammatory lipids e.g. free fatty acids, lysolecithins and oxidized lipids are released leading to increased adhesion molecules and cytokines promoting coagulation.[20]

1.1.6 The role of Apo B and treatment targets

Apolipoprotein B values reflect the number of circulating atherogenic lipoproteins including LDL, VLDL, IDL and Lp(a) and therefore are a good measure of the total burden of atherogenic lipid particles.[13]

1.1.7 Treatment gaps in diabetes with dyslipidemia

The National Cholesterol Education Program (NCEP) Evaluation Project highlighted treatment gaps in controlling diabetic dyslipidemia. Only 55% of diabetics reached their LDL target. In the hypertriglyceridemia diabetics without CVD cohort only a quarter reached their LDL and non-HDL cholesterol targets while 33% of those with established CVD reached both targets.[25, 27]

1.1.8 Studies on glycemic control and effect on CVD

Although one would expect improved glycemic control to translate into reductions in cardiovascular disease in patients with type 2 diabetes it has been difficult to prove this hypothesis in short and medium-term clinical trials. For instance, in the ACCORD and ADVANCE studies improved glycemic control did not reduce cardiovascular event rates and was even associated with increased mortality in the ACCORD study – possibly due to increased hypoglycemia with tight glycemic control. These trials did confirm the importance of glycemic control to reduce microvascular complications, and reminded all those treating patients with diabetes to not only focus on glycemic control but to be aware of other factors contributing to atherosclerosis such as dyslipidemia, hypertension and hypercoagulability.[28] Significant limitations of the studies are that they enrolled mainly patients with established cardiovascular disease, long-standing diabetes, multiple comorbidities and were relatively short-term.[28]

In long-term follow-up studies improved glycemic control early in the natural history of either type 1 [29] or type 2 diabetes [30] was associated with reduced cardiovascular event rates. Good glycemic control soon after diagnosis thus pays dividends many years later while attempting to control glucose tightly once cardiovascular disease is clinically evident is not a sucessful strategy.

1.1.9 Statins, Fibrates, PCSK-9 inhibitors, Ezetimibe and Omega 3 Fatty acids effect on CVD

Statins are effective in reducing cardiovascular risk in patients with type 2 diabetes.[13, 16, 31] The Cholesterol Treatment Trialists (CTT) collaboration meta-analysis showed that each mmol/L LDL-C reduction by statins in diabetics reduce myocardial infarctions and stroke by 21%.[32]

The importance of LDL reduction in cardiovascular risk reduction is highlighted by a series of studies in which LDL-C was progressively reduced to ever lower levels by addition of further LDL lowering therapies to statin-based lipid-lowering therapy.

The IMPROVE IT trial showed that lowering LDL-C by a further 24% through the addition of ezetimibe to simvastatin-based therapy resulted in a further reduction in cardiovascular events, with most of the benefits seen in patients with diabetes.[5, 33] Apart from LDL-C this combination (statin with ezetimibe) will also lower remnant cholesterol, but further studies to evaluate the outcome in patients with elevated remnant cholesterol have not been conducted.[23]

In the FOURIER trial PCSK-9 inhibition with evolocumab reduced cardiovascular outcomes significantly in diabetics and non-diabetics with established stable atherosclerotic cardiovascular disease [34], but no mortality benefit was seen in this trial of relatively short duration.[35]

In the Odyssey Outcome trial alirocumab significantly reduced LDL-C and non-HDL-C cholesterol and improved cardiovascular outcomes in patients with a recent acute coronary syndrome[36]. The absolute reduction in major adverse cardiovascular events was significantly larger in patients with diabetes compared to non-diabetics with an absolute reduction of 2.3%, (95%CI 0.4-4.2).[37]

It has been difficult to show that lipid-lowering drugs that do not predominantly target LDL reduce cardiovascular risk. For instance, the early fibrate monotherapy trials, Coronary Drug Project and World Health Organization (WHO) clofibrate trial failed to show a positive outcome on cardiovascular events.[31]

Several fibrate monotherapy trials (Helsinki Heart Study, Bezafibrate Infarction Prevention trail and FIELD) identified cardiovascular benefit in subgroups of patients whose baseline lipid profile was characterized by both triglyceride elevation and low HDL cholesterol. This subgroup was also the only subgroup to benefit in a sub-analysis of the ACCORD lipid trial.

The ACCORD lipid trial is the only published large trial of a fibrate added to statin-based therapy in patients with diabetes and added cardiovascular risk published to date. The overall trial hypothesis was that adding fenofibrate to simvastatin in patients with type 2 diabetes would increase plasma HDL-C and reduce TG and therefore improve cardiovascular outcomes. The trial included 5518 men and women with type 2 diabetes already on simvastatin who were then randomized to fenofibrate or placebo. The primary outcome was the occurrence of major adverse cardiovascular events. The hazard ratio was 0.92 (95% CI 0.79-1.08) P=0.32 for the primary outcome, thus the primary hypothesis was not confirmed. Subgroups were defined by sex, baseline HbA1C (above or below 8.0%), baseline lipids, and a group with both high TG (upper third) and low HDL (lower third). There was heterogeneity in treatment effect according to gender as men had a 16% lower primary event rate with added fenofibrate while women a 38% higher event rate. In the group with elevated TG and low HDL fenofibrate treatment was associated with a 30% reduction in cardiovascular event rates.[9, 31]

Approximately 58% of participants in the REDUCE IT trail were diabetic. In this trial 4g of the omega-3 fatty acid preparation icosapent ethyl (pure EPA preparation) added to baseline statin therapy in high-risk patients with a fasting triglyceride level of 1.52 to 5.63 mmol/L and a low-density lipoprotein cholesterol level of 1.06 to 2.59 mmol/L showed a 25% reduction in CVD .[38] In contrast to this in the STRENGTH Trial the addition of omega-3 FA (EPA + DHA) to a similar patient population did not reduce major adverse cardiovascular events.[39]

1.1.10 South African and European triglyceride management guideline

Current South-African guidelines suggest that triglycerides should be <2.3mmol/L but triglycerides are not a specified treatment target and specific TG lowering therapy is thus not routinely recommended but can be considered in high-risk patients with hypertriglyceridemia and adequate LDL-C control. Statin + fibrate therapy can be considered if TG > 2.3mmol/L but this is not a high-grade recommendation due to limited evidence. [5] Statins remain the

primary therapy for cardiovascular risk reduction. TG > 10 mmol/L are treated with fibrates to reduce the risk of pancreatitis.

The European Atherosclerosis Society Consensus Panel advises intervention in high and very-high risk patients with triglycerides >1.7mmol/L and HDL-C < 1mmol/L. The first interventions should be lifestyle modifications, review of medication compliance and exclusion of secondary causes. Thereafter fibrates, icosapent ethyl or intensifying LDL-C therapy can be considered.[40]

Thus, with the evolving evidence to suggest the importance of focusing on triglycerides (and therefore remnant cholesterol) to minimize the residual cardiovascular risk, especially in high risk population like diabetics further trials are currently ongoing.[41] The PROMINENT phase III multicenter trial is evaluating cardiovascular outcome in diabetic patients with both low HDL-C and high TG with Pemafibrate vs placebo over a 5 year period and should finally clarify the clinical utility of statin + fibrate combinations in those with atherogenic dyslipidaemia.[42] This trial was recently halted early for futility. (https://pace-cme.org/2022/04/11/phase-3-cv-outcomes-study-with-pemafibrate-stopped-early - accessed 26 June 2022)

1.1.11 Effect of diabetic agents on lipogram

Antidiabetic agents like insulin, sulphonylureas and metformin also reduces fasting and post prandial triglycerides.[19, 41] Improved glycemic control lowers triglycerides and increase HDL-C levels in patients with type 1 diabetes, but in type 2 diabetes the lipid abnormalities do not resolve fully.[43]

1.1.12 Studies conducted to show the effect of diabetic control on the lipogram

In 1983 Lopes-Virella [44] and associates subjected insulin dependent diabetics (sic) to 3 weeks of intensive in-hospital insulin therapy (Table 1). They observed a decrease in LDL cholesterol, VLDL cholesterol, total cholesterol, VLDL triglyceride and Apo B levels, while HDL-C and ApoA1 levels increased. The increase in HDL-C and ApoA1 was seen only in males. In patients admitted with very poor glycemic control (HbA1C >11%) a marked decrease in total LDL-C, total VLDL-C, total triglyceride and VLDL triglyceride was seen, whereas in those patients with a slightly lower admission HbA1C(<11%) decreases were only noted for total triglyceride and VLDL triglycerides.[44] The study illustrates the metabolic effect of improved glycemic control with insulin, although the study participants most likely had type 1 diabetes and were of younger age ranging from 5 to 52, with a mean(SD) age of 17(6) years.

Table 1 - 1983 Lopes-Virella variable summary of 55 diabetics before and after 3 weeks intensive insulin.

Parameter (mg/dl)	Admission	Discharge	P value
N	55	55	
Total cholesterol	215 ±61	180±44	P<0.001
LDL-C	144±47	119±43	P <0.001
Apolipoprotein B	143±58	117±54	P<0.001
HDL-C	38±10	44±13	P<0.001
Apolipoprotein A1	104±29	109±28	P<0.05
Triglycerides	189±201	88±44	P<0.001
VLDL triglycerides	148±192	59±38	P<0.01

VLDL Cholesterol	33±35	16±10	P<0.001
Glucose	244±127	115±65	P<0.001
HbA1c	13.1±3.7	10.3±2.6	P<0.001

Pfeifer et al treated 49 untreated diabetics without familial hypertriglyceridemia with sulphonylureas or insulin for three months. At one and three month follow up a significant linear relationship was established between HbA1C reduction and decreased triglyceride and cholesterol concentration, even after correction for change in ideal body weight.[45]

Agardh et al postulated that the lipogram provides additional information regarding diabetic control. Twenty-six patients with diabetes, both controlled and uncontrolled, were initiated on insulin. Over a period of 3-4 months an improved HbA1C was associated with significantly decreased plasma cholesterol and triglyceride levels as well as an increase in LPL activity.[46]

1.1.13 Previous descriptive studies in hypertriglyceridemia

Due to the paucity of large population-based studies describing lipid profiles in hypertriglyceridemia the recently published Very Large Database of Lipids (2019) analyzed 116925 samples with elevated triglycerides from a large population database and classified them using the Fredrickson-Levy-Lee classification. The authors identified 5 Type I, 20144 type IIb, 2151 type III, 85 540 Type IV and 246 Type V samples. Apart from Type I, all other phenotypes had high levels of RLP-C. This study did not provide information on diabetic status.[47]

1.1.14 Motivation for current study

There are no descriptive studies of a population of diabetic patients with hypertriglyceridemia attending a specialized lipid clinic with detailed baseline information including detailed lipoprotein phenotyping and review of their baseline and follow up variables.

Hypothesis

In the presence of insulin resistance and diabetes lipid abnormalities are common and improved glycemic control will correlate positively with improved triglycerides, lower remnant cholesterol and increased HDL-C levels on a background of treatment with statins and/or fibrates.

Objectives / Aim

Testing the hypothesis utilizing data from the Groote Schuur Hospital (GSH) lipid clinic.

1.1.15 Objectives

1. Clinical description of 100 diabetic patients with increased triglycerides attending the Groote Schuur Lipid Clinic.
2. Description of laboratory results of above patients.
3. Describe lipid and glycemic control after two years of follow-up.
4. Evaluate factors that correlate with lipids, especially triglyceride control.

1.1.16 Primary Aim

The primary aim is to identify and describe a proportion of the population of patients with type 2 diabetes and hypertriglyceridemia of more than 2.5 mmol/L at presentation, attending the Lipid Clinic at Groote Schuur Hospital who have at least two years of follow-up data. Data that will be collected includes baseline variables (demographics, lipid profile, apolipoproteins, calculated remnant cholesterol, electrophoresis, medications, etc.) and follow up lipid variables (total cholesterol, LDL cholesterol, triglycerides, calculated remnant cholesterol, HDL-C, HbA1c and medication (lipid lowering and diabetic).

1.1.17 Secondary Aim

The secondary aim is to investigate whether there are correlations between glycemic control, lipid modifying therapy and hypoglycemic therapy with lipid outcomes, specifically LDL cholesterol, remnant cholesterol, triglycerides, and HDL-C.

Chapter 2: Methods

Study Setting

The study was performed at the Lipid Clinic of Groote Schuur Hospital, Cape Town, South Africa. The Division of Lipidology provides clinical services on an outpatient basis but also offer consultation service for in patients with severe disorders of lipoprotein metabolism. The division is run from the Cape Heart Centre, formed in 1997 by uniting the Ischemic Heart Disease Unit, Cardiovascular Research Unit in Cardiothoracic Surgery and more recently the Hatter Institute, to promote cardiovascular research. The lipid lab offers specialized investigations for dyslipidemia that are generally not available elsewhere in the country. Groote Schuur Hospital is an academic health center, providing tertiary and quaternary care for patients from the Western Cape and beyond. The hospital operates in the Cape Town Central Health District of the Cape Metro Region with an estimated population 4 130 565 million people.

The referral criteria to the Groote Schuur Lipid Clinic are:

1. Total cholesterol levels persisting above 7.5mmol/L or below 2.5 mmol/L (after dietary management),
2. Low density lipoprotein levels (LDL-C) above 5mmol/L or below 1.5 mmol/L,
3. High density lipoprotein (HDL-C) cholesterol concentration above 2.5mmol/L or below 0.6mmol/L or below 0.8mmol/L if the triglyceride concentration is below 2.5mmol/L.
4. Hypertriglyceridemia of more than 5 mmol/L.
5. Xanthomata of the skin or tendons irrespective of lipid levels, with the exception of xanthelasma.
6. Premature atherosclerosis.
7. Statin intolerance once hypothyroidism and other disorders have been excluded.
8. Unusual metabolic defects that involve lipid metabolism or promote atherosclerosis, including homocystinuria.

Study design.

The study design is a descriptive medical record review of 100 patients with type 2 diabetes and hypertriglyceridemia attending the Groote Schuur Hospital Lipid clinic with a first visit to the clinic prior to September 2017 and with at least two years of follow up.

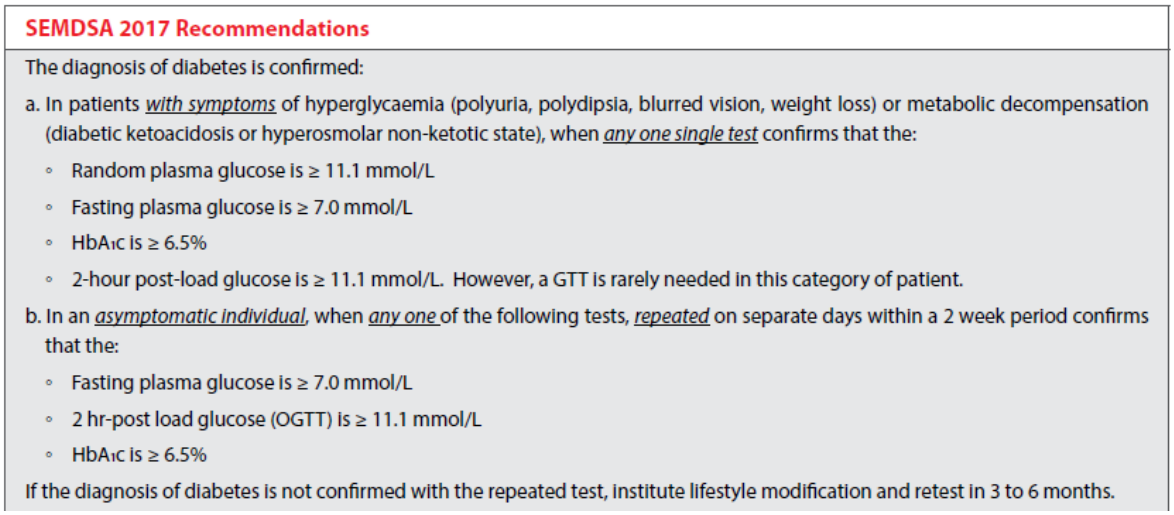
2.1.1 Study Population

The following criteria were used to define the study population.

2.1.1.1 Inclusion Criteria

1. Known diabetic patients already on oral hypoglycemic agents or insulin, or
2. Newly diagnosed type 2 diabetes at the first clinic attendance according to SEMDSA recommendations 2017[3], (Figure 2).

3. Triglyceride level ≥2.5mmol/L at presentation, and
4. Minimum of two years of follow up at the clinic.



SEMDSA 2017 Recommendations

The diagnosis of diabetes is confirmed:

a. In patients *with symptoms* of hyperglycaemia (polyuria, polydipsia, blurred vision, weight loss) or metabolic decompensation (diabetic ketoacidosis or hyperosmolar non-ketotic state), when *any one single test* confirms that the:

- Random plasma glucose is ≥ 11.1 mmol/L
- Fasting plasma glucose is ≥ 7.0 mmol/L
- HbA_{1c} is ≥ 6.5%
- 2-hour post-load glucose is ≥ 11.1 mmol/L. However, a GTT is rarely needed in this category of patient.

b. In an *asymptomatic individual*, when *any one* of the following tests, *repeated* on separate days within a 2 week period confirms that the:

- Fasting plasma glucose is ≥ 7.0 mmol/L
- 2 hr-post load glucose (OGTT) is ≥ 11.1 mmol/L
- HbA_{1c} is ≥ 6.5%

If the diagnosis of diabetes is not confirmed with the repeated test, institute lifestyle modification and retest in 3 to 6 months.

Figure 2 - SEMDSA 2017 Recommendations: Diagnostic Criteria Diabetes

2.1.1.2 Exclusion Criteria

Less than 2 years follow up at the clinic.

2.1.1.3 Sample size

The sample size of 100 patients was chosen to allow completion of the project within the limited time available for a MMed project. As this is a descriptive study no sample size calculations were performed.

2.1.1.4 Recruitment and Enrollment

This study reviewed existing information captured form patient folders at the Lipid Clinic at Groote Schuur Hospital. Routine clinic follow-up information and results was obtained from stored duplicate Lipidology Clinic folders. During a manual folder review data was captured into a database created for this study.

The clinic has an established database (Paradox) with baseline information of all patients seen at the lipid clinic in the last 30 years. Although the database contains detailed information on the first visit it does not contain follow-up information. The initial lipid clinic database search focused on identifying patients with triglycerides of more than 2.5mmol/L at presentation who first visited the clinic before September 2017 (to allow for a minimum of two years of follow-up). In a subsequent step all patients who did not have diabetes at presentation (either newly diagnosed or not known at presentation) or did not meet the inclusion criteria were excluded during a manual folder review. The database search was then extended backwards to patients who presented earlier than September 2017 followed by a manual folder review of these folders. Recruitment terminated once 100 patients were included to ensure feasibility for an MMed project.

Routine (six monthly) follow up clinic visit information is documented on continuation sheets within the duplicate lipidology folders. During manual retrospective folder review patient information was captured into a REDCap database created for this study.

2.1.2 Ethics

The study was reviewed and approved by UCT FHS HREC. The committee waived the need for participant consent as this was a retrospective study using de-identified patient data.

2.1.3 Study Investigators

Professor Dirk Blom is the principal investigator and student supervisor. The sub investigator is Dr Barbara Vermooten, a qualified medical doctor with sufficient clinical post graduate experience, and skilled in computer data capture. No other investigators were involved with the study.

2.1.4 Data Handling

During data collection only the PI and sub-investigator accessed clinical records. Data was captured from the medical records into a custom created and secured REDCap database. Data was captured in a deidentified format.

2.1.5 Data sets

Patient variables at first presentation and four consecutive visits following presentation were captured. Additionally, the visit at which triglycerides were the lowest was also captured if this did not occur during the first four follow-up visits. The data capture sheet can be found in the addendum.

2.1.5.1 Patient variables at first presentation

1. Gender (male/female)
2. Weight (kg)
3. Height (m)
4. Ethnicity
5. Date of birth
6. BMI (kg/m²)
7. Waist circumference (cm)
8. Hip circumference (cm)
9. HbA1c (%)
10. Fasting glucose (mmol/L)
11. Lipids: Total cholesterol, Total triglyceride, HDL-C, LDL-C (mmol/L)
12. Electrophoresis: agarose gel and acrylamide gradient gel electrophoresis.
    a. Agarose gel electrophoresis:
        - Type I
        - Type IIA
        - Type IIB

        - Type III
        - Type IV
        - Type V
    b. Acrylamide gradient gel electrophoresis:
        - Origin
        - Mid
        - LDL Species
            - LDL A
            - LDL AI
            - LDL I
            - LDL I/B
            - LDL B
            - LDL B Post
            - No species
13. Lipoprotein (a): mg/dL or nmol/L
14. Apo B (g/L)
15. Apo A1(g/L)
16. Medication details:
    - Lipid-lowering drugs
    - Diabetic therapy
17. Renal function(eGFR) if noted renal impairment. (60mL/min/1.73m²)
18. Thyroid function (mIU/L)
19. Alcohol use (units)
20. Smoker: former, current, never

2.1.5.2 Derived variable

The following variable was derived: Remnant cholesterol (mmol/L), based on the calculation Remnant Cholesterol = Total cholesterol - LDL-C - HDL-C.

2.1.5.3 Follow up variables

1. Date of follow up visit,
2. Glucose (mmol/L),
3. HBA1C (%),
4. Lipid Drugs:
    - Statin (drug and dose). Documented as
    - Fibrate (drug and dose)
    - Omega 3
    - Other
5. Diabetic Drugs:
    - Metformin,
    - Sulphonylureas
    - Insulin: daily dose including regime: basal/basal bolus/premixed)
6. Alcohol use,
7. Smoking: current, former, never,
8. HDL Cholesterol,
9. Triglyceride,
10. LDL cholesterol, and
11. Remnant cholesterol: with calculation.

2.1.5.4 Diabetic medication calculation

The dosage of metformin and sulphonylureas prescribed to our patient population was annotated by using the fraction of the maximal daily dosage according to the South African package insert. Sulphonylureas used were Glimeperide or Gliclazide, maximal dosages of 4mg and 320mg daily respectively. Metformin maximal dosage used was 2550mg daily.

Laboratory methods for specific variables

The following test principles are defined by the National Health Laboratory Service Manual

Table 2 - National Health Laboratory Service Manual/Assay and Reference Ranges for different analytes measured.

Analyte	**Machine**	**Test principle**
Lp (a)	Roche/Hitachi cobas c 501 Analyzer	2-Point End
Total Cholesterol	Roche/Hitachi cobas c Analyzer	1-Point Enzymatic colorimetric test.
Triglyceride	Roche/Hitachi cobas c Analyzer	1-Point Enzymatic colorimetric test.
HDL	Roche/Hitachi cobas c Analyzer	2-Point End Homogeneous enzymatic colorimetric test.
LDL	Calculated using the – Friedewald equation if TG < 4.5 mmol/L	
Agarose gel electrophoresis	Sebia Hydragel Lipoprotein(E)	Agarose gel electrophoreses on 8.5% buffered agarose gel. Separated lipoproteins are stained with Sudan Black stains. After excess stain is removed with an alcoholic solution, evaluation can be done visually or by densitometry.
Gradient gel electrophoresis		Sudan Black stained apolipoprotein B containing lipoproteins are separated on a 2-8% polyacrylamide gel plate at 4°C overnight. Major lipoproteins are separated into known subclasses with larger lipoproteins staining proximally at the cathodal end while the smaller lipoprotein migrate towards the anodal end of the gel. The gels are reported using an in-house classification in which A designates large LDL species and B designates small LDL species. Intermediate species are designated I and LDL-species that fall between these major sizes are designated AI, I/B and B post respectively. Origin (chylomicrons) Mid (VLDL) LDL Species:

		LDL A LDL AI LDL I LDL I/B LDL B LDL B Post [48].
Apo lipoprotein A1	Roche/Hitachi Cobas C3111, cobas c 501/502	2-Point End
Apo lipoprotein B	Roche/Hitachi Cobas C3111, cobas c 501/502	2-Point End
TSH	Cobas e411 analyzer	Sandwich principle
HBA1C	Roche/Hitachi Cobas C3111, cobas c 501/502	Turbidimetric inhibition immunoassay
Fasting Glucose	Roche/Hitachi Cobas C3111, cobas c 501/502	Enzymatic reference method with hexokinase
Creatinine	Roche/Hitachi Cobas C3111, cobas c 501/502	Enzymatic

Chapter 3: Results

Study subject selection

During the initial database search 400 potential study participants were identified; following manual folder review only 59 met all inclusion and exclusion criteria. After the database search was extended to patients who had presented earlier, 343 additional potential participants were identified. We included 41 of these patients to meet our enrolment target of 100 patients.

Demographics

100 patients were included in this study, 37 (37%) male and 63 (63%) females. Most of the cohort were of Mixed Ancestry (81%), the rest of the ethnic groups were White 9%, Black African 7% and Indian/Asian 3 %. The mean (SD) age at presentation was 50.87 (10.44) years ranging from 15.96 to 70.26 years (Table 3).

Table 3 - Baseline Demographics

Variable	Valid N	Mean (SD)	Median	Minimum	Maximum
Age at presentation	100	50.87 (10.44)	51.71	15.96	70.26
Height (m)	98	1.62 (0.09)	1.61	1.28	1.85
Weight (kg)	100	85.68 (16.98)	83.65	50.00	140.00
BMI (kg/m2)	98	32.76 (5.64)	32.55	19.05	50.81
Waist circumference (cm)	99	103.16 (12.73)	102.00	75.00	168.00
Hip circumference (cm)	99	105.95 (11.82)	104.00	86.00	156.00

Anthropometry

The mean (SD) BMI was 32.76 (5.64) kg/m² (P=0.957) (Table 3) and did not differ significantly between males (32.80 kg/m2) and females (32.74 kg/m2) (Table 5). About two-thirds (66.32%) of all patients were obese with a BMI >30 kg/m² (Table 6). The mean (SD) waist circumference was 103.16 (12.73) cm and did not differ significantly according to gender. The mean (SD) waist circumference was 103.16 (12.73) cm (Table 3), with a waist circumference of 103.81 (9.21) cm in females while waist circumference in males was 102.77 (14.49) cm (P=0.697) (Table 5). Mean waist circumferences for males and females exceeded the NECP and IDF definitions for metabolic syndrome (Table 4).

Table 4 - NCEP ITP III and IDF 2005 definitions for Metabolic syndrome.[49]

Guideline	Waist Circumference (cm) Males	Waist Circumference (cm) Female
The National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP II) 2001	≥102 cm	≥88cm
International Diabetes federation (IDF) 2005	≥94cm	≥80cm

Table 5 - Male: Female Baseline Demographics

Variable	**Male Mean (SD)**	**Female Mean (SD)**	**p**
Age at presentation	48.41 (8.78)	52.32 (11.11)	0.070
Height (m)	1.68 (0.10)	1.58 (0.06)	**0.000**
Weight (kg)	92.86 (15.44)	81.47 (16.52)	**0.0001**
BMI (kg/m2)	32.80 (5.17)	32.74 (5.93)	0.957
Waist circumference (cm)	103.81 (9.21)	102.77 (14.49)	0.697
Hip circumference (cm)	103.43 (7.47)	107.45 (13.62)	0.102
Alcohol amount (g/week)	87.50 (104.42)	42.91 (38.33)	0.193

Table 6 - Frequency table: BMI Categories at presentation: (kg/m²).

BMI categories	**N**	**%**
15-20	1	1.02
20-25	3	3.06
25-30	29	29.59
30-35	36	36.73
35-40	20	20.41
40-45	6	6.12
45-50	2	2.04
50-55	1	1.02
55-60	0	0
Totals	98	100

Habits

The mean (SD) reported age of starting smoking was 18 (4.36) years, N=35. Thirty-one patients were previous smokers, 34 had never smoked and 35 were current smokers. The mean (SD) amount cigarettes per day in smokers was 17(9.1). Females started smoking at a mean age of 18.83 years, males at 16.18 years (P=0.067). Mean reported cigarettes smoked per day was 18.0 for females and 16.6 for males (P=0.27).

Most participants (75%) reported that they did not consume any alcohol. The mean (SD) estimated weekly alcohol consumption in the 25% (14 males, 11 females) of participants who reported consuming alcohol was 67.88 g (83.83g). The mean (SD) reported alcohol intake among females was 42.91(38.3) g/week, with males consuming more alcohol at 87.50 (104.42) g/week (P=0.193).

Lipid results at presentation: Triglycerides and Remnant Cholesterol

3.1.1 Triglycerides at baseline

Our study included patients with a wide range of hypertriglyceridemia with baseline triglycerides ranging from 2.6 mmol/L to 63.2 mmol/L (Table 12). The median baseline TG value was 4.64 mmol/L with a mean (SD) of 10.47 (12.57) mmol/L (Table 12). The higher mean value results from multiple outliers with very severe hypertriglyceridemia as can be seen in the box and whiskers plot in Figure 3. 26% of patients had severe hypertriglyceridemia of ≥10.5mmol/L as seen in Table 7. Table 7 shows the frequency distribution of TG values, 58 patients had TG levels below 5.5 mmol/L (58%), 16 between 5.5-10.5 mmol/L (16%), 8 between 10.5 and 15.5 mmol/L (8%), 3 between 15.5-20.5 mmol/L (3%) and 15 patients had TG levels more than 20.5 mmol/L (15%).

As would be expected given the variation in TG values the total cholesterol was also highly variable ranging from 2.7 mmol/L to 24.25 mmol/L (Table 12). The mean (SD) and median TC values were 8.33 (3.18) and 7.45 mmol/L. respectively. As would be expected in a hypertriglyceridemia population the mean (SD) HDLC was low at 1.01 (0.34) mmol/L with a wide range from 0.4 mmol/L to 2.21 mmol/L (Table 12). The HDLC results should be interpreted with caution as certain assays may overestimate HDLC in the presence of significant hypertriglyceridemia. The laboratory at Groote Schuur Hospital does not utilize a direct LDLC assay but reports LDLC calculated according to the Friedewald equation. Baseline LDLC was thus only available in 48 participants. The mean (SD) LDLC at baseline was 4.26 (1.45) mmol/L with a range of 0.5 to 7.6 mmol/L (Table 12). Lp (a) (reported in mg/dl and nmol/l measurements as the laboratory changed its methodology during this study) was elevated, mean (SD) values in mg/dL were 54.15 (71.65) mg/dL with a range from 0 – 420 mg/dl; the mean (SD) value in nmol/L was 90.49 (120.19) nmol/L with a range from 0 – 675 nmol/l (Table 12). Further baseline laboratory results are shown in Table 12.

Apo B(g/l) had a mean (SD) of 1.36(0.45) g/l ranging from 0.43 – 2.74g/l(Table 12,Figure 9), in females the mean (SD) was 1.43 (0.48), and in males the mean (SD) was 1.25 (0.43)P=0.055 (Table 18). Apo A1 (g/l) had a mean (SD) of 1.38(0.30) g/l ranging from 0.44 – 2.35 g/l (Table 12, Figure 10), females had a higher Apo A1 level mean (SD) 1.47 (0.26) compared to males mean (SD) of 1.25 (0.43) g/l, p=0.000 (Table 18).

Table 7 - Frequency Table. Baseline Triglycerides at presentation

Triglycerides (mmol/L)	N	Percentage (%)
<5.5	58	58
5.5 -10.5	16	16
10.5-15.5	8	8
15.5-20.5	3	3
>20.5	15	15
Totals	100	100

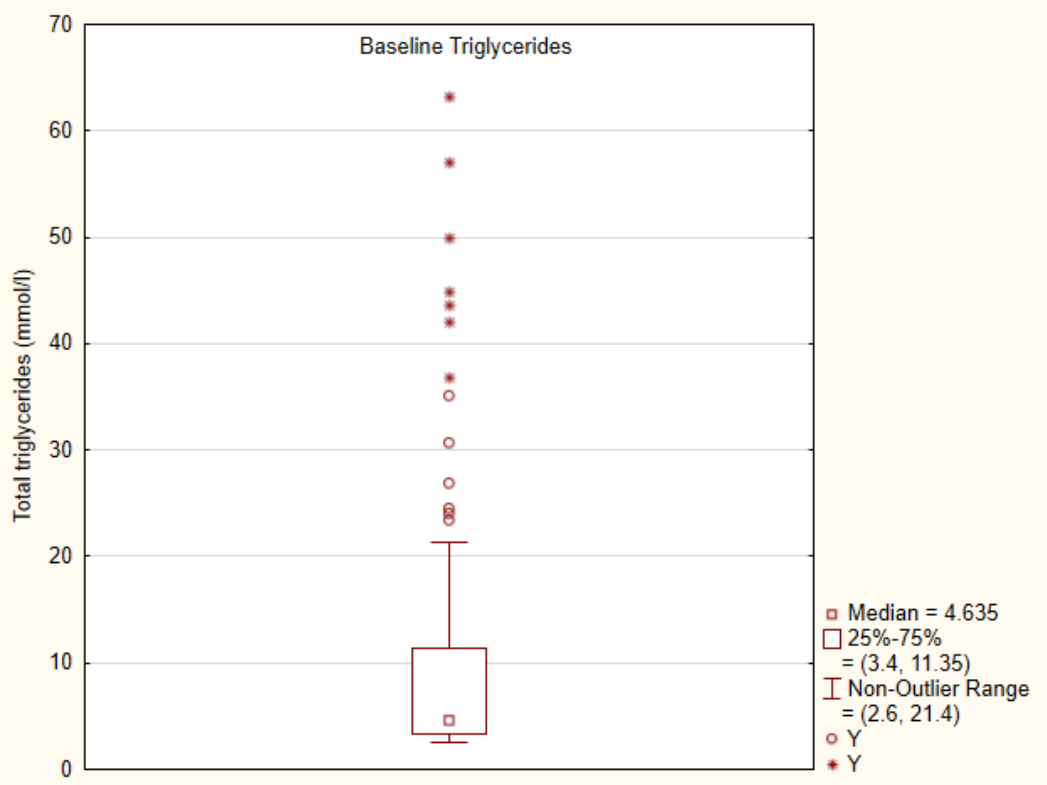


Figure 3 - Box Plot Triglycerides (mmol/L)

3.1.2 Lowest Triglycerides during follow-up

During the entire follow-up period at the clinic 30 patients reached TG levels below 1.7 mmol/L, 54 patients reached levels below 2.3 and the rest never reached any of these targets (Table 8).

Table 8 - Frequency table lowest on-treatment TG

Triglyceride range(mmol/L)	N	Cumulative count	Percent
0.7-≤0.8	3	3	0.56
0.8-≤0.9	1	4	0.19
0.9-≤1.0	2	6	0.37
1.0-≤1.1	2	8	0.37
1.1≤1.2	2	10	0.37
1.2-≤1.3	4	14	0.78
1.3 - ≤ 1.4	7	21	1.30
1.4 - ≤ 1.5	5	26	0.93
1.5 - ≤ 1.6	4	30	0.74
1.6 - ≤ 1.7	7	37	1.30
1.7 - ≤ 1.8	0	37	0.00
1.8 - ≤ 1.9	5	42	0.93
1.9 - ≤ 2.0	3	45	0.56
2.1 - ≤ 2.20	1	50	0.19
2.2 – ≤ 2.3	4	54	0.74
2.3 - ≤ 2.4	3	57	0.56
2.4 - ≤ 2.5	2	59	0.37
2.5 - ≤ 2.6	3	62	0.56
2.6 - ≤ 2.7	3	65	0.56
2.7 - ≤ 2.8	1	66	0.19
2.8 - ≤ 2.9	4	70	0.74
2.9 - ≤ 3	2	72	0.37
3 - ≤ 3.1	1	73	0.19
3.1 - ≤ 3.2	0	73	0.00
3.2 - ≤ 3.3	1	74	0.19
3.3 - ≤ 3.4	1	75	0.19
3.4 - ≤ 3.5	2	77	0.37
3.5 - ≤ 3.6	1	78	0.19
3.6 - ≤ 3.7	2	80	0.37
3.7 - ≤ 3.8	2	82	0.37
3.8 - ≤ 3.9	0	82	0.00
3.9 - ≤ 4	1	83	0.19
4 - ≤ 4.1	0	83	0.00
4.1 - ≤ 4.2	3	86	0.56
4.2 - ≤ 4.3	0	86	0.00
4.3 - ≤ 4.4	0	86	0.00
4.4 - ≤ 4.5	0	86	0.00
4.5 - ≤ 4.6	0	86	0.00
4.6 - ≤ 4.7	0	86	0.00
4.7 - ≤ 4.8	0	86	0.00
4.8 - ≤ 4.9	1	87	0.19
4.9 - ≤ 5	0	87	0.00
5 - ≤ 5.1	1	88	0.19

5.1 - ≤ 5.2	3	91	0.56
5.2 - ≤ 5.3	0	91	0.00
5.3 - ≤5.4	1	92	0.19
5.4 - ≤ 5.5	0	92	0.00
5.5 - ≤ 5.6	1	93	0.19
5.6 - ≤ 5.7	0	93	0.00
5.7 -≤ 5.8	0	93	0.00
5.8 - ≤ 5.9	0	93	0.00
5.9 - ≤ 6	0	93	0.00
6 -≤ 6.1	0	93	0.00
6.1 -≤ 6.2	0	93	0.00
6.2 - ≤ 6.3	0	93	0.00
6.3 - ≤ 6.4	0	93	0.00
6.4 - ≤ 6.5	0	93	0.00
6.5 - ≤ 6.6	0	93	0.00
6.6 - ≤ 6.7	0	93	0.00
6.7 - ≤ 6.8	0	93	0.00
6.8 - ≤ 6.9	1	94	0.19
6.9 - ≤ 7	0	94	0.00
7 - ≤ 7.1	0	94	0.00
7.1 - ≤ 7.2	0	94	0.00
7.2 - ≤ 7.3	0	94	0.00
7.3 - ≤ 7.4	0	94	0.00
7.4 - ≤ 7.5	0	94	0.00
7.5 - ≤ 7.6	0	94	0.00
7.6 - ≤ 7.7	0	94	0.00
7.7 - ≤ 7.8	0	94	0.00
7.8 - ≤ 7.9	0	94	0.00
7.9 - ≤ 8	0	94	0.00
8 - ≤ 8.1	0	94	0.00
8.1 - ≤ 8.2	0	94	0.00
8.2 - ≤ 8.3	0	94	0.00
8.3 - ≤ 8.4	1	95	0.19
8.4 - ≤ 8.5	0	95	0.00
8.5 - ≤ 8.6	0	95	0.00
8.6 - ≤ 8.7	0	95	0.00
8.7 - ≤ 8.8	0	95	0.00
8.8 - ≤ 8.9	0	95	0.00
8.9 - ≤ 9	0	95	0.00
9 - ≤ 9.1	0	95	0.00
9.1 - ≤ 9.2	0	95	0.00
9.2 - ≤ 9.3	0	95	0.00
9.3 - ≤ 9.4	0	95	0.00
9.4 - ≤ 9.5	0	95	0.00
9.5 - ≤ 9.6	0	95	0.00
9.6 - ≤ 9.7	0	95	0.00

9.7 - ≤9.8	0	95	0.00
9.8 - ≤ 9.9	0	95	0.00
9.9 - ≤ 10	1	96	0.19
10 - ≤ 10.1	0	96	0.00
10.1 - ≤ 10.2	0	96	0.00
10.2 - ≤ 10.3	0	96	0.00
10.3 - ≤ 10.4	0	96	0.00
10.4 - ≤ 10.5	0	96	0.00
10.5 - ≤ 10.6	0	96	0.00
10.6 - ≤ 10.7	0	96	0.00
10.7 - ≤ 10.8	0	96	0.00
10.8 - ≤ 10.9	0	96	0.00
10.9 - ≤ 11	0	96	0.00
11 - ≤ 11.1	0	96	0.00
11.1 - ≤ 11.2	0	96	0.00
11.2 - ≤ 11.3	0	96	0.00
11.3 - ≤ 11.4	0	96	0.00
11.4 - ≤ 11.5	1	97	0.19
11.5 - ≤ 11.6	0	97	0.00
11.6 - ≤ 11.7	0	97	0.00
11.7 - ≤ 11.8	0	97	0.00
11.8 - ≤ 11.9	0	97	0.00
11.9 - ≤ 12	0	97	0.00
12 - ≤ 12.1	0	97	0.00
12.1 - ≤ 12.2	0	97	0.00
12.2 - ≤ 12.3	0	97	0.00
12.3 - ≤ 12.4	0	97	0.00
12.4 - ≤ 12.5	0	97	0.00
12.5 - ≤ 12.6	0	97	0.00
12.6 - ≤ 12.7	1	98	0.19
12.7 - ≤ 12.8	0	98	0.00
12.8 - ≤ 12.9	0	98	0.00
12.9 - ≤ 13	1	99	0.19
13 - ≤ 13.1	0	99	0.00
13.1 - ≤ 13.2	0	99	0.00
13.2 - ≤ 13.3	0	99	0.00
13.3 - ≤ 13.4	0	99	0.00
13.4 - ≤ 13.5	0	99	0.00
13.5 - ≤ 13.6	0	99	0.00
13.6 - ≤ 13.7	0	99	0.00
13.7 - ≤ 13.8	0	99	0.00
13.8 - ≤ 13.9	0	99	0.00
13.9 - ≤ 14	0	99	0.00
14 - ≤ 14.1	0	99	0.00
14.1 - ≤ 14.2	0	99	0.00
14.2 - ≤ 14.3	0	99	0.00

14.3 - ≤ 14.4	0	99	0.00
14.4 - ≤ 14.5	0	99	0.00
14.5 - ≤ 14.6	0	99	0.00
14.6 - ≤ 14.7	0	99	0.00
14.7 - ≤ 14.8	0	99	0.00
14.8 - ≤ 14.9	0	99	0.00
14.9 - ≤ 15	0	99	0.00
15 - ≤ 15.1	0	99	0.00
15.1 - ≤ 15.2	0	99	0.00
15.2 - ≤ 15.3	0	99	0.00
15.3 - ≤ 15.4	1	100	0.19
15.4 - ≤ 15.5	0	100	0.00

3.1.3 Triglycerides during two years of follow-up

The lowest TG mean (SD) was 3.07(2.86) mmol/L ranging from 0.70 to 15.31 mmol/L and the highest follow-up TG mean (SD) was 8.87 (9.55) mmol/L with a wider range 1.50 to 48.78 mmol/L (Table 9). Figure 4 points out the high number of outliers especially in the highest TG group, P=0.000 (Table 10).

Table 11 displays the TG levels at visit one to visit 5. Repeat instance 5 is the visit recorded as having the lowest triglycerides if not recorded in the first 4 consecutive visits. 37 patients had their lowest TG level at this visit with the mean (SD) lowest triglycerides 2.33(1.64) mmol/L, ranging from 0.70 to a maximum of 8.3mmol/L.

Table 9 - Descriptive Stats Lowest to Highest Triglycerides during two years of follow-up

Variable	Valid N	Mean(SD)	Median	Mode	Frequency (of Mode)	Minimum	Maximum	Lower (Quartile)	Upper (Quartile)	Percentile (10.00000)	Percentile (90.00000)
Lowest TG (mmol/L)	99	3.07 (2.86)	2.21	Multiple	4	0.70	15.31	1.46	3.43	1.19	5.30
Highest TG (mmol/L)	99	8,87 (9.55)	4.90	3.40	5	1.50	48.78	3.40	10.70	2.30	20.10

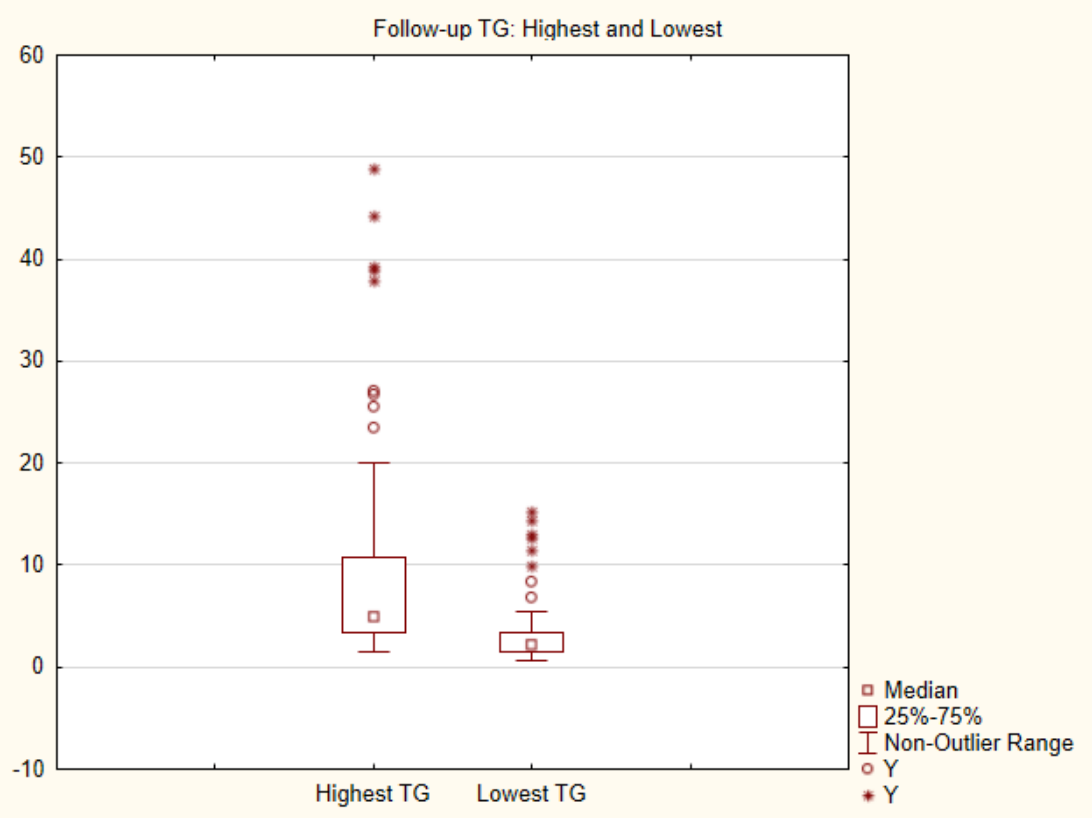


Figure 4 - Box and whisker plot Triglycerides.

Table 10 - T-test for independent Samples. Group 1: Lowest Triglyceride, Group 2 Highest Triglyceride

Variable	Mean (SD) (Group 1)	Mean (Group2)	t-value	Df	P	Valid N (Group 1)	Valid N (group 2)	F-ratio (Variances)	P(variances)
Lowest TG vs Highest TG	3.07 (2.86)	8.87(9.55)	-5.7	196	0.00	99	99	11.180	**0.00**

Table 11 - Triglycerides at Visit 1 to Visit 5

Variable	Repeat Instance	Valid N	Mean (SD)	Minimum	Maximum
Triglycerides (mmol/L)	1	97	5.85 (5.93)	1.130000	35.20
Triglycerides (mmol/L)	2	100	6.16 (6.82)	0.730000	44.20
Triglycerides (mmol/L)	3	98	6.71 (8.58)	1.00	39.84
Triglycerides (mmol/L)	4	100	6.12 (7.78)	0,90	48.78
Triglycerides (mmol/L)	5	37	2.33 (1.64)	0.70	8.30

Remnant Cholesterol

Derived remnant cholesterol could be calculated in 48 patients, with a mean (SD) 1.55 (0.24) mmol/L, median 1.59mmol/L, ranging from 1.1mmol/L to 2mmol/L. (Table *12*, Figure 5). Not unexpectedly, Figure 6 shows a very tight correlation between triglycerides and calculated remnant cholesterol. The caveat is that this is only in patients with triglycerides less than 4.5 in which a Friedewald calculation could be performed.

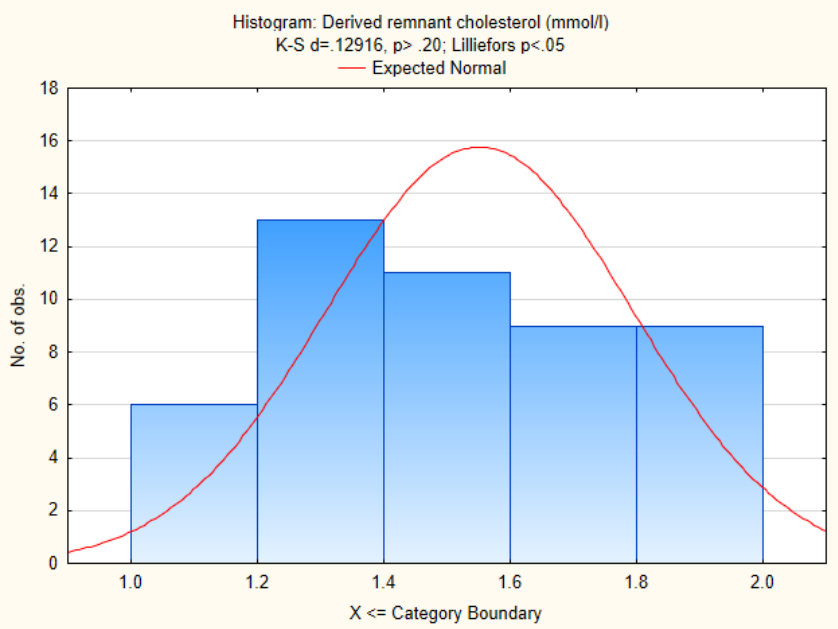


Figure 5 - Histogram: Derived Remnant Cholesterol (mmol/L)

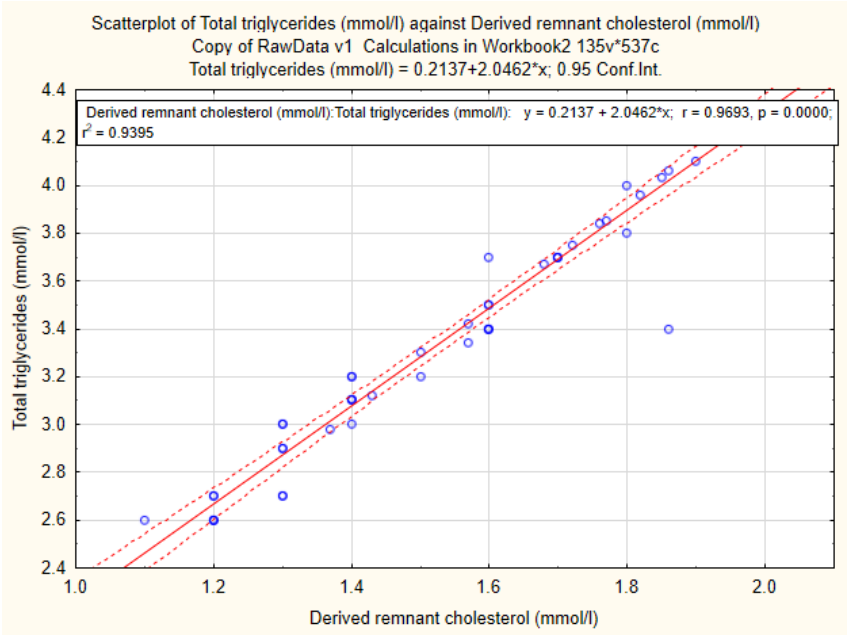


Figure 6 - Total Triglycerides (mmol/L) against derived remnant cholesterol (mmol/L)

Baseline Lipid results

The variables identified in table 12 were used.

Table 12 - Description of baseline laboratory variables

Variable	Valid N	Mean (SD)	Median	Minimum	Maximum
Total cholesterol (mmol/L)	100	8.33(3.18)	7.45	2.7	24.35
HDL-C (mmol/L)	100	1,01 (0.34)	0.96	0.4	2.21
LDL-C (mmol/L)	48	4.26 (1.45)	4.22	0.5	7.60
Triglycerides (mmol/L)	100	10.47 (12.57)	4.64	2.6	63.2
Derived remnant cholesterol (mmol/L)	48	1.55 (0.24)	1.59	1.1	2
Apo B (g/l)	97	1.36 (0.45)	1.28	0.43	2.74
Apo A1 (g/l)	96	1.38 (0.30)	1.37	0.44	2.35
Lp (a) level (mg/dl)	60	54.15 (71.65)	35.5	0.0	420
Lp (a) level (nmol/l)	36	90.49 (120.19)	65.5	0.0	675

In most of the cases the agarose gel electrophoresis pattern was type IIB (51.4%). the rest were Type V (19.6%), Type IV (18.7%), Type III (10.3%). The Acrylamide gradient gel electrophoresis findings are summarized in Table 13.

Table 13 - Descriptive statistics: Agarose Gel Electrophoresis.

Variable	N=107	Percentage (%)
Type I	0	0
Type II A	0	0
Type II B	55	51.4
Type III	11	10.3
Type IV	20	18.7
Type V	21	19.6

Table 14 - Descriptive Stats Acrylamide Gradient Gel Electrophoresis

	N
Origin	17
Mid	77
LDL Species	64
LDL A	2
LDL AI	1
LDL I	12
LDL I/B	20
LDL B	25
LDL B Post	4

Patients who show staining at the origin (indicating the presence of chylomicrons) have much higher baseline triglyceride values than those that do not have staining at the origin (Figure 7, Table 15). Those with very high triglycerides often do not have visible LDL species (Figure 8, Table 17). This phenomena is due to inefficient lipolysis. Most of the patients in this study have small dense LDL particles (smaller than 1) because of the high triglycerides.

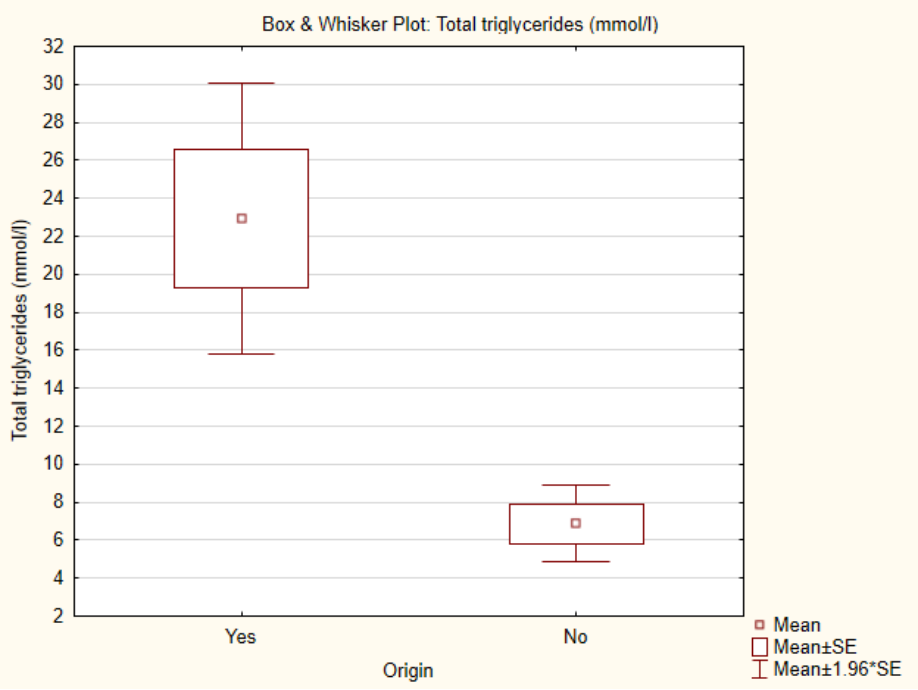


Figure 7 - Total Triglycerides(mmol/L) vs Origin staining.

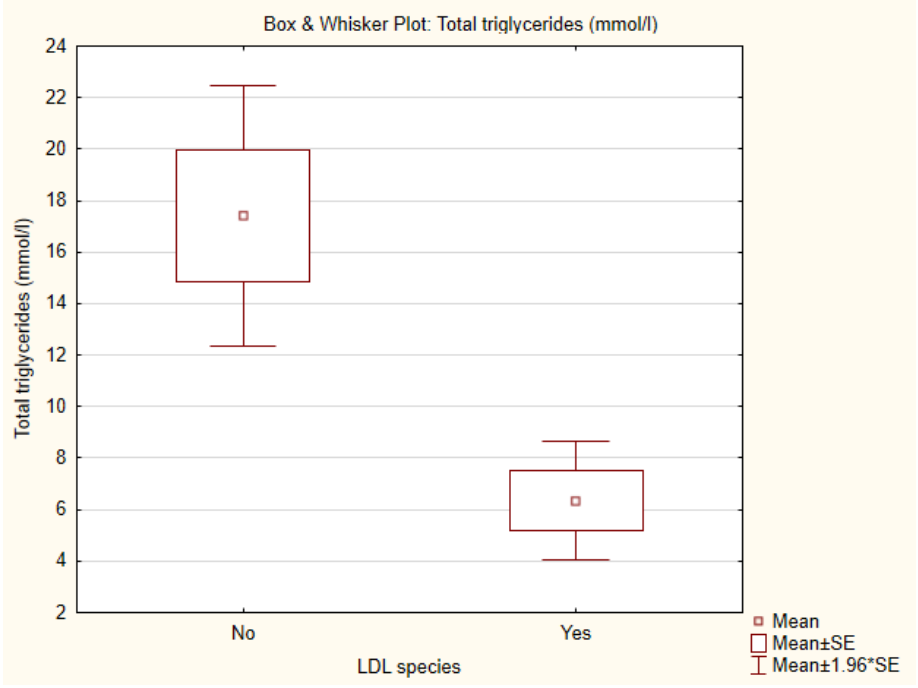


Figure 8 - Total Triglycerides mmol/L vs LDL species

Table 15 - Origin Group 1 : yes. Group 2: No

Variable	Mean (SD) Group 1 YES	Mean (SD) Group 2 NO	T value	df	P	N Group1 Yes	N Group 2 No	F ratio Variance	P (variance)
Tot TG (mmol/L)	22.91 (15.87)	6.86 (9.02)	5.85	93	0.00	19	76	3.10	**0.00**

Table 16 - Mid species: group 1 No. group 2 Yes

Variable	Mean (SD) Group 1 No	Mean (SD) Group 2 Yes	T value	df	P	N Group1 No	N Group 2 Yes	F ratio Variance	P (variance)
Tot TG mmol/L	10.64 (12.49)	9.94 (12.50)	0.21	93	0.83	18	77	1.00	1.00

Table 17 - LDL species: Group 1 No. Group 2 Yes

Variable	Mean (SD) Group 1 NO	Mean (SD) Group 2 YES	T value	df	P	N Group1 No	N Group 2 Yes	F ratio Variance	P (variance)
TG mmol/L	17.41 (14.58)	6.34 (9.29)	4.50	93	0.00	32	63	2.46	**0.00**

Figure 11 and Figure 12 on the next page show the distribution of Apo A and Apo B.

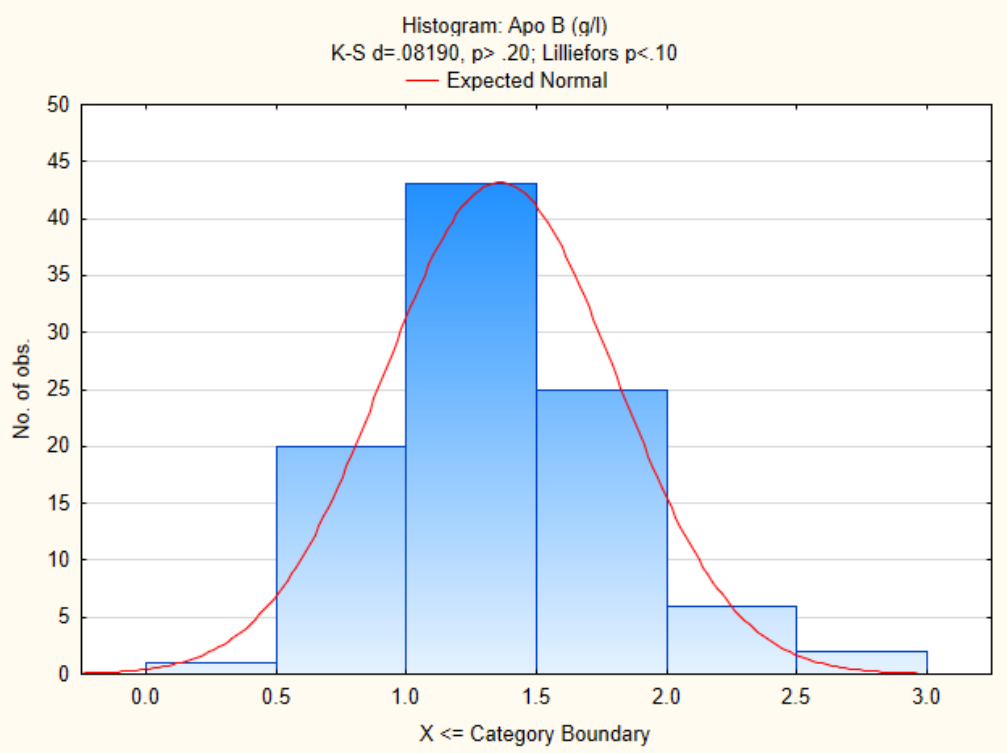


Figure 9 - Histogram: Apo B (g/l)

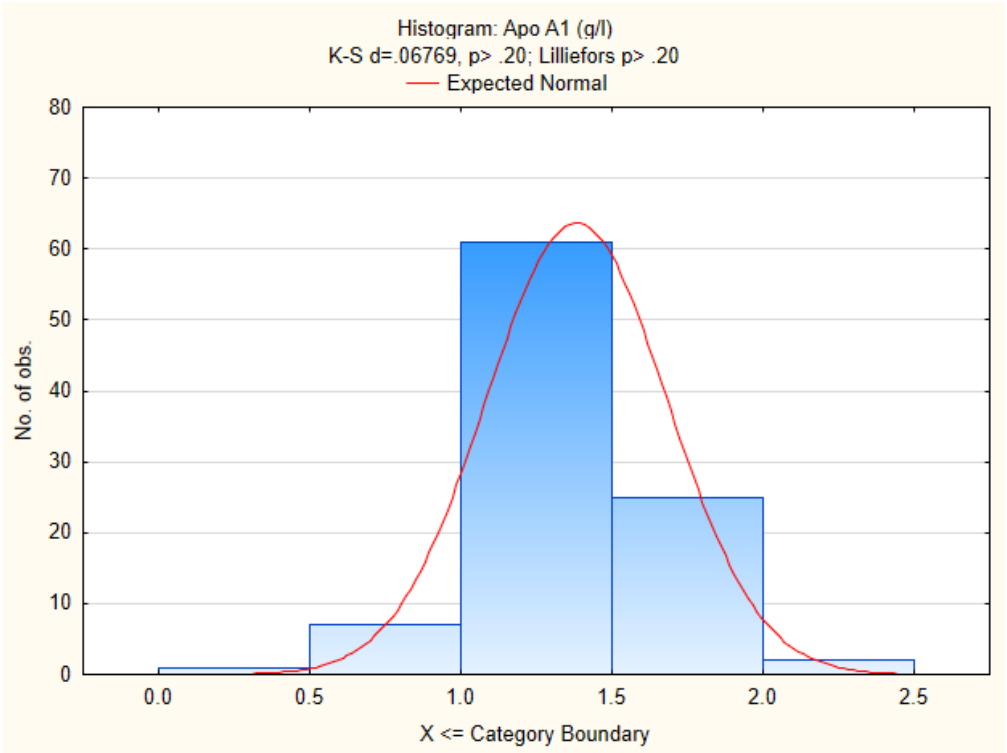


Figure 10 - Histogram Apo A1 (g/l)

Lipid results at presentation comparing males to females

The mean (SD) HDL-C was higher in females measuring 1.09 (0.36) mmol/L compared to 0.88 (0.26) mmol/L in males, P=0.002, (Table 18). The mean (SD) total cholesterol for both females and males were elevated. with minimal difference between the genders, females measuring 8.25 (3.12) mmol/L and males 8.47 (4.81) mmol/L (Table 18). The mean (SD) derived remnant cholesterol showed minimal difference between females and males with measurements of 1.43 (0.48) mmol/L and 1.53 (0.43) mmol/L respectively, (Table 18). The mean (SD) Apo A1 was statistically significantly higher in females 1.47 (0.26) g/l compared to males 1.23 (0.31) g/l, P=0.000, (Table 18). The Lp(a) level in mg/dl was higher in females 68.94 mg/dl compared to the lower value in males 28.6 (30.96) mg/dl. The measurement in nmol/l did not reflect this, but on the contrary showed a lower mean (SD) in females 87.14 (83.84) mg/dl in females compared to males 95.77(177.14) nmol/l, P=0.837, (Table 18).

Table 18 - Male: Female Baseline Laboratory Variables.

Variable	**Mean (Male) (SD)**	**Mean (Female) (SD)**	**p**
Total cholesterol (mmol/L)	8.47 (4.81)	8.25 (3.12)	0.78
HDL-C (mmol/L)	0.88 (0.26)	1.09 (0.36)	**0.002**
LDL-C (mmol/L)	3.47 (1.07)	4.47 (1.48)	0.051
Triglycerides (mmol/L)	12.91 (13.93)	9.04 (11.57)	0.138
Derived remnant cholesterol (mmol/L)	1.52 (0.27)	1.56 (0.24)	0.705
Apo B (g/l)	1.25 (0.43)	1.43 (0.48)	0.055
Apo A1 (g/l)	1.23 (0.31)	1.47 (0.26)	**0.000**
Lp (a) level (mg/dl)	28.6 (30.96)	68.94 (83.84)	**0.034**
Lp (a) level (nmol/l)	95.77 (177.14)	87.14 (67.97)	0.837

Ethnicity: Descriptive lipid results

Table 19 shows baseline demographic and lipid variables according to ethnicity. As all ethnic groups apart from the mixed ancestry group contained less than 10 individuals, we did not perform any statistical analysis according to patient ethnicity.

Table 19 - Ethnicity Descriptive Stats: Baseline Variables and Laboratory Results

Variable	**Mixed (n=81) Mean (SD)**	**Black (n=7) Mean (SD)**	**White (n=9) Mean (SD)**	**Indian/Asian (n=3) Mean (SD)**
Age at presentation	50.79 (10.33)	47.21 (10.24)	52.47 (12.88)	56.92 (5.19)
Height (m)	1.62 (0.10)	1.58 (0.06)	1.62 (0.10)	1.58 (0.08)
Weight (kg)	86.76 (16.05)	80.60 (13.22)	86.58 (25.35)	65.80 (11.56)
BMI (kg/m2)	33.06 (5.5)	32.37 (4.37)	32.66 (7.55)	26.11 (2.40)
Waist circumference (cm)	103.98 (12.04)	99.29 (10.05)	103.67 (19.31)	89.00 (6.56)
Hip circumference (cm)	105.23 (9.34)	113.00 (22.79)	110.89 (17.92)	93.83 (5.97)

Alcohol amount (g/week)	72.24 (89.02)	25.00 (7.07)	65.00 (77.78)	
Total cholesterol (mmol/L)	8.33 (4.06)	10.15 (2.97)	7.07 (1.27)	7.76 (2.28)
Total HDL-C (mmol/L)	1.01 (0.31)	1.11 (0.57)	0.91 (0.39)	1.31 (0.54)
Total LDL-C (mmol/L)	4.25 (1.48)	5.30 (1.52)	3.75 (1.00)	3.20
Total triglycerides (mmol/L)	10.72 (13.18)	14.34 (13.80)	6.34 (5.62)	7.11 (4.74)
Derived remnant cholesterol (mmol/L)	1.54 (0.25)	1.66 (0.08)	1.64 (0.27)	1.20
Apo B (g/l)	1.34 (0.44)	1.48 (0.47)	1.38 (0.52)	1.54 (0.50)
Apo A1 (g/l)	1.37 (0.29)	1.58 (0.42)	1.35 (0.34)	1.43 (0.34)
Lp (a) level (mg/dl)	57.91 (74.53)	40.00	15.00 (10.00)	18.50 (13.44)
Lp (a) level (nmol/l)	80.79 (137.13)	57.40 (46.14)	164.40 (47.84)	129.00
Baseline fasting glucose (mmol/L)	10.76 (3.64)	12.81 (8.67)	11.77 (6.71)	18.60 (8.41)
HbA1c (%)	9.64 (2.36)	10.38 (2.54)	8.57 (1.99)	9.82 (3.56)
TSH level	3.50 (11.47)	2.61 (0.91)	1.77 (1.62)	1.83 (0.63)

Correlations with Apo B

There was no correlation between Apo B (g/l) and remnant cholesterol, p=0.1668, Figure 13. There is a positive correlation between Apo B(g/l) and LDL-C, p=0.000 (Figure 14) as well as between Apo B(g/l) and total cholesterol (Figure 15).

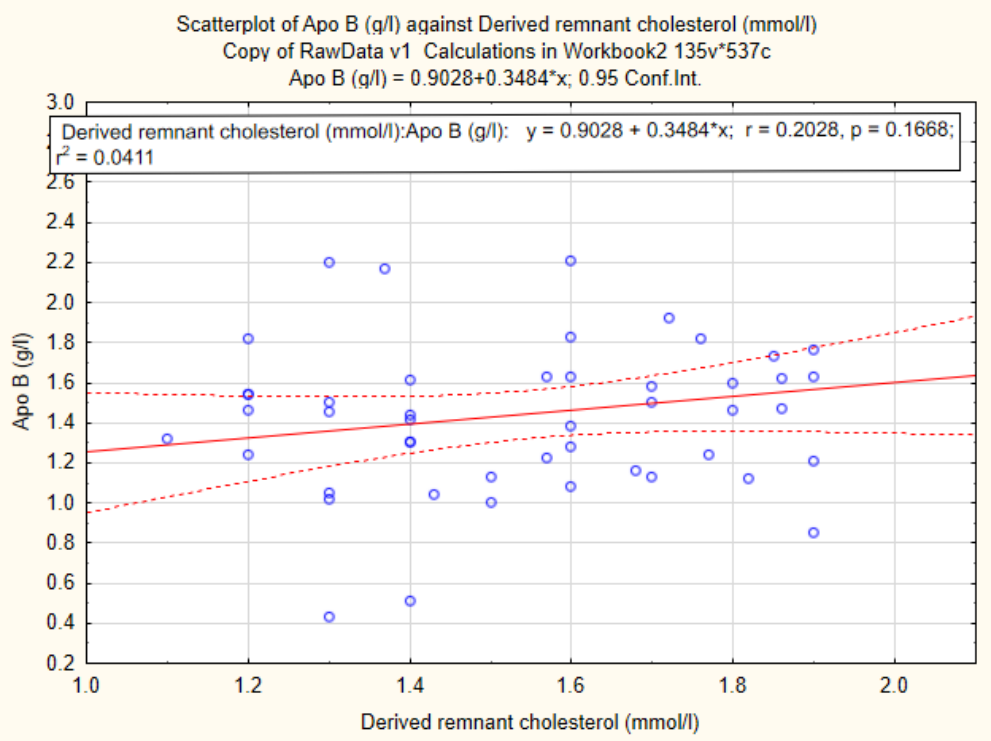


Figure 11 - Correlation between Apo B (g/l and remnant Cholesterol mmol/L)

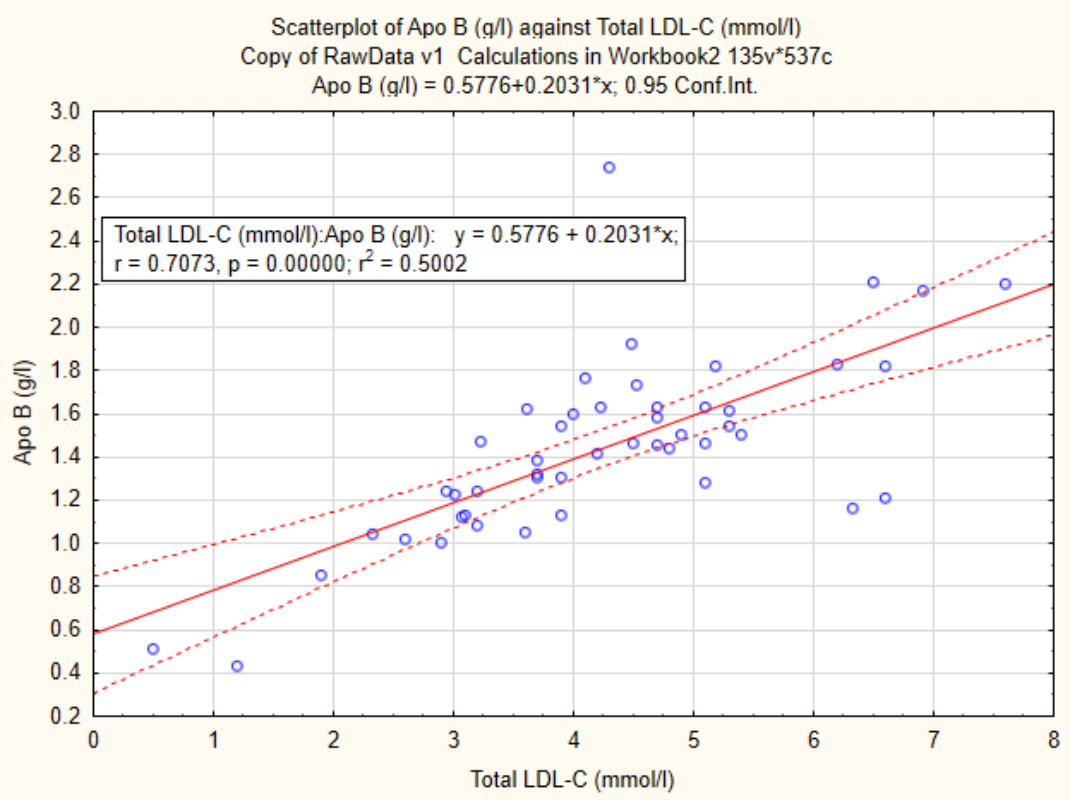


*Figure 12 - Correlation Apo B (g/l) against Total LDL-C (mmol/L_*

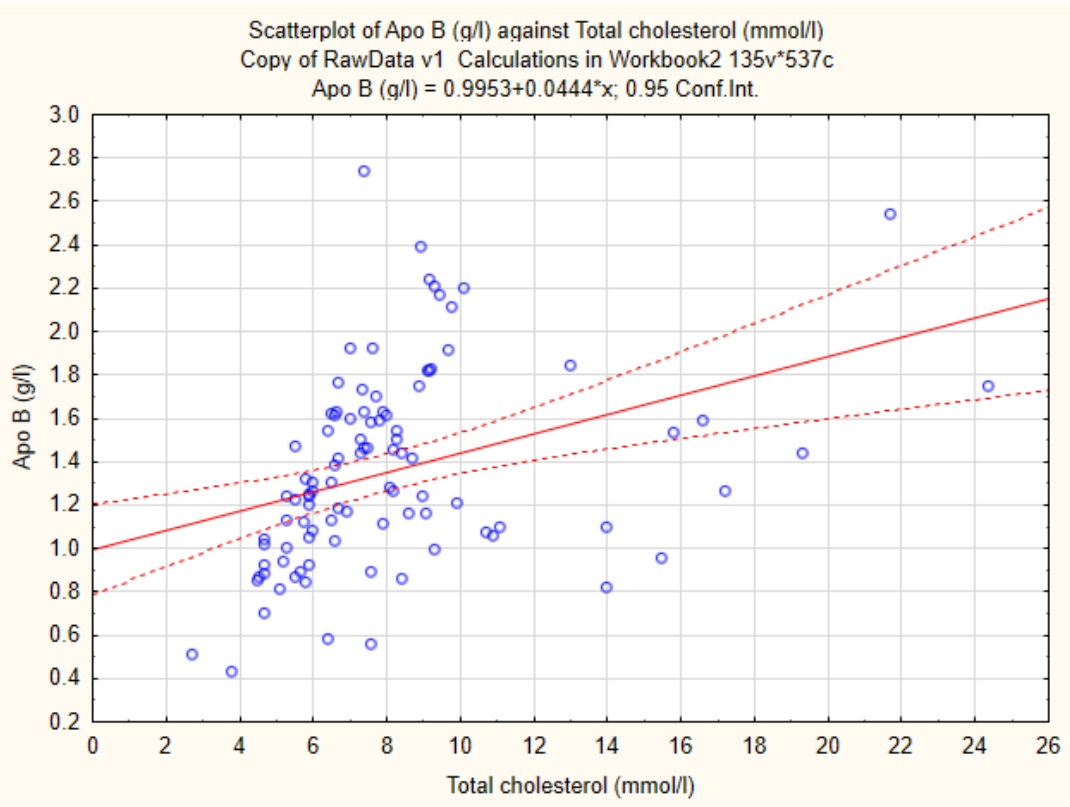


Figure 13 - Correlation Apo B against Total Cholesterol (mmol/L)

LDL Cholesterol: Lowest LDL-C (evaluation if LDL-C target is reached)

Not all patients have a LDL-C value, this is because some patients never had triglycerides that were low enough to enable calculation of LDL-C. As seen in Figure 16 and Table 20 among 85 evaluable patients the mean (SD) best LDL-C was 2.44 (0.88) mmol/L, ranging from 0.51 mmol/L to 5.11 mmol/L. Only 4.1% of all patients with a calculable LDL-C achieved values below 1.8mmol/L (Table 21).

Table 20 - Descriptive stats Best(lowest) LDL C

Variable	N	Mean(SD)	Median	Minimum	Maximum	Lower quartile	Upper quartile
Best LDL C	85	2.44 (0.88)	2.39	0.51	5.11	1.80	2.90

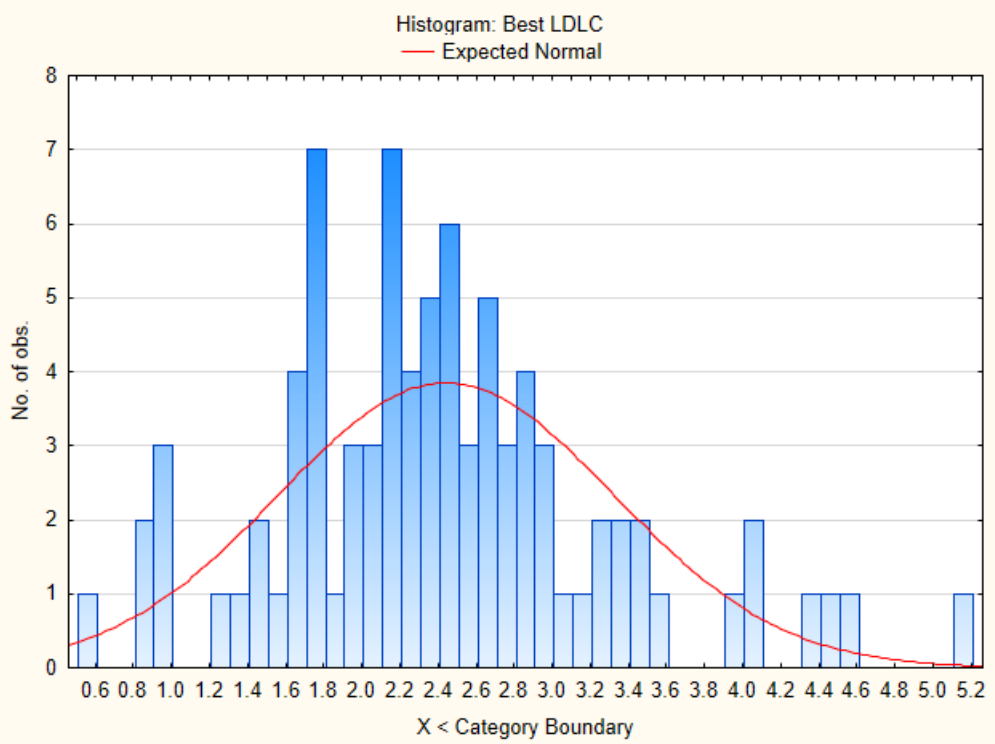


Figure 14 - Histogram Best LDL C

Table 21 - Frequency Table Best LDL C

LDL categories	Count	Cumulative Count	Percent	Cumulative Count
0.51 <=x<0.61	1	1	0.18622	0.1862
0.61 <=x<0.71	0	1	0.00000	0.1862
0.71 <=x<0.81	0	1	0.00000	0.1862
0.81 <=x<0.91	2	3	0.37244	0.5587
0.91 <=x<1.01	3	6	0.55866	1.1173
1.01 <=x<1.11	0	6	0.00000	1.1173
1.11 <=x<1.21	0	6	0.00000	1.1173
1.21 <=x<1.31	1	7	0.18622	1.3035

1.31 <=x<1.41	1	8	0.18622	1.4898
1.41 <=x<1.51	2	10	0.37244	1.8622
1.51 <=x<1.61	1	11	0.18622	2.0484
1.61 <=x<1.71	4	15	0.74488	2.7933
1.71 <=x<1.81	7	22	1.30354	4.0968
1.81 <=x<1.91	1	23	0.18622	4.2831
1.91 <=x<2.01	3	26	0.55866	4.8417
2.01 <=x<2.11	3	29	0.55866	5.4004
2.11 <=x<2.21	7	36	1.30354	6.7039
2.21 <=x<2.31	4	40	0.74488	7.4488
2.31 <=x<2.41	5	45	0.93110	8.3799
2.41 <=x<2.51	6	51	1.11732	9.4972
2.51 <=x<2.61	3	54	0.55866	10.0559
2.61 <=x<2.71	5	59	0.93110	10.9870
2.71 <=x<2.81	3	62	0.55866	11.5456
2.81 <=x<2.91	4	66	0.74488	12.2905
2.91 <=x<3.01	3	69	0.55866	12.8492
3.01 <=x<3.11	1	70	0.18622	13.0354
3.11 <=x<3.21	1	71	0.18622	13.2216
3.21 <=x<3.31	2	73	0.37244	13.5940
3.31 <=x<3.41	2	75	0.37244	13.9665
3.41 <=x<3.51	2	77	0.37244	14.3389
3.51 <=x<3.61	1	78	0.18622	14.5251
3.61 <=x<3.71	0	78	0.00000	14.5251
3.71 <=x<3.81	0	78	0.00000	14.5251
3.81 <=x<3.91	0	78	0.00000	14.5251
3.91 <=x<4.01	1	79	0.18622	14.7114
4.01 <=x<4.11	2	81	0.37244	15.0838
4.11 <=x<4.21	0	81	0.00000	15.0838
4.21 <=x<4.31	0	81	0.00000	15.0838
4.31 <=x<4.41	1	82	0.18622	15.2700
4.41 <=x<4.51	1	83	0.18622	15.4562
4.51 <=x<4.61	1	84	0.18622	15.6425
4.61 <=x<4.71	0	84	0.00000	15.6425
4.71 <=x<4.81	0	84	0.00000	15.6425
4.81 <=x<4.91	0	84	0.00000	15.6425
4.91 <=x<5.01	0	84	0.00000	15.6425
5.01 <=x<5.11	0	84	0.00000	15.6425
5.11 <=x<5.21	1	85	0.18622	15.8287
Missing	452	537	84.17132	100.0000

3.1.4 Diabetic control: Fasting glucose. HbA1C and correlation with Triglycerides

The mean (SD) baseline fasting glucose for males and females was elevated at 11.24 (4.76) mmol/L with a wide range from 1.8 to 30 mmol/L (Table 22). The baseline HbA1C was only available for 57 patients and was a mean (SD) of 9.61 (2.34) % ranging from 6.2 to 15.9% (Table 22). Of these 57 patients 82.46% had an HbA1C above 7% (Table 24). The mean (SD) baseline glucose and HbA1C for males and females were both elevated, with minimal difference between the two groups (Table 23). Baseline fasting glucose mean (SD)for males was 10.86 (4.26) mmol/L and 11.47 (5.05) mmol/L for females. The baseline mean (SD) HbA1C in males was 9.47 (2.63) % and 9.7 (2.16) % in females (Table 23).

There was no correlation between baseline triglycerides and HbA1C at presentation (Figure 17). There was no correlation between baseline triglyceride vs baseline Fasting Glucose (Figure 18).

HbA1C was captured 411 times in the follow-up data as seen in Table 25. The mean (SD) was 8.94 (2.18) ranging from 4.2 to 16.2 %. Of all the measured HbA1Cs 23.84% were below 7% and the rest of measured HBA1Cs values were above 7% (Table 26).

There was no correlation at baseline and follow up between the highest TG and the highest HbA1C level as well as the lowest TG and lowest HbA1C levels (Figure 19 and Figure 20).

Table 22 - Baseline Variables: Baseline Fasting glucose. HbA1c and TSH

Variable	N	Mean (SD)	Median	Minimum	Maximum
Baseline fasting glucose (mmol/L)	97	11.24 (4.76)	9.9	1.8	30
HbA1c %	57	9.61 (2.34)	9.5	6.2	15.9
TSH level (mIU/l)	93	3.212 (10.25)	1.9	0.00	100

Table 23 - Male: Female Baseline Laboratory Variables: Baseline fasting glucose, HbA1C and TSH

Variable	Mean (Male) (SD)	Mean (Female) (SD)	P value
Baseline fasting glucose (mmol/L)	10.86 (4.26)	11.47 (5.05)	0.547
HbA1c (%)	9.47 (2.63)	9.7 (2.16)	0.715
TSH level (mIU/l)	4.99 (16.55)	2.14 (1.75)	0.196

Table 24 - Frequency Table HbA1C at Baseline

HbA1C categories (%)	N	Cumulative percentage
<6.5	4	7.02
<7.0	6	10.53
>7.0	47	82.46
7-10	22	38.60
>10	25	43.86
Totals	57	

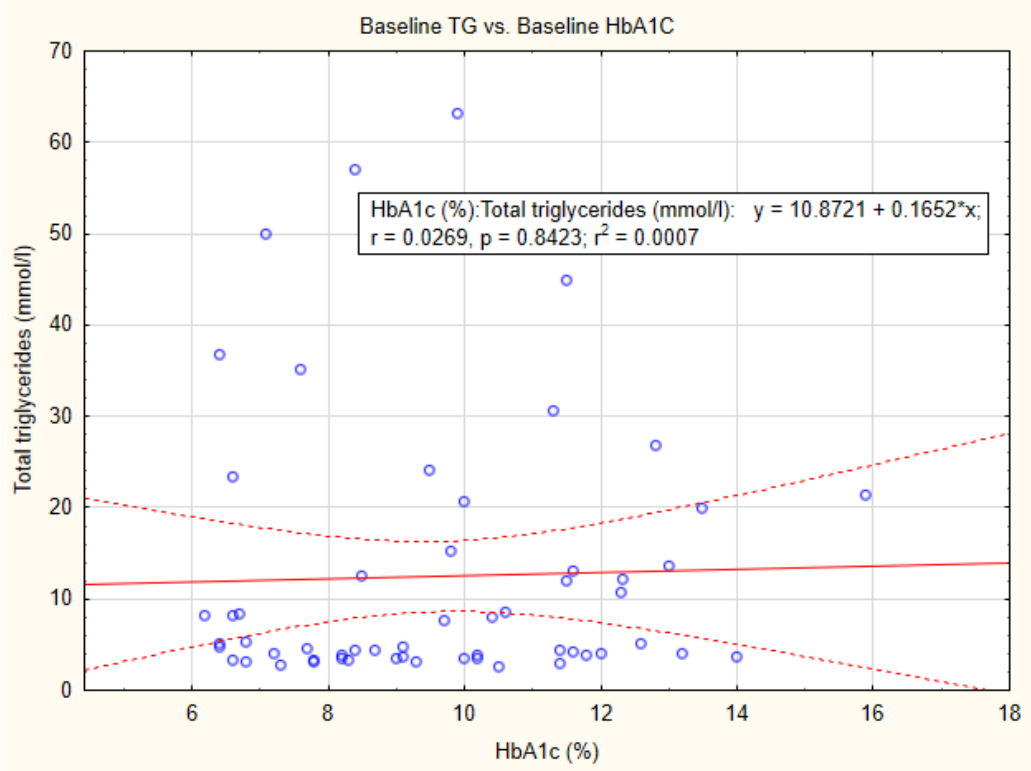


Figure 15 - Scatterplot baseline TG vs HbA1C at baseline presentation

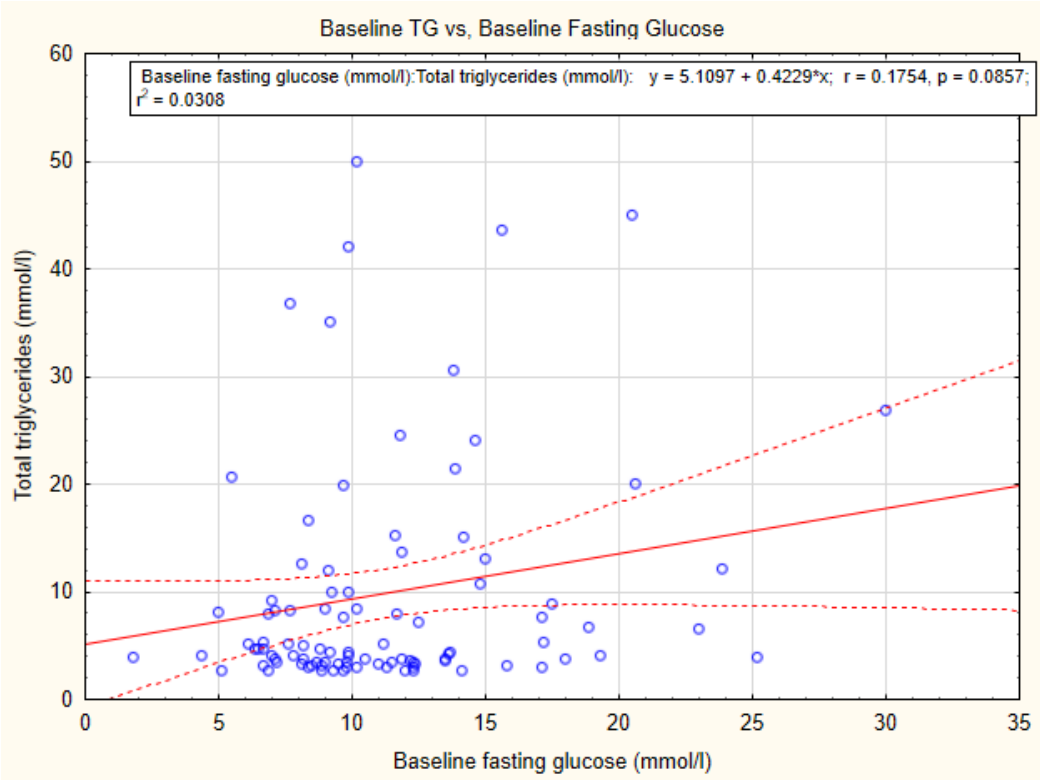


Figure 16 - Baseline Triglyceride vs Baseline Fasting Glucose

Table 25- Descriptive stats HbA1C all visits.

VARIABLE	N	MEAN (SD)	MEDIAN	MIN	MAX	LOWER QUARTILE	UPPER QUARTILE	RANGE	QUARTILE (RANGE)
HbA1c (%)	411	8.94 (2.18)	8.6	4.2	16.2	7.1	10.3	12.0	3.2

Table 26 - HbA1C Categories of all documented HBA1Cs over all visits.

CATEGORY HbA1C %	COUNT	CUMULATIVE COUNT	Percent (of Valid)	Cumulative % of valid
2-3	0	0	0	0
3-4	0	0	0	0
4-5	1	1	0.24	0.24
5.-6	9	10	2.19	2.43
6-7	88	98	21.41	23.84
7-8	67	165	16.30	40.15
8-9	75	240	18.25	58.39
9-10	59	299	14.36	72.75
10-11	35	334	8.52	81.27
11-12	36	370	8.76	90.02
12-13	21	391	5.11	95.13
13-14	14	405	3.41	98.54
14-15	2	407	0.49	99.03
15-16	3	410	0.73	99.76
16-17	1	411	0.24	100.0000
Missing	126	537	30.66	

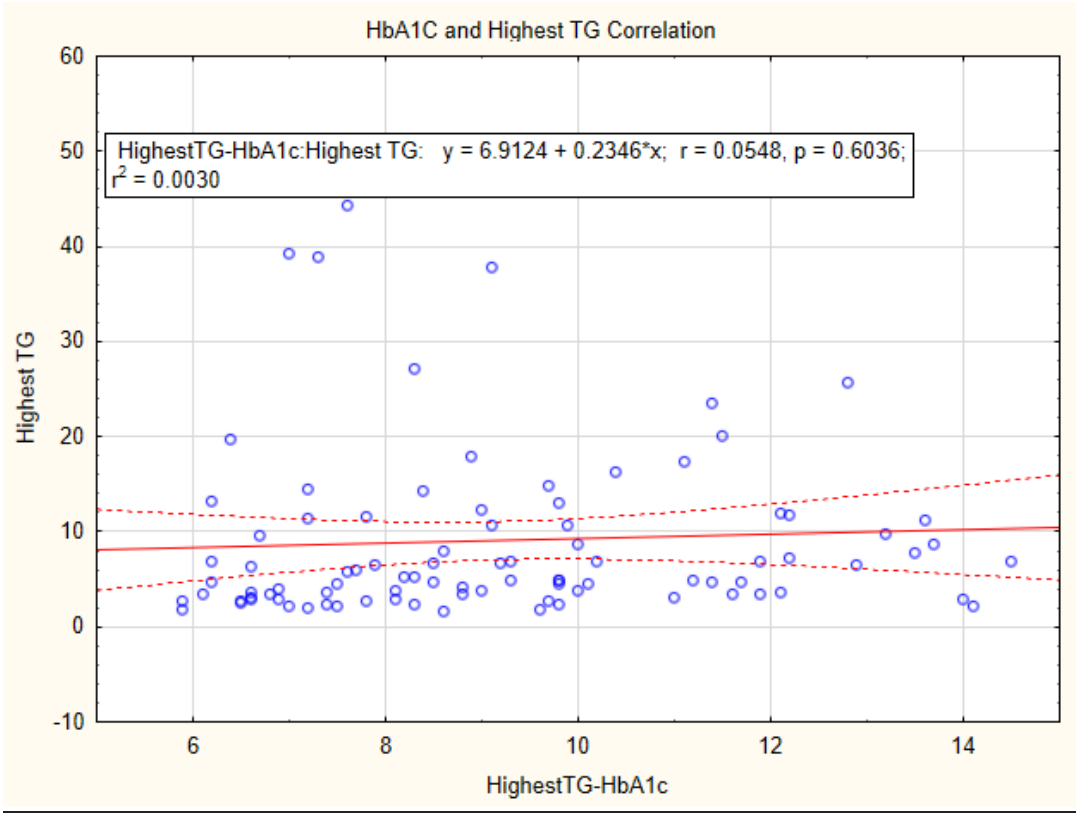


Figure 17 - HbA1C and highest Triglyceride correlation

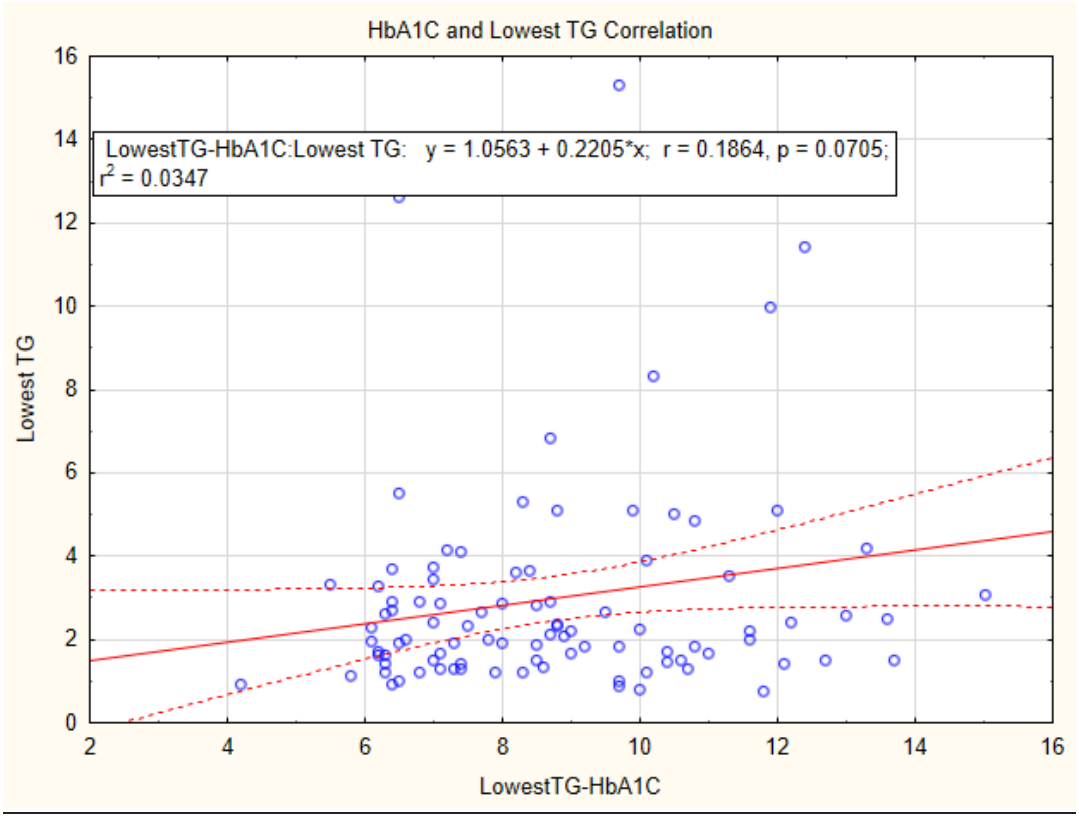


Figure 18 - HbA1C and lowest Triglyceride Correlation

3.1.5 Alcohol correlation with triglyceride

We identified a positive correlation between triglycerides and alcohol intake in those that consume alcohol (Figure 21).

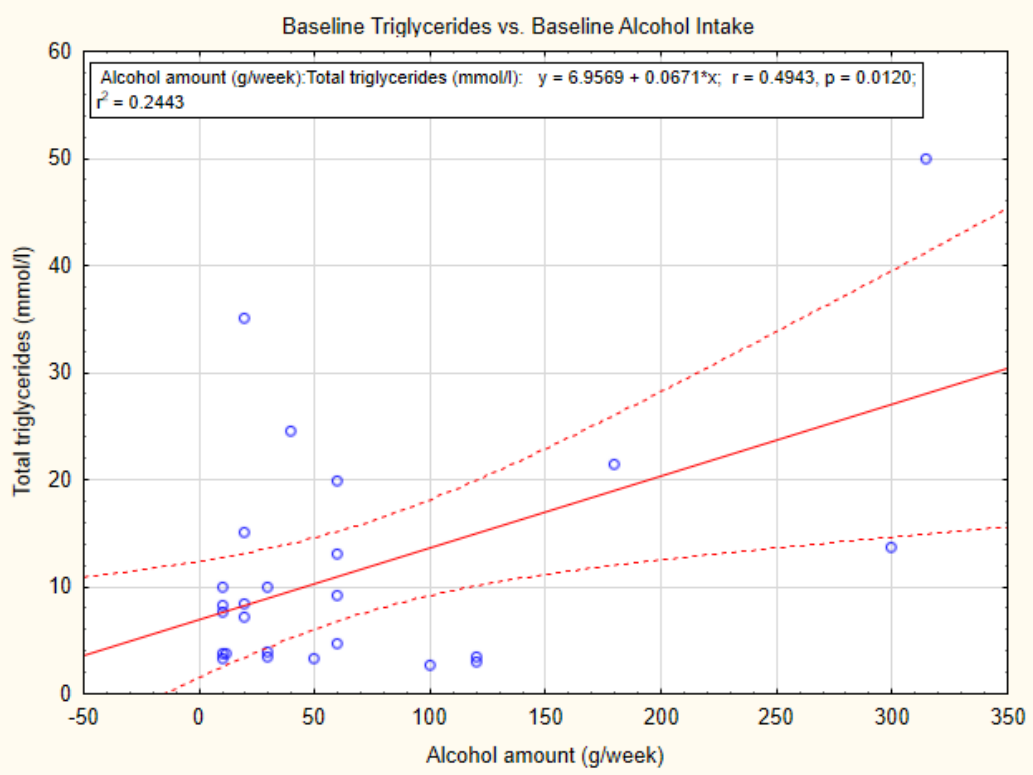


Figure 19 - Baseline Triglycerides vs Baseline Alcohol Intake

3.1.6 Thyroid function

There were 5 patients with a TSH more than the upper limit of normal, but only one patient had a markedly raised TSH of >100 secondarily to undiagnosed hypothyroidism.

The TSH was documented in 93 participants with a mean (SD) 3.21 (10.25) mIU/l with a wide range of 0 – 100 mIU/l.

The thyroid function in males were more variable in males with a mean (SD) TSH of 4.99 (16.55) mIU/l as the patient with the TSH > 100 was male. Mean (SD) TSH values in females were 2.14 (1.75).

3.1.7 BMI and waist circumference correlation to Triglycerides.

There was no correlation between baseline triglycerides (mmol/L) and BMI (Figure 22).
There was also no correlation between baseline triglyceride and baseline waist circumference (Figure 23).

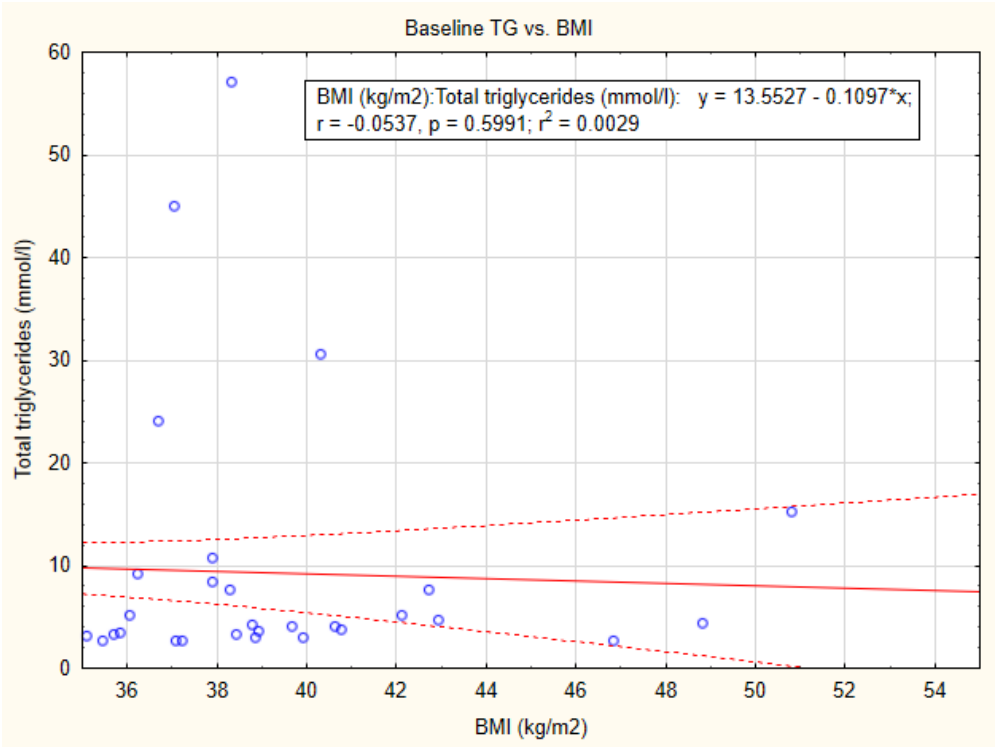


Figure 20 - Scatterplot of baseline total Triglycerides (mmol/L) vs BMI

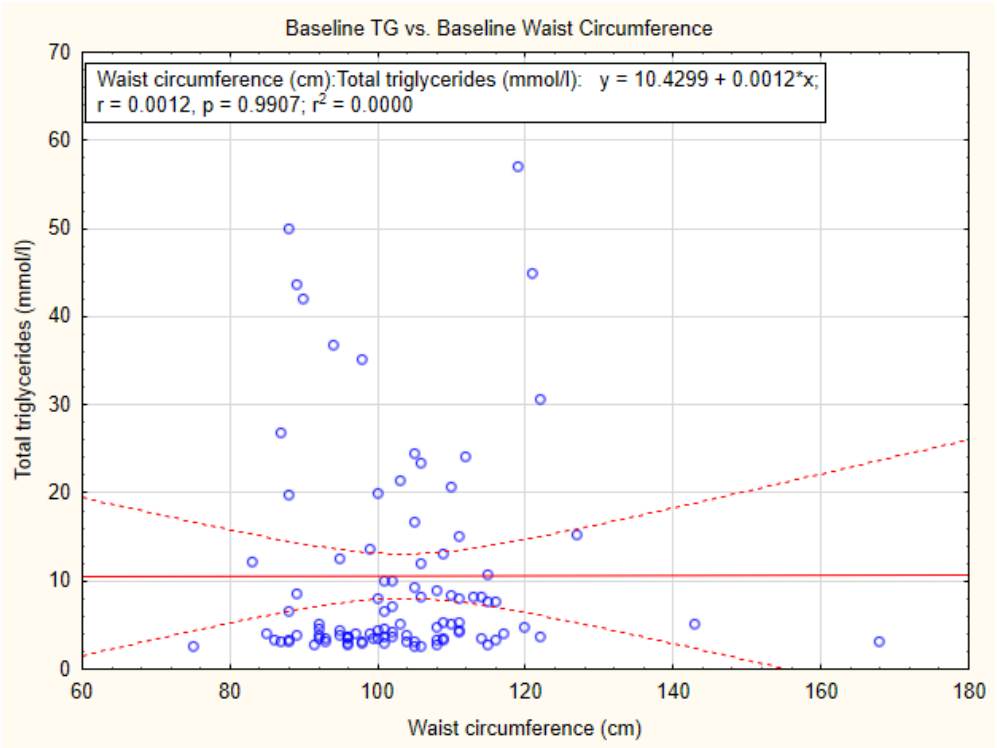


Figure 21 - Baseline Triglyceride vs Baseline Waist Circumference

3.1.8 Correlation between baseline Triglycerides and Total Cholesterol

There was a positive correlation between baseline triglycerides and total cholesterol (Figure 24)

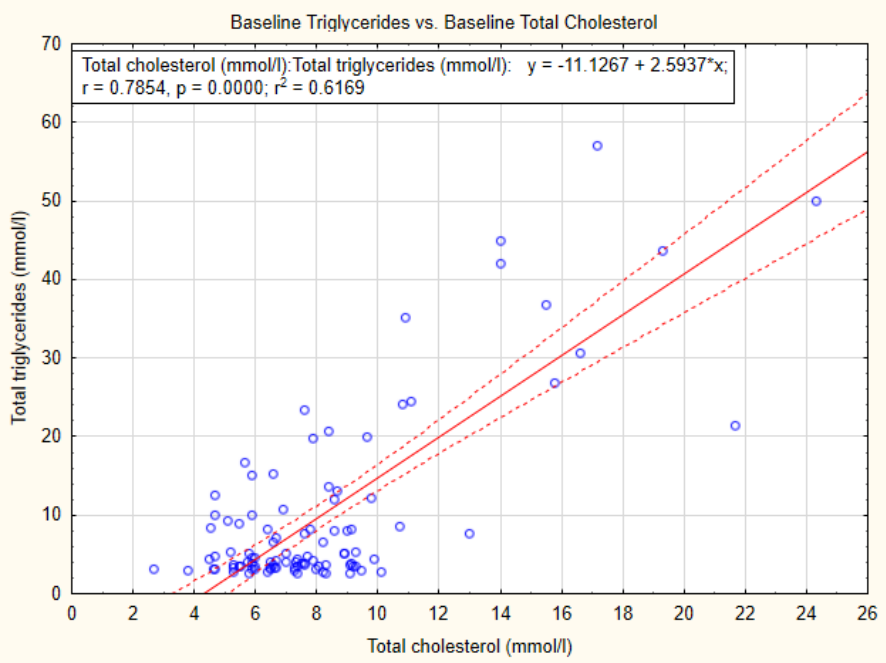


Figure 22 - Baseline TG vs Baseline Cholesterol

3.1.9 Correlation between baseline Triglycerides and HDL-C

There was no correlation between baseline triglycerides and HDL-C (Figure 25).

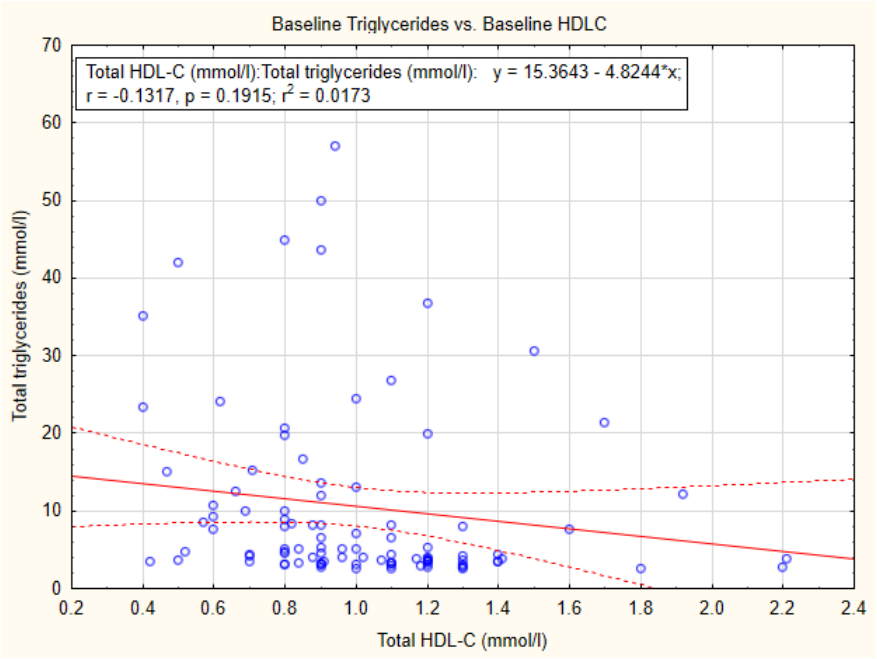


Figure 23 - Baseline Triglycerides vs HDL-C

Follow up visit results

The laboratory variables from visit 1 to 5 are summarized in Table 27, Table 28, Table 29, Table 30 and Table 31. Visit 5 is the visit at which the lowest TG level was captured if the lowest TG was not within the initial 4 visits. Table 32 compares laboratory visits at year one and year 2 visits, with no significant differences in the variables.

Table 27 - Descriptive stats Visit 1

Variable	N	Mean(SD)	Median	Min	Max	Lower quartile	Upper quartile
HbA1c (%)	93	8.94(2.17)	8.50	5.90	14.00	7.00	10.40000
Total HDL-C (mmol/L)	99	0.99(0.33)	0.90	0.45	2.30	0.76	1.20
Total LDL-C (mmol/L)	51	3.19(1.18)	2.90	0.97	6.24	2.50	3.90
Total triglycerides (mmol/L)	100	6.16(6.82)	3.89	0.73	44.20	2.45	6.55
Random blood glucose (mmol/L)	90	10.92(4.54)	10.60	3.30	27.80	7.40	13.00
HbA1c (%)	93	8.94(2.17)	8.50	5.90	14.00	7.00	10.40
Total HDL-C (mmol/L)	99	0.99(0.33)	0.90	0.45	2.30	0.76	1.20

Table 28 - Descriptive stats Visit 2

Variable	N	Mean(SD)	Median	Min	Max	Lower quartile	Upper quartile
HbA1c (%)	93	8.78(2.28)	8.30	5.80	16.20	7.00	10.30
Random blood glucose (mmol/L)	89	10.82(4.92)	9.50	3.50	28.00	7.20	12.80
Total cholesterol	99	6.11(1.87)	5.70	2.66	12.80	4.90	7.04
HDL-C (mmol/L)	97	0.98(0.31)	0.92	0.41	2.10	0.80	1.15
LDL-C (mmol/L)	59	3.27(1.27)	3.00	1.10	7.50	2.35	3.80
Triglycerides (mmol/L)	97	5.85(5.93)	3.59	1.13	35.20	2.40	6.23
HbA1c (%)	93	8.78(2.28)	8.30	5.80	16.20	7.00	10.30

Table 29 – Descriptive stats Visit 3

Variable	N	Mean	Median	Min	Max	Lower quartile	Upper quartile
HbA1c (%)	96	8.80(2.01)	8.75	5.80	15.20	7.10	9.80
Random blood glucose (mmol/L)	95	10.58(4.59)	9.20	4.40	26.40	7.00	12.90
Total cholesterol	99	6.14(2.34)	5.60	3.06	16.50	4.70	6.70
HDL-C (mmol/L)	98	0.99(0.33)	0.95	0.15	2.50	0.80	1.16
LDL-C (mmol/L)	57	3.15(1.28)	2.90	0.92	6.94	2.23	3.70
Triglycerides (mmol/L)	98	6.71(8.58)	3.84	1.00	39.84	2.30	6.30

Table 30 - Descriptive Stats Visit 4

Variable	N	Mean(SD)	Median	Min	Max	Lower quartile	Upper quartile
HbA1c (%)	95	9.25(2.29)	8.90	5.90	15.30	7.30	11.00
Total HDL-C (mmol/L)	99	1.03(0.34)	1.00	0.16	2.10	0.80	1.20
Total LDL-C (mmol/L)	63	3.09(1.29)	2.83	0.86	7.20	2.30	3.61
Total triglycerides (mmol/L)	100	6.12(7.78)	3.45	0.90	48.78	2.13	6.51
Random blood glucose (mmol/L)	93	11.19(4.58)	10.50	2.40	29.50	8.10	13.30

Table 31 - Descriptive Stats Visit 5

Variable	N	Mean(SD)	Median	Min	Max	Lower quartile	Upper Quartile
HbA1c (%)	34	8.87(2.06)	8.90	4.20	13.00	7.10	10.20
Random blood glucose (mmol/L)	34	9.73(4.87)	7.90	4.30	21.60	5.70	12.10
Total cholesterol	37	4.82(1.17)	4.63	2.84	7.86	4.04	5.32
HDL-C (mmol/L)	37	1.12(0.36)	1.10	0.60	2.49	0.88	1.28
LDL-C (mmol/L)	33	2.75(1.11)	2.79	0.51	5.38	2.10	3.10
Triglycerides (mmol/L)	37	2.33(1.64)	1.88	0.70	8.30	1.33	2.52

Table 32 - Comparing outcomes between one year and two years of follow-ups. Group 1: visit 2. Group 2: visit 4

Variable	Mean Group 1	Mean Group 2	t-value	df	P	Valid N Group 1	Valid N Group 2	Std dev Group 1	Std dev Group 2	F-ratio	P (variance)
Random blood glucose (mmol/L)	10.9	11.19	-0.39	181	0.70	90	93	4.54	4.58	1.02	0.94
HbA1c (%)	8.94	9.25	-0.96	186	0.34	93	95	2.17	2.29	1.12	0.60
Total cholesterol	6.17	6.10	0.25	198	0.80	100	100	1.97	2.12	1.15	0.49
Total HDL-C (mmol/L)	0.99	1.03	-0.90	196	0.37	99	99	0.33	0.34	1.11	0.60
Total LDL-C (mmol/L)	3.19	3.09	0.44	112	0.66	51	63	1.18	1.29	1.19	0.52
Total triglycerides (mmol/L)	6.16	6.12	0.03	198	0.97	100	100	6.82	7.78	1.30	0.19

The mean (SD) reduction in HbA1C from presentation to the lowest subsequently documented level was 0.94 (1.64)% ranging from either an increase of 1.7% to a decrease of 7.8% [95% CI (1.44 – 1.91%)].

The mean (SD) change in HDL from Visit 1 to the highest documented post initial visit value was 0.15 (0.23)mmol/L, ranging from either a decrease of 0.27mmol/L to an increase of 1.2 mmol/L. 95% CI (0.21-0.27).

At the visit where TG was the lowest, the captured mean(SD) change in total cholesterol was a decrease of 1.35(1.65)mmol/L, ranging from an increase of 4.02mmol/L to a decrease by 8.7 mmol/L, [95 % CI 1.45 – 1.93]. The mean (SD) change in LDL-C from visit one to the visit at which it was the lowest was 0.83(1.08)mmol/L, ranging from an increase by 2.69 mmol/L to a decline of 3.60 mmol/L, 95% CI 0.91 – 1.35mmol/L.

The change in total triglycerides from initial presentation to the follow-up visit at which it was the lowest was a mean(SD) decrease of 2.91 (4.98)mmol/L, ranging from either no change to a decrease of 32.68mmol/L, 95% CI4.36-5.80. (Table 33)

The majority of patients (74.75%) showed a decrease in TG in the range from 0 to 10mmol/L by visit one, while 6 showed an increase from 0 – 10mmol/L at visit one (Table 34). The mean (SD) change in TG from the baseline to the highest level over the first four follow up visits was -7.30(12.49) with a median of -2,5mmol/L (meaning a mean of 2,5mmol/L elevation of TG) (table 23), with multiple outliers seen in Figure 26.

The mean(SD) change in TG from baseline to the highest follow up was an elevation of 1.51 (12.03)mmol/L, with a median elevation of 0.02 mmol/L, ranging from an increase of 51.3mmol/L to a decline of 39.9 mmol/L (Table 36 and Figure 27).

Table 33 Descriptive Stats, Change from Visit 1 to Visit 4 and to the lowest

Variable	Valid N	Mean (SD)	Median	Minimum	Maximum	Lower (Quartile)	Upper (Quartile)	Percentile (10.00000)	Percentile (90.00000)	Confidence SD (-95.000%)	Confidence SD (+95.000%)	Coef. Var.
Change HbA1cV1-V4	90	-0,36 (2.22)	-0.25	-6.40	7.30	-1.80	0.70	-3.10	2.15	1.94	2.60	-609.60
Change HbA1c V1 to lowest	97	0.94(1.64)	0.20	-1.70	7.80	0.00	1.10	0.00	3.10	1.44	1.91	174.92
Change in HDL V1-V4	96	0.04(0.23)	0.00	-0.41	1.08	-0.10	0.14	-0.20	0.30	0.20	0.27	581.87
Change in HDL V1 to highest	97	0.15(0.23)	0.10	-0.27	1.20	0.00	0.20	0.00	0.40	0.21	0.27	158.45
Change in TC V1-V4	99	-0.09(2.13)	0.00	-9.61	4.67	-0.99	1.20	-2.57	2.68	1.87	2.47	-2466.35
Change in TC v1 to lowest	98	1.35(1.65)	0.95	-4.02	8.70	0.24	2.01	0.00	3.40	1.45	1.93	122.72
Change in LDLC V1-V4	46	0.21(1.18)	0.00	-2.25	3.60	-0.50	1.00	-1.00	1.90	0.98	1.49	565.94
Change in LDLC V1-best	50	0.83(1.08)	0.53	-2.69	3.60	0.13	1.40	0.00	2.40	0.91	1.35	130.54
Change TG V1-V4	97	-0.35(6.82)	0.20	-33.47	17.21	-1.40	1.31	-5.50	6.60	5.97	7.94	-1930.13
Change TG V1 to best	97	2.91(4.98)	1.10	0.00	32.68	0.20	2.80	0.00	8.70	4.36	5.80	170.91

Table 34 - Frequency Table Change in Triglyceride from baseline to Visit one

Category	N	Percentage
-70 000 - -60 000	0	0
-60 000 - -50 000	2	2.02
-50 000 - -40 000	3	3.03
-40 000 - -30 000	3	3.03
-30 000 - -20 000	3	3.03
-20 000 - -10 000	8	8.08
-10 000 – 0.00	74	74.75
0.00 – 10.00	6	6.06
Total	99	

Best Response

The median change in TG from baseline to the lowest level was -2.5mmol/L.

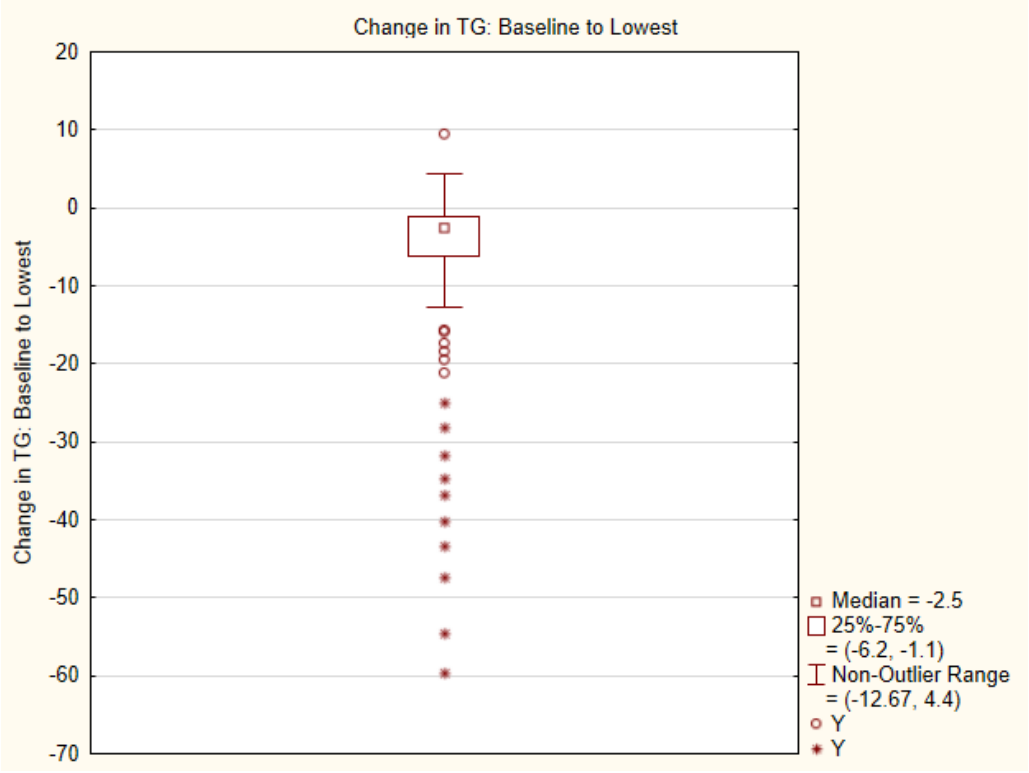


Figure 24- Change in TG Baseline to Lowest

Table 35 - Change in Triglyceride Baseline to lowest follow up (based on 4 follow-up visits).

Variable: change in TG	**N**	**Mean**	**Median**	**Minimum**	**Max**	**Lower Quartile**	**Upper Quartile**	**Percentile (10 00000)**	**Percentile (90 00000)**
Baseline to lowest	99	-7.30	-2.5	-59.70	9.50	-6.2	-1.1	-24.90	-0.40

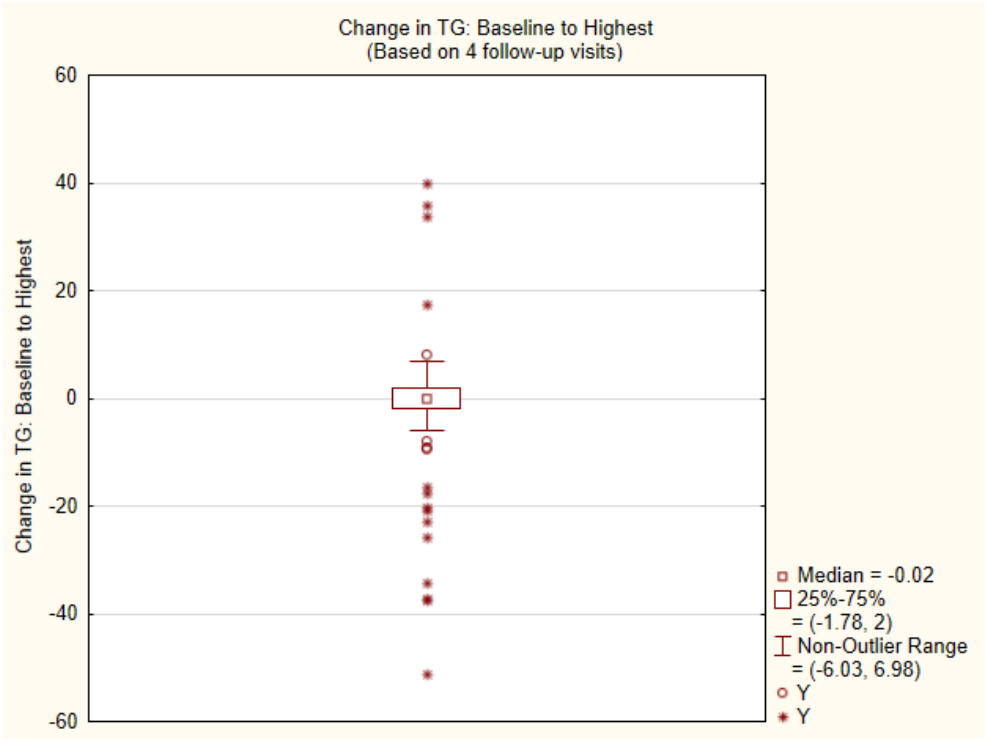


Figure 25 - Change in Triglyceride: Baseline to Highest. Based on 4 follow-up visits.

Table 36 - Change in Triglyceride Baseline to highest follow up

Change in TG	N	Mean(SD)	Median	Min	Max	Lower (quartile)	Upper (quartile)	Percentile. (10 00000)	Percentile (90 00000)
Baseline to Highest	99	-1.51(12.03)	-0.02	-51.30	39.90	-1.78	2.00	-16.55	4.55

The median change in triglyceride from baseline to the highest levels during four follow up visits was only -0.02 mmol/L was minimal. The box and whiskers also illustrates that during follow up triglycerides often still increase from baseline.

Table 37 - Frequency table Baseline triglyceride to highest

Category	N	Percentage
-80 000 - -60 000	0	0
-60 000 - -40 000	1	1.01
-40 000 - -20 000	7	7.07
-20 000 - 0 000	45	45.45
0 000 - 20 000	43	43.43
20 000 - 40 000	3	3.03
Total	99	

Although overall triglycerides values decreased on follow-up the change was neither uniform nor consistently maintained (Table 37). Only 53 at the worst TG measurement were below their baseline, while 46 were above their baseline. Only half of all patients had consistently lower TG at follow up than baseline.

Linked analysis

The HbA1c value corresponding to the instances when the triglycerides were either lowest [8.77(2.25), Mean(SD)] or highest [9.20(2.26), Mean(SD)] were compared, with no statistical significance due to a high variability (P=0.96), (Figure 28, Figure 29, Figure 30, Table 38, Table 39, Table 40, Table 41). Although the mean HbA1C is lower when the triglycerides are at their lowest, it is not statistically significant due to a very high variability.

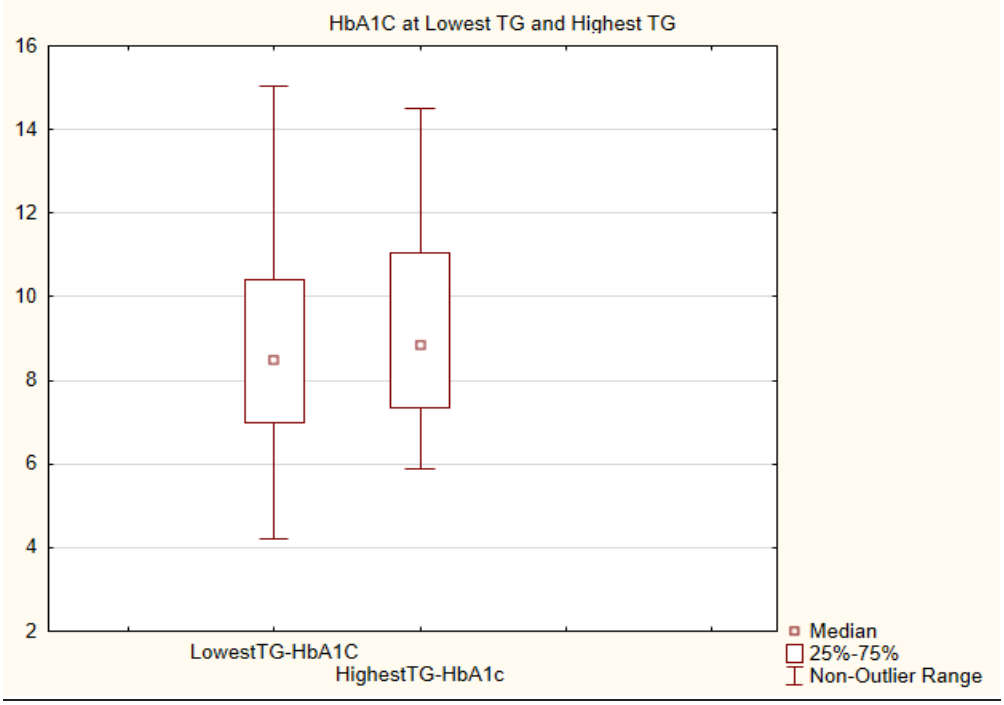


Figure 26- HbA1C at lowest Triglyceride and highest Triglyceride

Table 38 - Group 1: lowest Triglyceride vs HbA1C. Group 2: Highest Triglyceride vs HbA1C

Group 1 vs Group2	Mean (Group 1) (SD)	Mean (Group 2) (SD)	t-value	df	p	Valid N (Group 1)	Valid N (Group 2)	F-ratio (Variances	p (Variances	Mean 1 (-Mean 2)	Confidence (-95.000%)	Confidence (+95.000%)
LowestTG-HbA1c vs. HighestTG-HbA1c	8.77 (2.25)	9.20 (2.26)	-131	185	0,19	95	92	1,01	0,96	-0,43	-1,08	0,22

Table 39 - HbA1C at highest and lowest Triglycerides

Variable	N	Mean	Median	Min	Max	Lower quartile	Upper quartile	Std dev
LowestTG-HbA1c	95	8.77	8.50	4.20	15.03	7.00	10.40	2.25
HighestTG-HbA1c	92	9.20	8.85	5.90	14.50	7.35	11.05	2.26

Table 40 - Lowest TG <1.7mmol/L

Variable	Mean	Std dev	Min	Max	N	Missing cases
LowestTG-HbA1c	8.51	2.28	4.20	13.70	34	3

Table 41 - Highest TG: more than 5mmol/L

Variable	Mean	Std dev	Min	Max	N	Missing cases
HighestTG-HbA1c	9.62	2.31	6.20	14.50	47	3

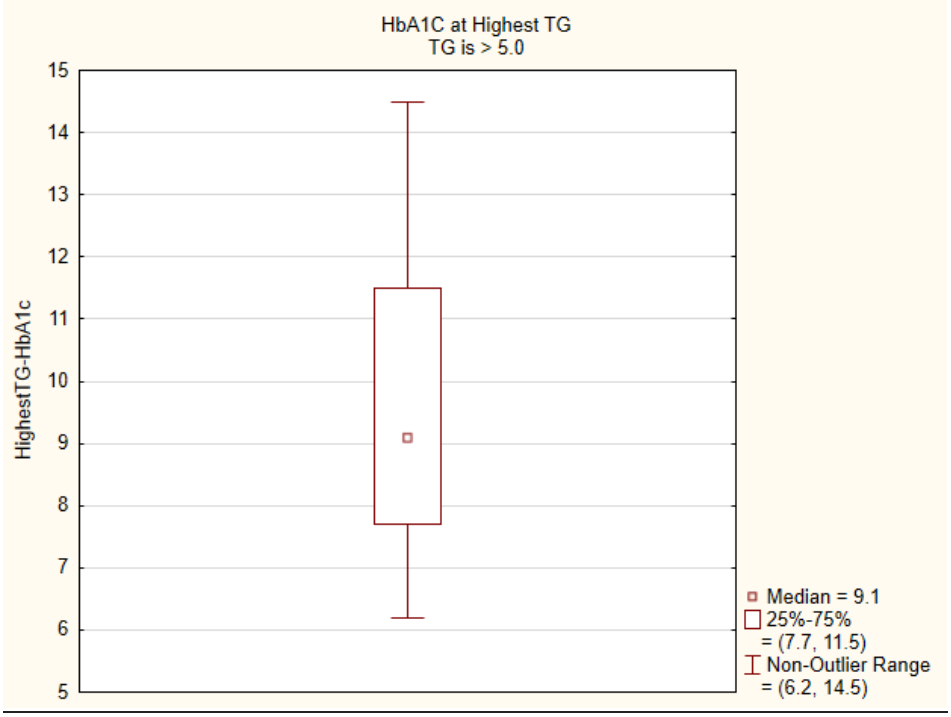


Figure 27 - HbA1C at Highest TG >5mmol/L

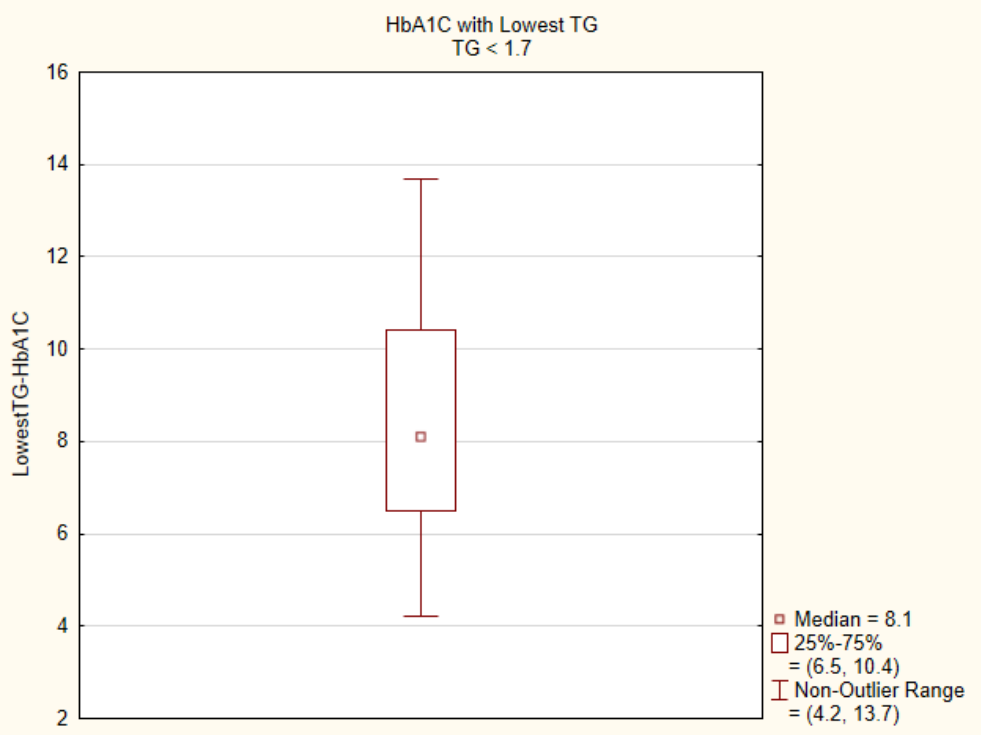


Figure 28 - HbA1C at Lowest TG

3.1.10 Lipid-lowering medication and antidiabetic medication at baseline

At baseline 2 % of patients were on Atorvastatin, with a dose ranging from 10-40mg, one patient was on 10mg, and the other on 40 mg (Table 42).

At baseline 72 % of patients were prescribed simvastatin, 32 patients were on 10mg, 33 on 20mg and 7 on 40 mg (Table 42).

At baseline 5% of patients were on Bezafibrate 400mg (Table 42).

Metformin: 70 patients were on metformin, mean (SD) dosage 1516,42 (550.60 mg) ranging from 500mg to 2550mg.

44 patients were prescribed a sulphonylurea with a mean (SD) 0.72 (0.28) fraction of maximal dosage, the minimum fraction of the total dosage was 0.25 and maximal 1.30.

Table 42 - Baseline data on lipid drug use at baseline

Drug	N	Percentage	Dose/mg 10	Dose/mg 20	Dose/mg 40	Dose/mg 400
Atorvastatin	2	2%	1	0	1	
Simvastatin	72	72%	32	33	7	
Bezafibrate	5	5%				5
Other statins	0	0	0	0	0	

3.1.11 Follow up data for lipid-lowering medication

On follow up 62 patients were prescribed Atorvastatin (Table 43), the dosage categories are summarised in Table 44. 21 patients received simvastatin (Table 45), with dosages described in Table 46. A fibrate was added to a statin in 43 % of patients (Table 47).

Table 43 - Atorvastatin

Drug	N	Mean	Median	Min	Max	Std Dev
Atorvastatin	62	45	40	5	80	22.86

Table 44 - Atorvastatin Dosage/mg

Atorvastatin Dose mg	N	Percentage
5	2	3
10	5	8.1
20	5	8.1
40	33	53.23
50	1	1.61
60	1	1.61
80	15	24.19

Table 45 - Simvastatin

Drug	N	Mean	Median	Min	Max	Std Dev
Simvastatin Dose	21	24.76	20	10	40	10.3

Table 46 - Simvastatin dosage/mg

Simvastatin Dose mg	N	Percentage
10	2	9.52
20	13	61.90
40	6	28.57

Table 47 - Fibrate added to statin on final visit

Fibrate added	N	Percentage
Yes	43	43
No	56	56
Missing	1	1

3.1.12 Fibrate and TG data

We compared the outcomes in the patients receiving a fibrate (Y) (Table 48) with those who were not prescribed a fibrate (N) (Table 49).

The mean (SD) baseline TG in those patients where a fibrate was added was 18,56(15,48)mmol/L compared to a lower mean (SD) of 4.11(1,85) mmol/L when a fibrate was not added, P=0.000. The mean (SD) lowest TG on a fibrate was 4,15 (3,54) mmol/L compared to 2,24 (1,82) mmol/L when a fibrate was not added, p=0.00. The mean (SD) highest TG on treatment for the group where fibrates was added was14.09(12.25) mmol/L compared to a mean (SD) of 4,85 (3.17) mmol/L where a fibrate was not added, P=0.00. The mean(SD) change in TG from visit one to the lowest TG level when a fibrate was added was 4.45 (6.94) mmol/L compared to 1.74(2.07) when a fibrate was not added, P=0.01.(Table 51)

The mean (SD) change in TG from baseline to highest level when a fibrate was added was -4.44 (17.76) mmol/L and 0.75 (2.31) mmol/L when a fibrate was not added, P = 0.000, (Table 50). The mean (SD) TG from baseline to the lowest levels in the group receiving fibrates vs patients not receiving fibrates were -14.38(16.44)mmol/L and -1.87 (1.52)mmol/L respectively, P=0.000 (Table 50).

Table 48 - Group 1: Fibrate added: Y

Variable	N	Mean(SD)	Median	Min	Max	Lower quartile	Upper quartile
Baseline TG	44	18.56(15.48)	12.34	3.10	63.20	7.90	24.30
Lowest TG	43	4.15 (3.54)	2.88	0.73	15.31	1.90	5.10
Highest TG	43	14.09 (12.25)	10.70	1.88	48.78	4.80	19.60

Table 49 - Group 2: Fibrate not Added: N

Variable	N	Mean(SD)	Median	Min	Max	Lower quartile	Upper quartile
Tot TG	56	4.11(1.86)	3.59	2.60	13.60	3.05	4.30
Lowest TG	56	2.24(1.82)	1.66	0.70	12.92	1.30	2.49
Highest TG	56	4.86(3.17)	3.75	1.50	17.90	2.70	6.40

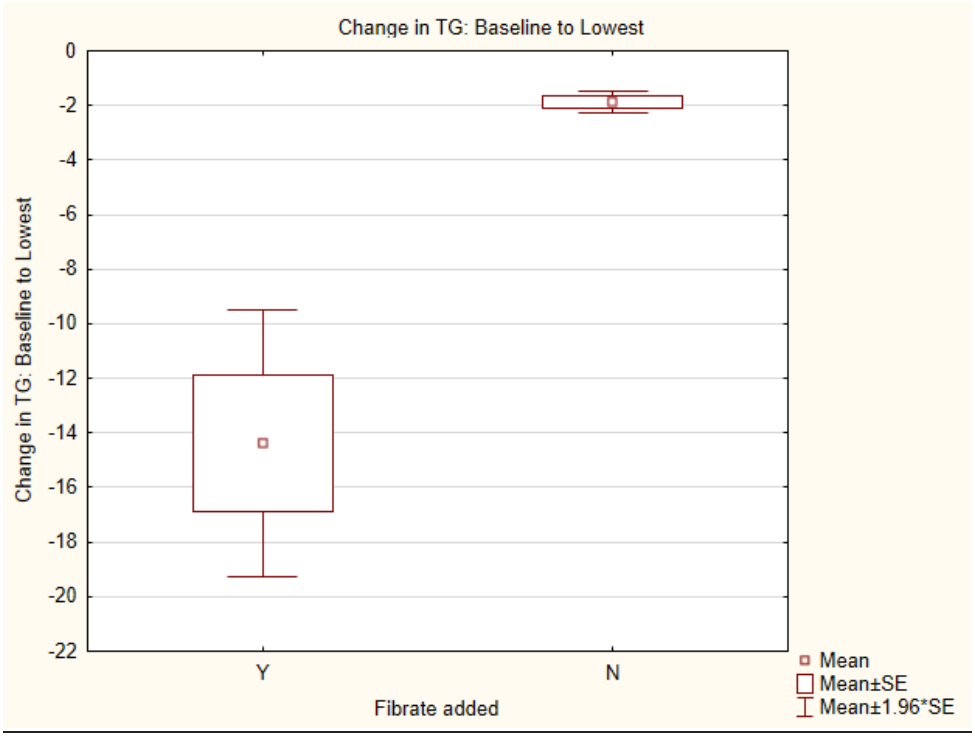


Figure 29 - Change in triglyceride baseline to Lowest when fibrate added

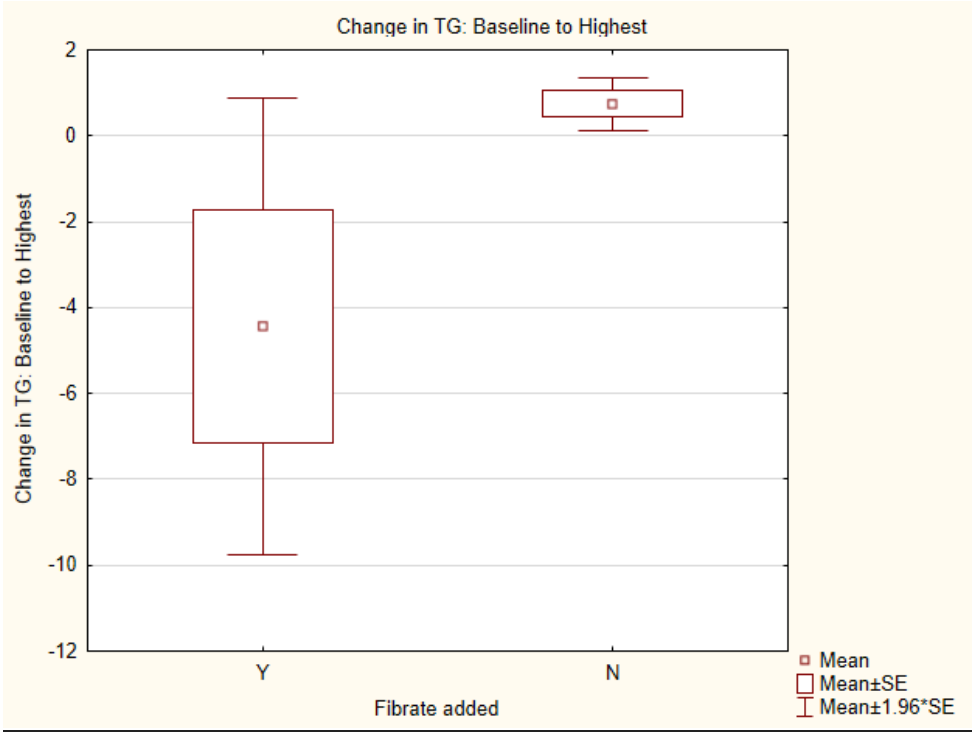


Figure 30 - Change in Triglyceride Baseline to highest when fibrate added

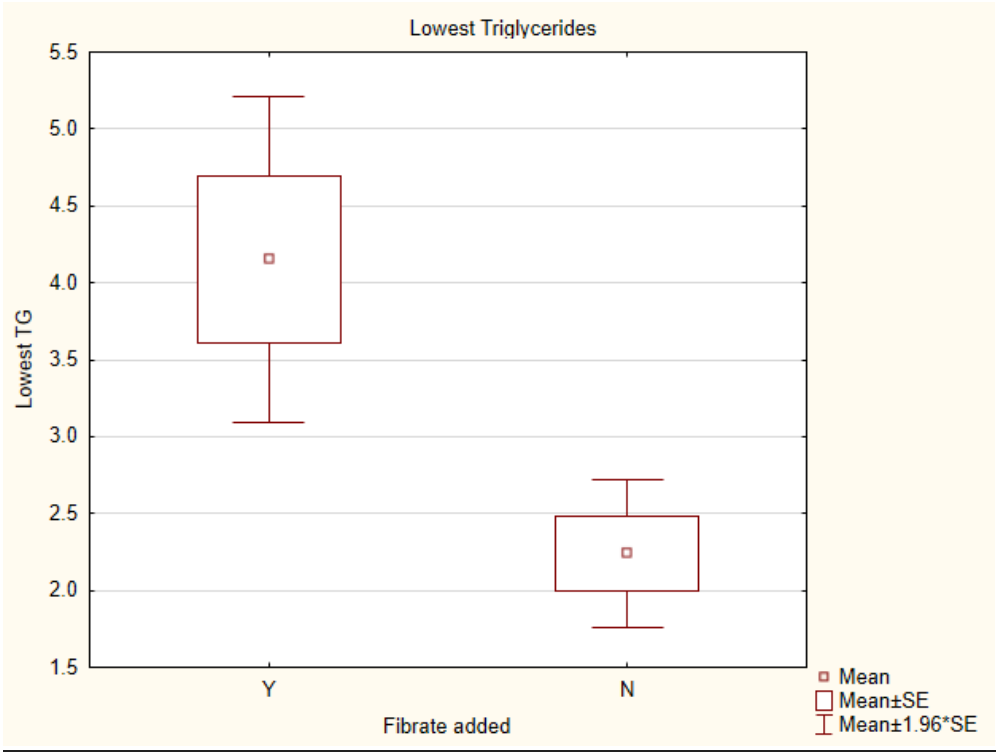


Figure 31 - Lowest triglyceride when fibrate added

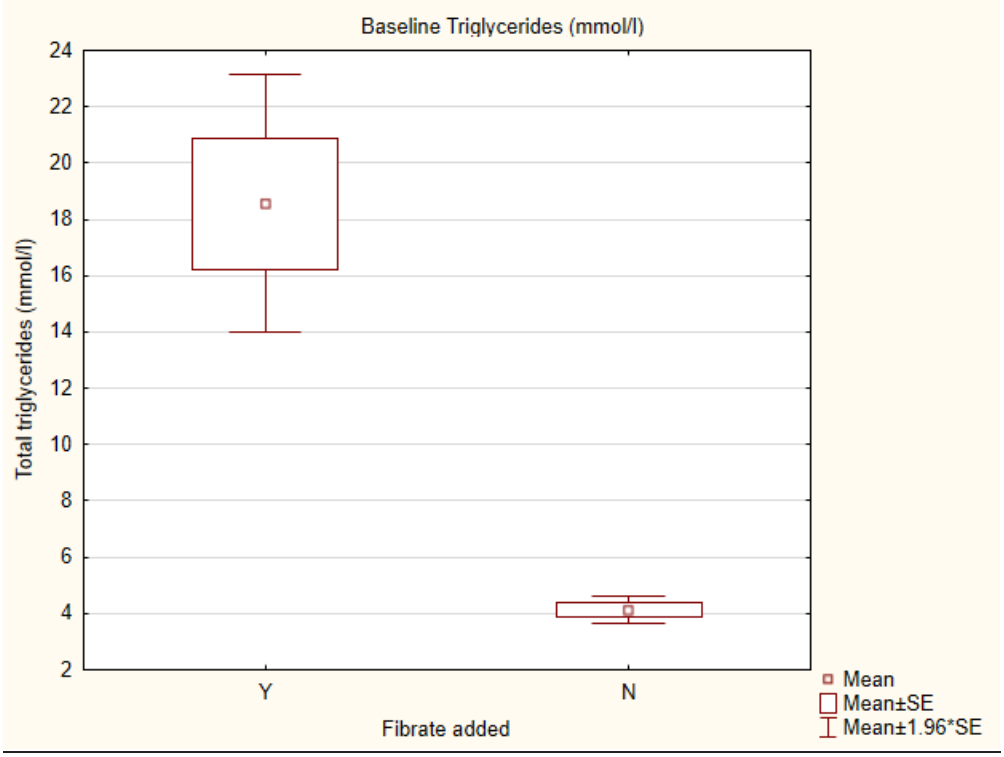


Figure 32 - Baseline Triglyceride when fibrate added

Table 50 - T Tests, Grouping, Fibrate added or not. Group 1 Y Fibrate added. Group 2:N Fibrate not added

Variable: Change in TG:	**Mean (SD) Y**	**Mean (SD) N**	**t-value**	**df**	**P**	**Valid N Y**	**Valid N N**	**F-ratio (Variance)**	**P**
Baseline to Highest	-4.44 (17.76)	0.75(2.31)	-2.16	97	**0.03**	43	56	58.64	0.00
Baseline to Lowest	-14.38 (16.44)	-1.87(1.52)	-5.67	97	**0.000**	43	56	115.97	0.00

Table 51 - T Tests Grouping. Group 1 Y: Fibrate Added. Group 2 N: Fibrate not added

Variable mmol/L	**Mean(Y) (SD)**	**Mean(N) (SD)**	**t-value**	**df**	**P**	**Valid N(Y)**	**Valid N (N)**	**F-ratio**	**p (variance)**
Total Triglycerides	18.56(15.48)	4.11(1.85)	6.93	98	**0,000**	44	56	69.40	0.000
Lowest TG	4.15(3.54)	2.24(1.82)	3.48	97	**0.00**	43	56	3.79	0.000
Highest TG	14.09(12.25)	4.85(3.17)	5.41	97	**0.00**	43	56	14.90	0.000
Change in TG V1 to best	4.45(6.94)	1.74(2.07)	2.75	95	**0.01**	42	55	11.23	0.000

Insulin

On average insulin was increased by a median of 18 Units (Figure 35).

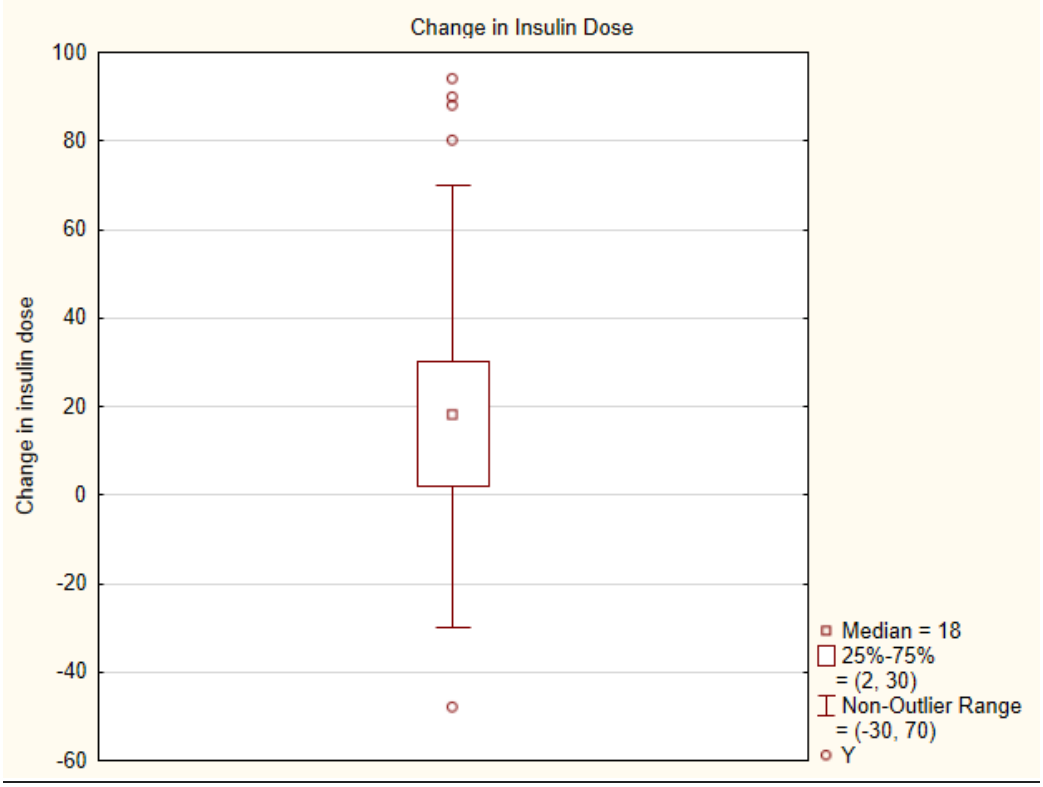


Figure 33 - Change in Insulin dosage

Chapter 4: Discussion

In this study we describe 100 diabetic patients with triglycerides of > 2.5 mmol/L at their first visit who attended a specialised lipid clinic at a tertiary level referral hospital for at least two years. The majority of the study population had clinical and biochemical features compatible with a diagnosis of metabolic syndrome. Baseline lipids were in keeping with the atherogenic lipid phenotype in most patients, although approximately a quarter of patients had severe hypertriglyceridaemia. Diabetes was mostly poorly controlled and control remained poor in many patients despite escalation of therapy.

Triglyceride and remnant cholesterol values at presentation/baseline

At presentation triglycerides were highly variable ranging from 2.6 mmol/L to 63.2 mmol/L with a median baseline TG value of 4.64 mmol/L with a mean (SD) of 10.47 (12.57) mmol/L (Table 12). Derived remnant cholesterol could be calculated in 48 patients, with a mean (SD) 1.55 (0.24) mmol/L, median 1.59mmol/L, ranging from 1.1mmol/L to 2mmol/L. (Table 12, Figure 5).

Table 52 Triglyceride levels below 5.5mmol, moderate to severe hypertriglyceridemia.

Triglyceride level	**N**
TG levels below 5.5mmol/l.	58 (58%)
Moderate hypertriglyceridemia (5.5-10.5mmol/l)	16 (16%)
Severe Hypertriglyceridemia (>10.5mmol/l)	26 (26%)

Baseline TG was highly variable and a quarter of the study population had severe hypertriglyceridaemia of > 10.5 mmol/L that could potentially result in acute pancreatitis. Approximately 60% of the population had TG levels < 5.5 mmol/L where the major concern would be increased cardiovascular risk mediated by high remnant cholesterol. For patients with TG levels 5.5-10.5 mmol/L cardiovascular risk remains relevant, but pancreatitis could also occur with further elevation of TG levels by for example poor diabetic control, a high fat diet or excess alcohol intake. Only 5% of patients were on a fibrate at presentation to the lipid clinic despite 26% having triglycerides more than 10.5 mmol/L. The low fibrate prescription rate is likely due to the very restrictive drug formulary for public sector patients in the Western Cape which restricted the initiation of fibrate therapy to specialist lipid clinics for many years.

Changes in Triglycerides

In this population selected for elevated TG > 2.5 mmol/L at the first consultation only 30 patients reached levels below 1.7mmol/L and 54 patients reached levels below 2.3 mmol/L (Table 8) at least once during the entire 2 year follow up period. Current South African Guidelines suggest that TG should be <2.3mmol/L, but TG are not a treatment target and specific TG lowering therapy is thus not routinely recommended.

The change in total triglycerides from initial presentation to the follow-up visit at which it was the lowest was also variable with a mean(SD) decrease of 2.91 (4.98)mmol/L, ranging from either no change to 32.68mmol/L, 95% CI 4.36-5.80. (Table 33).

The majority of patients (74.75%) showed a decrease in TG in the range from 0 to 10mmol/L by visit 1, most likely due to the provision of dietary counselling and the addition of novel therapies.

Analysing change in TG from baseline to the highest follow up most patients did not show further increases as the median increase was only 0.02 mmol/L – similar to the baseline presenting value. However, the data also illustrates that TG in some patients may increase substantially from baseline despite attendance at a specialised lipid clinic with some patients increasing their TG by more than 50 mmol/L. Potential reasons for such dramatic increases could include loss of diabetic control, non-adherence to lipid-lowering medications, high oral fat intake, alcohol abuse or addition of other medications that increase TG levels such as steroids or retinoic acid. Patients with hypertriglyceridaemia should therefore continue with specialised follow-up and regular lipid monitoring as there is always the potential for TG levels to become uncontrolled. A 'fire-and forget' treatment strategy would be inappropriate in such patients.

Correlations with triglycerides

There was a strong direct correlation between baseline triglycerides and total cholesterol, Figure 24; p=0.000. This emphasizes how important it is to check the full lipogram in patients with high total cholesterol to see whether an excess of LDLC (e.g.as in a patient with Familial Hypercholesterolaemia), an excess of remnants (e.g. Type III), an excess of TGRL (high TG) or an accumulation of LpX (e.g. as seen in obstructive jaundice) accounts for the high TC.

There was a tight correlation between triglycerides and calculated remnant cholesterol in patients in whom a Frederickson calculation could be done (Triglycerides <4.5 mmol/L), p=0.000, Figure 6. If improved glycaemic control decreases triglyceride levels it would thus also be expected to decrease remnant cholesterol. This has also been found in other studies [6, 21,22,25] and emphasises triglyceride concentrations not only acts as an indication of insulin resistance, but also of the atherosclerosis prone metabolic milieu. We did not observe a correlation between baseline TG and HDL-C, P=0.1015 Figure 25.

As expected there was a positive correlation between triglycerides and alcohol intake, Figure 21, P=0.012).

4.1.1 Triglyceride levels and glycaemic control

The mean (SD) baseline glucose and HbA1C for males and females were both elevated, with minimal difference by gender (Table 23). The baseline fasting glucose had a wide range (1.8-30mmol/l) and the majority (82.46%) of baseline HbA1C readings were above 7%. After two years of follow-up, and on average 4 visits to the clinic, HBAIC values remained high with a median of 8.90% and a mean (SD) of 9.25(2.29).

This failure to obtain good glycaemic control is likely multifactorial. Due to limited resources most patients were not offered intensive diabetic education or the assistance of a diabetic educator with insulin dose titration. The hospital formulary restricted therapeutic options to metformin, sulfonylureas and human insulin in addition to limiting patients to approximately one home glucose test per day through a limited supply of test strips. The lipid clinic visits were also relatively infrequent and patients generally reported that they received very little input into their management when they attended at their local clinics between visits to the lipid

clinic. Additionally, control in type 2 diabetes often becomes more difficult with time as the pancreatic insulin secretory capacity decreases.

There was no correlation between baseline triglycerides and HbA1C at presentation (p=0.8423) and similarly no correlation between baseline triglyceride and baseline fasting glucose (p=0.0857) (Figure 17, Figure 18). The lack of correlation in this study could be due to multiple factors. Poor glycaemic control is just one of many factors that influences triglyceride metabolism. The genetic background may have a major influence (e.g. variations in polygenic score). Diet may also play a major role in patients with delayed catabolism and clearance of TGRL. Medications can also influence triglyceride metabolism usage. Given that glycaemia is only one factor amongst many, this study may not have had the statistical power to show a clear association.

To explore whether triglyceride levels and glycaemic control (as assessed by HbAIC) correlated during follow-up we evaluated correlations between triglycerides and HbAIC when triglycerides were either at their highest or lowest during follow-up. We did not find any correlation at the extremes of follow-up triglycerides, Figure 19 and Figure 20.

Possible explanations for this phenomenon include:

- Diabetic control generally remained unsatisfactory with a minority of patients reaching HbAIC levels below 6.5%. It is possible that tighter control (or even near normalization of glucose control such as can be achieved during in-patient studies in metabolic units) may be required to achieve lower TG.
- Glucose control is just one of multiple factors that may affect TG Levels. Diet may have changed following clinic attendance. Medication was prescribed and fibrates were preferentially given to those with high TG biasing the data.
- TG tend to be highly variable in those with hypertriglyceridemia making it difficult to demonstrate correlations in a small cohort.

In a further analysis we compared the HbA1c value corresponding to the instances when the triglycerides were either lowest [8.77(2.25),Mean(SD)] or highest [9.20(2.26), Mean(SD)]. Although, the mean HbAIC was numerically lower when the triglycerides were lowest, the difference was not statistically significant (P=0.96), (Figure 28, Figure 29, Figure 30, Table 38, Table 39, Table 40, Table 41). In some patients improved diabatic control likely contributed to triglyceride lowering, but our data also shows that triglycerides remain high in some patients despite improved (and in some instances good) diabetic control. Controlling glycaemia is thus not guaranteed to control triglycerides, but failure to control the diabetes also does not doom patients to persistent hypertriglyceridaemia in all cases.

4.1.2 Lipids in relationship to anthropometric, demographic variables and other clinical variables

4.1.2.1 BMI/waist circumference

66.32% of patients were obese with BMI > 30kg/m². Mean waist circumferences for males and females exceeded the NECP and IDF definitions for metabolic syndrome (Table 4). We did not find a correlation between baseline TG and BMI and there also was no correlation between baseline TG and baseline waist circumference, Figure 22 and Figure 23. Possible

explanations for the lack of correlation include lack of statistical power, genetic contributions to hypertriglyceridaemia, dietary contributions and the fact that the majority of the cohort was already obese.

4.1.2.2 Gender and lipids

There were no significant difference in BMI between males and females with a mean of 32.80kg/m² in males and 32.74kg/m² in females, P=0.967. There was also no significant difference in mean waist circumference according to gender, P=0.697. The mean (SD) weight in males [92.86kg (15.44kg)] was significantly more than in females mean(SD) [81.47kg(16.52kg), P=0.00].

The mean (SD) HDL-C was higher in females at 1.09 (0.36) mmol/L compared to 0.88 (0.26) mmol/L in males, P=0.002, (Table 18). Similarly, the mean (SD) Apo A1 was statistically significantly higher in females 1.47 (0.26) g/l compared to males 1.23 (0.31) g/l, P=0.000, (Table 18). As one would expect in a cohort of patients with diabetes and elevated triglycerides HDL-C was low in both females and males. The association of female gender with higher HDL-C was maintained despite the overall lowering in HDL-C. ApoA1 levels were not markedly low, using a reference range of > 1.4 g/l for females and > 1.2 g/l for males. There is therefore a disconnect between the effect of hypertriglyceridemia on HDL-C and apoA1, suggesting that HDL particle number is not markedly affected, but that the particles are small and relatively cholesterol poor.

The mean (SD) total cholesterol was elevated in both females and males with minimal difference between the genders; females 8.25 (3.12) mmol/L and males 8.47 (4.81) mmol/L (Table 18). The mean (SD) derived remnant cholesterol showed minimal difference between females and males with calculated values of 1.43 (0.48) mmol/L and 1.53 (0.43) mmol/L, respectively (Table 18). The mean (SD) LDL-C was lower in males compared to females 3.47(1.07) in males compared to 4.47(1.48) mmol/l in females, P=0.051. The Lp(a) level in mg/dl was higher in females 68.94 mg/dl compared to a mean (SD) of 28.6 (30.96) mg/dl in men. The measurement in nmol/l did not reflect this, but on the contrary showed a lower mean (SD) in females 87.14 (83.84) mg/dl in females compared to males 95.77(177.14) nmol/l, P=0.837, (Table 18).

4.1.2.3 Drugs

As one would expect we found that fibrates were more commonly prescribed to patients with higher TG levels, mean SD 18.56(15.48)mmol/L, compared to mean(SD) 4.11(1.85)mmol/L TG levels in patients who were not prescribed a fibrate (Table 48 and Table 49). Apart from lower baseline TG levels patients who were not prescribed fibrates achieved lower TG levels on follow-up. Given the lower baseline TG levels the absolute decrement in TG levels was also smaller in these patients. As bezafibrate was the only fibrate listed on the hospital formulary all patients treated with a fibrate were prescribed bezafibrate.

At baseline only 2% of patients were receiving atorvastatin. In the Western Cape public health care sector atorvastatin can only be initiated at lipid clinics. As the patients in this study all attended a lipid clinic, and would generally be considered to have very high cardiovascular risk status (diabetes and dyslipidaemia in all), atorvastatin was ultimately prescribed to 62 patients

during their follow-up period (Table 43). Forty-three % of patients received a statin combined with a fibrate (Table 47).

LDL-C at baseline

LDL-C is not available for all patients, this is because some patients never had triglycerides that were low enough to enable calculation of LDL-C. As seen in Figure 14 and Table 20 among 85 evaluable patients the mean (SD) best LDL-C was 2.44 (0.88) mmol/L, ranging from 0.51 mmol/L to 5.11 mmol/L. Only 4.1% of all patients with a calculable LDL-C achieved values below 1.8mmol/L (Table 21).

4.1.3 Changes in LDL-C

The majority of the study population did not reach LDL-C targets. Only 4.1% of all patients with a calculable LDL-C achieved values below 1.8mmol/L (treatment goal for patients with diabetes according to the South African guidelines at the time the study was conducted).

The mean (SD) change in LDL-C from visit one to the visit at which it was the lowest was 0.83(1.08)mmol/L, ranging from an increase of 2.69 mmol/L to a decline of 3.60 mmol/L, 95% CI 0.91 – 1.35mmol/L.

4.1.4 Correlations with LDL and comments on Apo lipoproteins

There was no correlation between ApoB and calculated remnant cholesterol, p=0.01668 Figure 13. There was a positive correlation between ApoB and LDL-C, p=0.000 as well as between Apo B and total cholesterol)(Figure 14 and Figure 15). In most humans HDL is a minor cholesterol carrier with apoB-containing lipoproteins (VLDL, IDL, LDL and Lp(a)) carrying the bulk of circulating cholesterol. It is therefore not unexpected to find a correlation between total cholesterol and apoB. Except in cases of severe hypertriglyceridaemia, where there is an excess of chylomicron and VLDL particles, the majority of apoB is found in LDL which usually is the predominant lipoprotein in humans. This explains why we found a strong correlation between LDL-C and apoB in our analysis which excluded patients with severe hypertriglyceridaemia as these patients did not have LDL-C values. We did not find a correlation between apoB and remnant cholesterol.

This analysis was once again confined to patients without severe hypertriglyceridaemia in whom LDL would be the main cholesterol carrier. LDL particles are smaller than remnant particles and thus generally carry less cholesterol per particle and would thus contribute more to the apoB reading. Remnant particles are also more variable in size then LDL particles. It is thus not surprising that we were not able to show a correlation between calculated remnant cholesterol and apoB in this relatively small dataset. ApoB was not checked on follow-up and could thus not be analysed further.

4.1.5 Glucose control

The majority of our study population had uncontrolled diabetes at presentation and did not reach HbA1C targets during follow up. At baseline the HbA1C was only available for 57 patients [mean (SD) 9,61(2.43)], with 82.46% of the documented HbA1C values above 7%, and on follow up only 23.84% of the HbA1C's were below 7%.

The mean (SD) reduction in HbA1C from presentation to the lowest subsequently documented level was 0.94 (1.64)% ranging from either an increase of 1.7% to a decrease of 7.8% [95% CI (1.44 – 1.91%)].

During the 2 years follow up on average insulin required a median increase by 18 Units in addition to increasing sulphonylureas and metformin dosage, displaying the need for not only lipid control with increasing lipid lowering therapy, but also close diabetic control with addition and ongoing revision of diabetic therapy at each visit.

4.1.6 Other major ASCVD risk factors

4.1.6.1 Smoking

Regrettably, smoking was highly prevalent in this cohort who were already at very high cardiovascular risk due to diabetes and dyslipidaemia. Only 35% of patients had never smoked before. In those who had either smoked before or were current smokers the average age at which patients started smoking was very young - females started smoking at a mean age of 18.83 years, males at 16.18 years (P=0.067). At presentation 35% of patients were still smokers, smoking a mean (SD) of 17 (9.1) cigarettes per day. As described in the literature, cigarette smoking has been associated with a two to four fold increase risk of coronary heart disease.[50]. Unfortunately, we are unable to comment on how many patients stopped smoking during their time at the clinic as this information was not collected and documented in a systematic fashion in the clinical notes.

4.1.6.2 Hypertension

As this project was completed in limited time and focussed on dyslipidaemia. We did not document or analyse blood pressure readings or antihypertensive therapy.

4.1.7 Pancreatitis risk

At presentation 26% of the study population had TG >10.5mmol/l placing them at high risk of pancreatitis. The TG was > 20 mmol/L in 15% of patients. Four patients never achieved TG levels of less than 10 mmol/L during their entire follow-up period. We unfortunately did not systematically collect data on pancreatitis during this study and are therefore unable to comment of the pancreatitis prevalence in this population.

Similar studies and comparisons with other studies done

Our study findings of poor diabetic control were also seen in a local study done in the Western cape by Haque et al [4] due to multiple patient, physician, and health system related reasons.

There is a paucity of large population-based studies describing lipid profiles in hypertriglyceridemia diabetic populations, and no descriptive studies describing diabetic patients in South Africa with their baseline demographic and laboratory variables.

We found no descriptive studies of a population of diabetic patients with hypertriglyceridemia attending a specialized lipid clinic with detailed baseline information, including detailed lipoprotein phenotyping and review of their baseline and follow up variables.

We also found no studies to evaluate the impact of diabetic and lipid outcomes in a hypertriglyceridaemic population over a period of time to compare all our parameters.

Below we compare aspects of our study with published data.

Comparison LDL targets: The poor LDL-C control in our study (only 4.1% of all patients with a calculable LDL-C achieved values below 1.8mmol/L) was also seen in the 2005 United States national survey including 4885 patients in The National Cholesterol Education Program (NCEP) Evaluation Project where only 55% of diabetics reached their LDL targets. [25, 27]. However, it is important to note that the NCEP Evaluation Project was a population survey, while our study focuses on a cohort of patients selected for severe dyslipidaemia.

Diabetic and lipid control at other tertiary hospitals in South Africa:
Our study can be compared to the recent 2019 retrospective observational study by Raal et al. at the Charlotte Maxeke Johannesburg Academic Hospital. In the study from Johannesburg 200 Type 2 DM patients on statin therapy were evaluated retrospectively and only 26.5% reached LDL-C targets, 66.5% reached TG targets, 73% reached total cholesterol targets and 56% reaching HDL-C targets. Patients requiring high intensity statins had a median LDL-C of 2.34mmol/L. Poor diabetic control had no influence of lipid levels. An HbA1C <7% was associated with lower TG levels.[51] Although the patients in the study by Raal et al attended a tertiary level facility the cohort was not specifically selected for dyslipidaemia at baseline which likely explains the higher, but still imperfect, lipid goal attainment.

Bulbulla et al did a retrospective cross sectional clinical audit in 2019 at the Helen Joseph Academic Hospital Diabetic Clinic in Johannesburg. The authors audited 321 type 2 diabetic patients receiving insulin. HbA1C was <7% in 15.3% of study participants, with the majority of patients having poorly controlled diabetes. Mean HbA1C was 9.5% (2.4%), ranging from 3.9-16.2%. The majority of patients (91.6%) was on a statin while only 2.2% of patients received a fibrate. LDL <1.8mmol/L was seen in 22.6% of patients. . The mean(SD) total cholesterol was 4.46 (1.09)mmol/L ranging from 2.05-9.28mmol/L. The mean(SD) LDL-C was 2.49 (0.91) ranging from 0.29-6.03mmol/L. HDL C mean(SD) was 1.07 (0.27)mmol/L ranging from 0.57-2.77mmol/L. TG also showed a high variability ranging from 0.43-11.70mmol/L, with a mean(SD) of 1.89 (1.27)mmol/L. [52]

We did not observe the same lipoprotein changes observed in 1983 Lopes-Virella [44] trial, where insulin dependent diabetics were subjected to 3 weeks on insulin therapy, see pages 1-12 for details. The difference is that out diabetic population where not subjected to intensive

inpatient insulin therapy while consuming standardized diets. However, the study by Lopes-Virella does highlight the important metabolic effect on lipoprotein metabolism of improved glycaemic control with insulin.

We did not find correlations between TG levels and HbA1C at baseline or on follow-up when TG levels were at their lowest or highest, Figure 19 and Figure 20. Pfeifer et al treated 49 untreated diabetics without familial hypertriglyceridemia with sulphonylureas or insulin for three months. They found a significant linear relationship between HbA1C reduction and decreased TG and Cholesterol concentration at one and three months, even after correction for change in ideal body weight [45]. As the patients in the study by Pfeifer et al were previously untreated they had a very high mean HbA1C of 14.0% which decreased to a mean of 11.4% after three months of treatment. The Pfeifer data is thus not directly comparable to our study as the starting glycaemic control was much worse and the HbA1C decrement achieved was much larger.

We also did not duplicate findings in Agardh et al [46] where 26 patients with diabetes were initiated on insulin, over a period of 3-4 month and the HbA1C improved from 14.3% to 9.4% which was associated with significant decreased plasma cholesterol and TG as well as an increase in LPL activity.

The recently published Very Large Database of Lipids (2019) is a large cross-sectional study that includes de-identified data from fasting samples of 979 539 patients analysed in a single laboratory using density gradient ultracentrifugation (VAP- vertical auto profile). In a sub analysis of this database 116925 (11.9%) were categorised into of the following Frederickson Levy Lee categories associated with elevated triglycerides – Type I, IIb, III, IV and V .[47]

Table 53: The Very Large Database of Lipids: FLLL phenotypes

FLL phenotype	**N**
Type I	5(<0.0004%)
Type IIa	94453(9.7%)
Type IIb	20144(2.1%)
Type III	2151(0.2%)
Type IV	85540(8.8%)
Type V	246 (0.03%)

Our study cohort specifically reviewed a diabetic population with hypertriglyceridemia and found 0% type I, 0% type IIA, 51.4% type IIB, 10.3% type III, 18.7% type IV and 19.6% type V.

Clinical relevance

Our study reveals the complex metabolic milieu in diabetes, the importance of looking at the whole lipid profile and consideration of adding fibrates, intensifying diabetic control and escalating lipid drugs. The aim for this study is to further define the problem and to identify treatment gaps to improve cardiovascular outcomes in high risk diabetic. This study also highlighted numerous reasons why diabetes is often poorly controlled.

Limitations and motivation for further studies

Our study has several significant limitations and the data should be interpreted taking these limitations into account.

The study was conducted at a specialised lipid clinic at a tertiary referral hospital and the patients seen, and treatment provided, may therefore not be fully representative of diabetic patients in South Africa. Additionally, we limited data collection to 100 patients and did not collect data on all variables, e.g., our study did not include data on cardiovascular disease, markers of subclinical atherosclerosis such as carotid intimal thickness or pancreatitis. Patients were also only seen once every six months and glycaemic control did not improve significantly in the whole cohort making it difficult to demonstrate the effect of glycaemia on lipids.

Chapter 5: Conclusion

In a population of patients (with uncontrolled diabetes) improved glycaemic control would be expected to result in decreased TG and remnant cholesterol levels. In this study patients attending a specialist lipid clinic at Groote Schuur Hospital presented with poorly controlled diabetes and although hypertriglyceridaemia and other lipid measures improved following intensification of lipid-lowering therapy most patients did not reach their lipid goals. Glycaemic control remained inadequate despite significant escalation of glycaemic therapies.

Our study highlights the heterogeneity of hypertriglyceridaemia and the difficulties of achieving good metabolic control in these patients. Our study also demonstrated that patients with hypertriglyceridaemia who were initially controlled can relapse readily and be at risk of acute pancreatitis highlighting the need for ongoing follow-up.

Bibliography

1. Erasmus, R.T., et al., *High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: baseline data of a study in Bellville, Cape Town.* S Afr Med J, 2012. **102**(11 Pt 1): p. 841-4.
2. Lin, X., et al., *Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025.* Sci Rep, 2020. **10**(1): p. 14790.
3. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*. Taylor & Francis: Abingdon.
4. Haque, M., et al., *Barriers to initiating insulin therapy in patients with type 2 diabetes mellitus in public-sector primary health care centres in Cape Town.* S Afr Med J, 2005. **95**(10): p. 798-802.
5. Klug, E., et al., *South African dyslipidaemia guideline consensus statement: 2018 update A joint statement from the South African Heart Association (SA Heart) and the Lipid and Atherosclerosis Society of Southern Africa (LASSA).* S Afr Med J, 2018. **108**(11b): p. 973-1000.
6. Kreisberg, R.A., *Diabetic dyslipidemia.* Am J Cardiol, 1998. **82**(12A): p. 67U-73U; discussion 85U-86U.
7. Ginsberg, H.N. and P.R. MacCallum, *The obesity, metabolic syndrome, and type 2 diabetes mellitus pandemic: Part I. Increased cardiovascular disease risk and the importance of atherogenic dyslipidemia in persons with the metabolic syndrome and type 2 diabetes mellitus.* J Cardiometab Syndr, 2009. **4**(2): p. 113-9.
8. Steyn, K., et al., *Risk factors associated with myocardial infarction in Africa: the INTERHEART Africa study.* Circulation, 2005. **112**(23): p. 3554-61.
9. Ginsberg, H.N., *The ACCORD (Action to Control Cardiovascular Risk in Diabetes) Lipid trial: what we learn from subgroup analyses.* Diabetes Care, 2011. **34 Suppl 2**: p. S107-8.
10. Liu, J., et al., *Joint distribution of non-HDL and LDL cholesterol and coronary heart disease risk prediction among individuals with and without diabetes.* Diabetes Care, 2005. **28**(8): p. 1916-21.
11. Rao Kondapally Seshasai, S., et al., *Diabetes mellitus, fasting glucose, and risk of cause-specific death.* N Engl J Med, 2011. **364**(9): p. 829-841.
12. Haffner, S.M., et al., *Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction.* N Engl J Med, 1998. **339**(4): p. 229-34.
13. Wu, L. and K.G. Parhofer, *Diabetic dyslipidemia.* Metabolism, 2014. **63**(12): p. 1469-79.
14. Stahel, P., et al., *The Atherogenic Dyslipidemia Complex and Novel Approaches to Cardiovascular Disease Prevention in Diabetes.* Can J Cardiol, 2018. **34**(5): p. 595-604.
15. Chehade, J.M., M. Gladysz, and A.D. Mooradian, *Dyslipidemia in type 2 diabetes: prevalence, pathophysiology, and management.* Drugs, 2013. **73**(4): p. 327-39.
16. Jialal, I., W. Amess, and M. Kaur, *Management of hypertriglyceridemia in the diabetic patient.* Curr Diab Rep, 2010. **10**(4): p. 316-20.
17. Watts, G.F. and F. Karpe, *Triglycerides and atherogenic dyslipidaemia: extending treatment beyond statins in the high-risk cardiovascular patient.* Heart, 2011. **97**(5): p. 350-6.

18. Schaefer, E.J., et al., *Elevated remnant-like particle cholesterol and triglyceride levels in diabetic men and women in the Framingham Offspring Study.* Diabetes Care, 2002. **25**(6): p. 989-94.
19. Eleftheriadou, I., et al., *The effects of medications used for the management of diabetes and obesity on postprandial lipid metabolism.* Curr Diabetes Rev, 2008. **4**(4): p. 340-56.
20. Goldberg, I.J., R.H. Eckel, and R. McPherson, *Triglycerides and heart disease: still a hypothesis?* Arterioscler Thromb Vasc Biol, 2011. **31**(8): p. 1716-25.
21. Jorgensen, A.B., et al., *Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction.* Eur Heart J, 2013. **34**(24): p. 1826-33.
22. Varbo, A., et al., *Remnant cholesterol as a causal risk factor for ischemic heart disease.* J Am Coll Cardiol, 2013. **61**(4): p. 427-436.
23. Varbo, A. and B.G. Nordestgaard, *Remnant cholesterol and ischemic heart disease.* Curr Opin Lipidol, 2014. **25**(4): p. 266-73.
24. Hong, L.F., et al., *Predictive value of non-fasting remnant cholesterol for short-term outcome of diabetics with new-onset stable coronary artery disease.* Lipids Health Dis, 2017. **16**(1): p. 7.
25. Peters, A.L., *Clinical Relevance of Non-HDL Cholesterol in Patients With Diabetes.* Clinical Diabetes, 2008. **26**(1): p. 3-7.
26. Marais, D., *Dysbetalipoproteinemia: an extreme disorder of remnant metabolism.* Curr Opin Lipidol, 2015. **26**(4): p. 292-7.
27. Davidson, M.H., et al., *Results of the National Cholesterol Education (NCEP) Program Evaluation ProjecT Utilizing Novel E-Technology (NEPTUNE) II survey and implications for treatment under the recent NCEP Writing Group recommendations.* Am J Cardiol, 2005. **96**(4): p. 556-63.
28. Dluhy, R.G. and G.T. McMahon, *Intensive glycemic control in the ACCORD and ADVANCE trials.* N Engl J Med, 2008. **358**(24): p. 2630-3.
29. Diabetes, C., I. Complications Trial /Epidemiology of Diabetes, and G. Complications Study Research, *Intensive Diabetes Treatment and Cardiovascular Outcomes in Type 1 Diabetes: The DCCT/EDIC Study 30-Year Follow-up.* Diabetes Care, 2016. **39**(5): p. 686-93.
30. Holman, R.R., et al., *10-year follow-up of intensive glucose control in type 2 diabetes.* N Engl J Med, 2008. **359**(15): p. 1577-89.
31. Wierzbicki, A.S., *FIELDS of dreams, fields of tears: a perspective on the fibrate trials.* Int J Clin Pract, 2006. **60**(4): p. 442-9.
32. Cholesterol Treatment Trialists, C., et al., *Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis.* Lancet, 2008. **371**(9607): p. 117-25.
33. Murphy, S.A., et al., *Reduction in Total Cardiovascular Events With Ezetimibe/Simvastatin Post-Acute Coronary Syndrome: The IMPROVE-IT Trial.* J Am Coll Cardiol, 2016. **67**(4): p. 353-361.
34. Sabatine, M.S., et al., *Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial.* Lancet Diabetes Endocrinol, 2017. **5**(12): p. 941-950.
35. Wong, N.D. and M.D. Shapiro, *Interpreting the Findings From the Recent PCSK9 Monoclonal Antibody Cardiovascular Outcomes Trials.* Front Cardiovasc Med, 2019. **6**: p. 14.

36. Schwartz, G.G., et al., *Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome.* N Engl J Med, 2018. **379**(22): p. 2097-2107.
37. Ray, K.K., et al., *Effects of alirocumab on cardiovascular and metabolic outcomes after acute coronary syndrome in patients with or without diabetes: a prespecified analysis of the ODYSSEY OUTCOMES randomised controlled trial.* Lancet Diabetes Endocrinol, 2019. **7**(8): p. 618-628.
38. Bhatt, D.L., et al., *Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia.* N Engl J Med, 2019. **380**(1): p. 11-22.
39. Nicholls, S.J., et al., *Effect of High-Dose Omega-3 Fatty Acids vs Corn Oil on Major Adverse Cardiovascular Events in Patients at High Cardiovascular Risk: The STRENGTH Randomized Clinical Trial.* JAMA, 2020. **324**(22): p. 2268-2280.
40. Chapman, M.J., et al., *Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management.* Eur Heart J, 2011. **32**(11): p. 1345-61.
41. Alexopoulos, A.S., et al., *Triglycerides: Emerging Targets in Diabetes Care? Review of Moderate Hypertriglyceridemia in Diabetes.* Curr Diab Rep, 2019. **19**(4): p. 13.
42. Pradhan, A.D., et al., *Rationale and design of the Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients with Diabetes (PROMINENT) study.* Am Heart J, 2018. **206**: p. 80-93.
43. O'Brien, T., T.T. Nguyen, and B.R. Zimmerman, *Hyperlipidemia and diabetes mellitus.* Mayo Clin Proc, 1998. **73**(10): p. 969-76.
44. Lopes-Virella, M.F., et al., *Effect of metabolic control on lipid, lipoprotein, and apolipoprotein levels in 55 insulin-dependent diabetic patients. A longitudinal study.* Diabetes, 1983. **32**(1): p. 20-5.
45. Pfeifer, M.A., et al., *The response of plasma triglyceride, cholesterol, and lipoprotein lipase to treatment in non-insulin-dependent diabetic subjects without familial hypertriglyceridemia.* Diabetes, 1983. **32**(6): p. 525-31.
46. Agardh, C.D., P. Nilsson-Ehle, and B. Schersten, *Improvement of the plasma lipoprotein pattern after institution of insulin treatment in diabetes mellitus.* Diabetes Care, 1982. **5**(3): p. 322-5.
47. Quispe, R., et al., *Characterization of lipoprotein profiles in patients with hypertriglyceridemic Fredrickson-Levy and Lees dyslipidemia phenotypes: the Very Large Database of Lipids Studies 6 and 7.* Arch Med Sci, 2019. **15**(5): p. 1195-1202.
48. Blom, D.J., et al., *Non-denaturing polyacrylamide gradient gel electrophoresis for the diagnosis of dysbetalipoproteinemia.* J Lipid Res, 2003. **44**(1): p. 212-7.
49. American Heart, A., et al., *Diagnosis and management of the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Executive summary.* Cardiol Rev, 2005. **13**(6): p. 322-7.
50. Lakier, J.B., *Smoking and cardiovascular disease.* Am J Med, 1992. **93**(1A): p. 8S-12S.
51. Naidoo, S. and F.J. Raal, *Pattern of dyslipidaemia in relation to statin use in patients with type 2 diabetes mellitus attending a tertiary care hospital.* Journal of Endocrinology, Metabolism and Diabetes of South Africa, 2020. **25**(1): p. 6-11.
52. Bulbulia, S., F. Variava, and Z. Bayat, *Are type 2 diabetic patients meeting targets? A Helen Joseph Hospital Diabetic Clinic Audit.* Journal of Endocrinology, Metabolism and Diabetes of South Africa, 2020. **25**(1): p. 12-17.

Appendices

a) Protocol
b) Data Capturing Sheet
c) Ethics Approval
d) DRPC approval

Appendix A: Research protocol FHS015

Purpose of study:

A descriptive study of the type 2 diabetic population with hypertriglyceridemia of more than 2.5mmol/l at presentation with subsequent analysis of their baseline and follow up variables.

Hypothesis: In the presence of insulin resistance and diabetes lipid abnormalities are common and improved glycemic control will correlate positively with improved triglycerides, lower remnant cholesterol and increased HDL-C levels on a background of treatment with statins and/or fibrates.

The primary aim is to identify and describe a subset of the population of patients with type 2 diabetes and hypertriglyceridemia of more than 2.5 mmol/L at presentation, attending the Lipid Clinic at Groote Schuur Hospital who have at least two years of follow-up data. Data that will be collected includes baseline variables (demographics, lipid profile, apolipoproteins, calculated remnant cholesterol, electrophoresis, medications, etc.) and follow up lipid variables (total cholesterol, LDL cholesterol, triglycerides, calculated remnant cholesterol, HDL-C, HbA1C and medication (lipid lowering and diabetic).

The secondary aim is to investigate whether there are correlations between glycemic control, lipid modifying therapy and hypoglycemic therapy with lipid outcomes, specifically LDL cholesterol, remnant cholesterol, triglyceride and HDL-C.

Background:

In 2019 South Africa had an estimated population of 58.78 million people, with 51.2% being female and 48.8% males. The population consists of different ethnic groups consisting of Black Africans (80.7%), Colored (8.8%), Indian/Asian (2.2%) and White (7.9%). The Western Cape has a high migration inflow noted from 2016-2021 with an estimated population of 6 844 272 people, 49.3% male and 50.7% female. [3]

The International Diabetes Federation (IDF) predicts a 140.0% projected increase in adults with diabetes by 2040 in Africa, and a concerning 126.4% projected increase in adults with impaired glucose tolerance in Sub-Saharan Africa. In 2015 there were 2286 million adults with diabetes (20-79 years of age), 61.1% of these were undiagnosed with a national prevalence of 7%[1]. Multiple ethnicities are found in the Western Cape. The majority of the population is of mixed ancestry with a high prevalence of metabolic syndrome and diabetes. Cardiovascular disease in diabetes is very common and macrovascular disease accounts for the majority of mortality in diabetes[2-4]. The INTERHEART AFRICA Study confirmed diabetes as a major risk factor for myocardial infarction with multiple mechanisms involved[5]. Dyslipidemia is an important component of the increased risk in diabetics but of course is not the sole factor[6, 7]. Type 2 diabetics are at very high risk for cardiovascular events[2, 3]. The hazard ratios for death in the presence of diabetes from any cause and vascular events were reported to be 1.90 (95% CI, 1.19-1.31) and 2.32(95% CI 2.11-2.56), respectively[8]. At the

[3] SATS SA: Statistical release P0302: Mid-year Population Estimates. Stats SA 2019,Pretoria. [Cited 2019 Dec 27]. Available from: http://www.statssa.gov.za/publications/P0302/P03022019.pdf

age of 50 a person with diabetes dies approximately 6 years earlier than a person without diabetes[8].

To emphasize the importance of aggressive risk factor management in diabetics without previous myocardial infarction (MI) compared to non-diabetics with MI over a seven-year period, Steven et al compared the incidence of coronary heart disease mortality of 1373 nondiabetic patients with 1053 diabetic patients. This cross-sectional follow-up study concluded that the hazard ratio for mortality from ischemic heart disease in patients with diabetes without prior MI compared to patients without diabetes with previous MI was 1.4 (95%CI 0.7-2.6) adjusted for age and sex. The hazard ratio remained close to 1 at 1.2(95%CI 0.6-2.4) after further adjusting for age, gender, smoking, high blood pressure, LDL-C, HDL-C and TG, thus suggesting that diabetics without prior MI are as high risk for the development of MI as non-diabetics[9].

A pooled prospective cohort not only confirmed the presence of diabetes as a strong risk for coronary artery disease, but also suggest that Non-HDL-C (Tot Chol minus HDL-C) is a stronger predictor thereof compared to LDL-C[10]. It emphasizes the critical role of VLDL triglyceride content[7, 11]. Non HDL-C l includes all atherogenic Apo B lipoproteins: LDL, IDL, VLDL and Lp(a)[11].

Lipoprotein abnormalities are already evident in patients with abnormal glycaemia who do not have diabetes yet[3]. Diabetic dyslipidemia is characterized by elevated fasting and postprandial triglycerides, low HDL-C cholesterol, normal/elevated LDL cholesterol, the presence of small dense LDL, elevated remnant lipoproteins and is also known as the atherogenic dyslipidemia complex[12-15]. In diabetes or insulin resistant states atherosclerotic dyslipidemia is often defined as triglyceride levels >2.2mmol/L and HDL-C <1mmol/l[16].
The atherogenicity of diabetic dyslipidemia is mediated by multiple factors, including the presence of small dense LDL particles, elevated remnant cholesterol which is associated with a 2.8 fold increased cardiovascular risk, and dysfunctional HDL[12].

Insulin resistance is characterized by increased secretion of triglyceride rich VLDL and inefficient and incomplete clearance resulting in higher concentrations of remnant lipoproteins. The high amount of circulating triglyceride-rich VLDL also has secondary effects such as leading to low HDL-C and small dense LDL particles via cholesteryl ester transfer protein(CETP) mediated exchanges[3, 16, 17]. Hepatic apo B100 production generally remains fairly constant at a level exceeding demand and any apoB100 that is not lipidated by triglycerides is subsequently degraded. Increased availability of triglycerides leads to most of the apoB being lipidated and thus secreted[16, 17]. Hypertriglyceridemia stimulates CETP activity leading to increased TG content of HDL-C and LDL[12, 18]. CETP facilitates exchange of cholesterol esters for triglyceride, leading to triglyceride-rich LDL-C which then becomes a substrate for LPL and hepatic lipase, ultimately leading to the formation of small dense LDL particles[3, 12, 18]. CETP exchanges cholesterol esters in HDL-C for triglyceride in triglyceride rich lipoproteins with more rapid catabolism of small triglyceride enriched HDL-C, leading to the decreased level of HDL-C and predominance of small HDL-C particles seen in diabetes[3, 16].

In insulin resistance Apo A1 production is not upregulated appropriately, and high tumor necrosis factor alpha(TNF alpha) levels seen in chronic inflammatory states also suppress HDL-C and Apo A1 production[14]. The decreased concentration of HDL-C in diabetes not only act as a marker for its impaired functionality, but is also a marker of the impaired

metabolism of triglyceride-rich apo B lipoprotein[12] - in fact HDL-C cholesterol has been called the 'HbA1c of triglyceride rich lipoprotein metabolism'.

Normally insulin inhibits Hormone Sensitive Lipase (HSL)[17]and thus also inhibits transcription of microsomal triglyceride transfer protein. Thus, in insulin resistant states more triglycerides are transferred to nascent apo B, with excess secretion of triglyceride-rich VLDL1, Apo C III and apo E, the former undergoing hydrolysis by Lipoprotein lipase(LPL) or Hepatic lipase yielding small dense LDL-C[12, 16].

Impaired Lipoprotein lipase (LPL) activity causes decreased VLDL clearance, delipidation and post prandial chylomicronemia with the high triglyceride concentration, leading to elevated remnant cholesterol levels[13]. The elevated free fatty acids causes LPL to detach from endothelial surfaces thus impairing its activity[12]. Saturation of LPL at VLDL triglyceride concentration above approximately 5-6mmol/L impairs chylomicron hydrolysis[16].
Apo C III expression is increased by hyperglycemia and deceased by insulin and inhibits LPL by impairing hepatic uptake of triglyceride by Apo B/E receptors[12]. Clearance of Chylomicron remnants by the liver LDL receptor and remnant receptor is negatively regulated by PCSK9(Proprotein convertase subtilisin-kexin type9)[13] .

Remnant cholesterol:
Remnant cholesterol represents the cholesterol carried in VLDL and chylomicron remnants. The role of remnant cholesterol in atherosclerotic cardiovascular disease has been evaluated in multiple studies[19-21]. In a Mendelian randomization study Nordestgaard et al. showed that each 1 mmol/l increase in non-fasting remnant cholesterol caused a 2.8 fold risk for IHD independent of HDL-C reduction[12, 20]. In another Mendelian randomization study, Nordestgaard et al evaluated the effects of a genetic variation in APOA5 and confirmed the elevated risk for myocardial infarction with elevated non-fasting TG and remnant cholesterol.

The hazard ratios for non-fasting TG, casual genetic odds ratio and observed calculated remnant cholesterol were 1.57(1.32-2.68), 1.94(1.2-1.85) and 1.67(1.38—2.02), respectively[21]. Remnant cholesterol contributes significantly to cardiovascular risk[22] but does not qualify as a prognostic biomarker in diabetics with new onset coronary artery disease in a time with high statin use and coronary revascularization[23]. Dysbetalipoproteinemia which results from mutations in apolipoprotein E, is characterized by the accumulation of cholesterol enriched remnants and serves as a natural genetic model to demonstrate the atherogenicity of remnant lipoproteins[24].

Remnant cholesterol is associated with increased inflammatory parameters such as: hsCRP(high-sensitivity C-reactive protein), fibrinogen and neutrophil count and higher adverse cardiovascular events[23]. In a multidirectional Mendelian randomized study of 60608 Danish participants a causal association was shown between elevated non-fasting remnant cholesterol and low-grade inflammation with an elevated risk of IHD. This inflammatory causality was not shown for LDL. Emphasis is placed on the remaining risk of atherosclerosis with the persistent inflammatory milieu when LDL-C targets are reached[25].

Hypertriglyceridemia is associated with atherosclerosis. It not only acts as an indication of insulin resistance, but also the atherosclerosis prone metabolic milieu. Triglyceride enriched small dense lipoproteins are able to infiltrate blood vessel walls, oxidize readily and are frequently ingested by macrophages[11]. The presence of high chylomicron remnants and small VLDL particles favors a gradient for arterial wall penetration[3]. With

hypertriglyceridemia TGRLPs (VLDL and IDL) are packed with cholesterol transferred from LDL, thus become very atherogenic[11].

In a review article by Goldberg two hypotheses regarding the pathogenicity of TGRL were postulated:
Remnant Hypothesis by Zilversmit: Remnant Lipoproteins were detected in human arterial walls. Triglyceride rich lipoproteins converted to remnants are carried into arterial walls, carrying both triglycerides and cholesterol[20, 21]. Increased influx mediated by LPL explains the atherogenicity of remnant cholesterol[16].
The Lipolytic Toxin hypothesis: VLDL lipolysis induces inflammation. With lipolysis of TGRL inflammatory lipids e.g. free fatty acids and lysolecithins and oxidized lipids are released leading to increased adhesion molecules and cytokines promoting coagulation[16].

Treatment
Improved glycemic control lowers triglycerides and increase HDL-C levels in patients with type 1 diabetes, but in type 2 diabetes the lipid abnormalities do not resolve fully[26]. In 1983 Lopes-Virella and associates subjected insulin dependent diabetics to 3 weeks intensive in-hospital insulin therapy. They observed a decrease in LDL cholesterol, VLDL cholesterol, total cholesterol, VLDL triglyceride and Apo B levels, while HDL-C and ApoA1 levels increased. The increase in HDL-C and ApoA1 was seen only in males. In patients admitted with very poor glycemic control (HBA1C >11%) a marked decrease in total LDL-C, total VLDL-C, total triglyceride and VLDL triglyceride was seen, whereas in those patients with a slightly better admission HBA1C(<11%) decreases were only noted for total triglyceride and VLDL triglycerides[27]. The study illustrates the metabolic effect of improved glycemic control with insulin, although the study participants most likely had diabetes type 1.

Pfeifer et al treated 49 untreated diabetics without familial hypertriglyceridemia with sulphonylureas or insulin for three months. At one and three month follow up a significant linear relationship was established between HBA1C reduction and decreased triglyceride and cholesterol concentration, even after correction for change in ideal body weight[28].

Agardh et al postulated that the lipogram provides additional information regarding diabetic control. Twenty-six patients with diabetes, both controlled and uncontrolled, were initiated on insulin. Over a period of 3-4 months an improved HBA1C was associated with significant decreased plasma cholesterol and triglyceride levels as well as an increase in LPL activity[29].

Although one would expect improved glycemic control to translate into reductions in cardiovascular disease in patients with type 2 diabetes it has been difficult to prove this hypothesis in short and medium-term clinical trials. For instance, in the ACCORD and ADVANCE studies improved glycemic control did not reduce cardiovascular events and was even associated with increased mortality in the ACCORD study[30]. Significant limitations of the studies are that they enrolled mainly patients with established cardiovascular disease, long-standing diabetes, multiple comorbidities and were relatively short-term. In long-term follow-up studies improved glycemic control early in the natural history of either type 1[31] or type 2 diabetes[32] is associated with reduced cardiovascular event rates.
Statins are effective in patients with type 2 diabetes.

The Cholesterol Treatment Trialists (CTT) collaboration meta-analysis provides evidence that each mmol/L LDL-C reduction by statins reduce myocardial infarctions and stroke by 21%[33]. The importance of LDL reduction in cardiovascular risk reduction is highlighted by

a series of studies in which LDL-C was progressively reduced to ever lower levels by addition of further LDL lowering therapies to statin-based lipid-lowering therapy.

The IMPROVE IT trial showed that lowering LDL-C by a further 24% through the addition of ezetimibe to simvastatin-based therapy resulted in a further reduction in cardiovascular events, with most of the benefits seen in patients with diabetes[34].

In the FOURIER trial PCSK-9 inhibition with evolocumab reduced cardiovascular outcomes significantly in diabetics and non-diabetics with established stable atherosclerotic cardiovascular disease, but no mortality benefit was seen in this trial of relatively short duration[34].

In the Odyssey Outcome trial alirocumab significantly reduced LDL-C and non-HDL-C cholesterol and improved cardiovascular outcomes in patients with a recent acute coronary syndrome[35].

It has been difficult to show that lipid-lowering drugs that do not predominantly target LDL reduce cardiovascular risk. For instance, the early fibrate monotherapy trials, Coronary Drug Project and World Health Organization clofibrate trial) failed to show a positive outcome on cardiovascular events[36].

Post-hoc reanalysis of several fibrate monotherapy trials that identify cardiovascular benefit in subgroups of patients whose baseline lipid profile was characterized by both triglyceride elevation and low HDL cholesterol. This subgroup was also the only subgroup to benefit in the ACCORD lipid trial. The ACCORD lipid trial is the only large trial of a fibrate added to statin-based therapy in patients with diabetes and added cardiovascular risk published to date[6, 17, 35, 36].

Methodology

Study design.
The study design will be a descriptive medical record review of 100 patients with type 2 diabetes attending the Groote Schuur Hospital Lipid clinic with a first visit to the clinic prior to September 2017 and with at least two years of follow up.

Objectives

- Clinical description of 100 diabetic patients with increased triglycerides attending the Groote Schuur Lipid Clinic.
- Description of laboratory results of above patients.
- Describe lipid and glycemic control after two years of follow-up.
- Evaluate factors that correlate with lipids, especially triglyceride control.

Characteristics of the study population:
Patients for this study will be recruited from the Groote Schuur Lipid clinic.
The referral criteria to the Lipid Clinic include:
*Total cholesterol levels persisting above 7.5mmol/L or below 2.5 mmol/L (after dietary management),
*Low density lipoprotein levels (LDL-C) above 5mmol/L or below 1.5 mmol/L,

*High density lipoprotein (HDL-C) cholesterol concentration above 2.5mmol/L or below 0.6mmol/L or below 0.8mmol/L if the triglyceride concentration is below 2.5mmol/L.
*Hypertriglyceridemia of more than 5 mmol/L.
*Xanthomata of the skin or tendons irrespective of lipid levels, with the exception of xanthelasma.
*Premature atherosclerosis,
*Statin intolerance once hypothyroidism and other disorders have been excluded.
*Unusual metabolic defects that involve lipid metabolism or promote atherosclerosis, including homocystinuria.

Patients meeting the inclusion criteria referred to this clinic will be included in the study.

Inclusion criteria for this study:

1. Known diabetic patients already on oral hypoglycemic agents or insulin, or
2. Newly diagnosed type 2 diabetes:
   SEMDSA recommendations 2017[1]:

SEMDSA 2017 Recommendations

The diagnosis of diabetes is confirmed:

a. In patients *with symptoms* of hyperglycaemia (polyuria, polydipsia, blurred vision, weight loss) or metabolic decompensation (diabetic ketoacidosis or hyperosmolar non-ketotic state), when *any one single test* confirms that the:
   - Random plasma glucose is ≥ 11.1 mmol/L
   - Fasting plasma glucose is ≥ 7.0 mmol/L
   - HbA_{1c} is ≥ 6.5%
   - 2-hour post-load glucose is ≥ 11.1 mmol/L. However, a GTT is rarely needed in this category of patient.

b. In an *asymptomatic individual*, when *any one* of the following tests, *repeated* on separate days within a 2 week period confirms that the:
   - Fasting plasma glucose is ≥ 7.0 mmol/L
   - 2 hr-post load glucose (OGTT) is ≥ 11.1 mmol/L
   - HbA_{1c} is ≥ 6.5%

If the diagnosis of diabetes is not confirmed with the repeated test, institute lifestyle modification and retest in 3 to 6 months.

3. Triglyceride level ≥2.5mmol/l
4. Age > 18 years

Exclusion criteria for this study:
Less than 2 years follow up at the clinic.

Due to the limited time available recruitment will proceed as follows:

1. The lipid clinic database will be reviewed for potentially eligible patients starting with patients who first visited the clinic in September 2017 (to allow for a minimum of two years of follow-up). The search will then be extended backwards to patients who presented earlier than September 2017. Recruitment will terminate once 100 patients have been included, to ensure feasibility for an MMed project. The subsequent evaluation of variables will include their documented follow up over the two year period.
2. Eligible patients will be included in the study consecutively

Vulnerability:
This study is an anonymized folder review, and no additional investigations or clinic visits will be required. No data from patients younger than 18 will be included in this study.
Further research regarding diabetes, insulin resistance and dyslipidemia is needed in South Africa, and thus justifies this inclusion.

Location of research:
This study will be performed from the UCT Lipid Clinic at Groote Schuur Hospital, Cape Town, South Africa. The Division of Lipidology provides clinical services on an outpatient basis but does offer consultation service for in patients with severe disorders of lipoprotein metabolism. The division is run from the Cape Heart Centre, formed in 1997 by uniting the Ischemic Heart Disease unit, Cardiovascular Research unit in Cardiothoracic Surgery and more recently the Hatter Institute, to promote cardiovascular research. The lipid lab offers specialized investigations for dyslipidemia that are generally not available elsewhere in the country. Groote Schuur Hospital is an academic health center, providing tertiary and quaternary care for patients from the Western Cape and beyond. The hospital operates in the Cape Town Central Health District of the Cape Metro Region with an estimated population 4 130 565 million people.

Recruitment and Enrollment:
This study will review existing information captured form patient folders in die Lipidology Clinic at Groote Schuur Hospital.

Research Procedures and Data Collection:
Baseline presenting information is routinely captured for all patients attending the Lipid Clinic.

The initial database search will focus on identifying patients with triglycerides of more than 2.5 mmol/L at presentation September 2017. In a subsequent step all patients who do not have diabetes at presentation (either newly diagnosed or not known at presentation) will be excluded. Recruitment will terminate once 100 patients have been included. Should an insufficient number of patients be identified the search will be extended backwards to patients who presented earlier than September 2017. The subsequent evaluation of variables will include their documented follow up over the two year period.

The list of patients so generated will then require manual review to identify all patients who meet the minimum duration of attendance criterion. Thereafter this study will review existing information captured within duplicate patient folders kept by the Division of Lipidology at UCT. Routine clinic follow-up information and results will be obtained from stored duplicated Lipidology Clinic folders. During a manual folder review data will be captured into a database created for this particular study. The database will be designed on REDCap.

Data will be stored anonymously. During data collection only the PI and sub-investigator will access clinical records with patient identification. Once the data has been captured in the REDcap database it will be in a deidentified format. The Principal Investigator will hold the key that will allow study identification numbers to be linked to specific patients.

Patient variables at first presentation include:
Refer to questionnaire within the addendum.

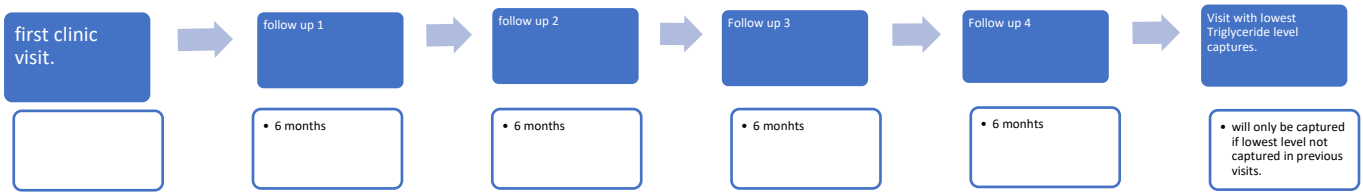
Different sections of Data:

- Gender
- Weight
- Height
- Ethnicity
- Date of birth
- BMI
- Waist circumference
- Hip circumference
- HbA1C
- Fasting glucose
- Lipids: Total cholesterol, Total triglyceride, HDL-C, LDL-C
- Electrophoresis: agarose gel and acrylamide gradient gel electrophoresis.
- Lipoprotein (a): mg/dL or nmol/L
- Apo B
- Apo A1
- Medication details:
    - Lipid-lowering drugs
    - Diabetic therapy
- Renal function(eGFR)
- Thyroid function
- Alcohol use
- Smoker: former, current, never

Derived Variables:
Remnant cholesterol.

Follow up:

1. Four consecutive visits following presentation will be captured. Additionally, the visit at which triglycerides were the lowest will also be captured if this did not occur during the first four follow -up visits.



2. Date of follow up visit.
3. Glucose
4. HBA1C
5. Lipid Drugs:
    - Statin (drug and dose)
    - Fibrate (drag and dose)
    - Omega 3,
    - Other.

6. Diabetic Drugs:
    - Metformin,
    - Sulphonylureas,
    - Insulin: daily dose including regime: basal/basal bolus/premixed)
7. Alcohol use
8. Smoking: current, former, never
9. HDL Cholesterol
10. Triglyceride
11. LDL cholesterol
12. Remnant cholesterol: with calculation

Research Procedures and Data Collection:
Routine (six monthly) follow up clinic visit information are documented on continuation sheets within the duplicate lipidology folder. During manual retrospective folder review patient information will be captured into a REDcap database for this study. Professor Dirk Blom is the principal investigator. The sub investigator is Dr Barbara Vermooten (MP0769380) a qualified medical doctor with sufficient clinical post graduate experience, and skilled in computer data capture. No other investigators will be involved with the study. The approximate total length of time for this study is 36 months.

Ethical approval for this study will be sought from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee.

Data Safety and Monitoring Plan:
The database for this study will be secured by password access, thus confidentiality will be maintained on the computer and hard drive, in keeping with the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil. October 2013).

Data Analysis:
The parameters discussed above will be incorporated into a database created specifically for this study. Guidance in the use of the data collection software (REDcap) by the principal investigator, who is skilled in the use of this software, will be offered to the sub investigator. The responsibility for precise data obtained lies with the principal investigator.

Analysis will include the following:

- Descriptive statistics of all variables (using mean and standard deviation or medians as appropriate according to data distribution).
- HBA1C/HDL-C/calculated remnant cholesterol levels and change over a period of time.
- Correlation of changes in lipid variables with specific diabetic or dyslipidemia drugs.
- Comparison of patients showing good reduction in triglyceride versus those with no reduction or increase (top tertile vs. bottom tertile).
- Correlation of HBA1C with triglyceride level, HDL-C and calculated remnant cholesterol level.

Description of risks and benefits:
Risks: Although all possible efforts will be undertaken to maintain confidentiality during the conduct of this study, the potential remains for unexpected access of personal or sensitive information, e.g. HIV status/infertility.

Risk classification: This study consists of a retrospective folder review, thus classifies under minimal risk for harm.

Minimizing risks: Access control and password gated data base assist to preserve confidential information within the period of folder review and data base capture.

Potential benefits: Advancement of knowledge and benefit to future and existing patients.

Alternatives to participation: There are no alternatives to participation in this study.

Harm: Benefit ratio: The potential benefit from obtaining this study results outweighs the potential harm.

Informed consent:
The investigators will request that the UCT FHS HREC waive the requirement for individual consent from participants whose folders will be included in this review.

Privacy and confidentiality:
Patient data captured into the database will be anonymous. No personal details of patients will be obtained. Data will be stored electronically on a hard drive, password secured.
Duplicate paper based lipidology folders are safely stored, and only made available to personnel involved in the study.

Reimbursement for participations:
Not applicable in this setting.

Emergency care and insurance for research related injuries:
Not applicable in this setting.

What happens at the end of a study:
The outcome and results of this study will be published online and in in a medical journal. A presentation of the results will also be given in a poster form.

Funding:
Not applicable.

References:

1. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*. Taylor & Francis: Abingdon.
2. Klug, E., et al., *South African dyslipidaemia guideline consensus statement: 2018 update A joint statement from the South African Heart Association (SA Heart) and the Lipid and Atherosclerosis Society of Southern Africa (LASSA).* S Afr Med J, 2018. **108**(11b): p. 973-1000.
3. Kreisberg, R.A., *Diabetic dyslipidemia.* Am J Cardiol, 1998. **82**(12A): p. 67U-73U; discussion 85U-86U.
4. Ginsberg, H.N. and P.R. MacCallum, *The obesity, metabolic syndrome, and type 2 diabetes mellitus pandemic: Part I. Increased cardiovascular disease risk and the importance of atherogenic dyslipidemia in persons with the metabolic syndrome and type 2 diabetes mellitus.* J Cardiometab Syndr, 2009. **4**(2): p. 113-9.
5. Steyn, K., et al., *Risk factors associated with myocardial infarction in Africa: the INTERHEART Africa study.* Circulation, 2005. **112**(23): p. 3554-61.
6. Ginsberg, H.N., *The ACCORD (Action to Control Cardiovascular Risk in Diabetes) Lipid trial: what we learn from subgroup analyses.* Diabetes Care, 2011. **34 Suppl 2**: p. S107-8.
7. Liu, J., et al., *Joint distribution of non-HDL and LDL cholesterol and coronary heart disease risk prediction among individuals with and without diabetes.* Diabetes Care, 2005. **28**(8): p. 1916-21.
8. Rao Kondapally Seshasai, S., et al., *Diabetes mellitus, fasting glucose, and risk of cause-specific death.* N Engl J Med, 2011. **364**(9): p. 829-841.
9. Haffner, S.M., et al., *Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction.* N Engl J Med, 1998. **339**(4): p. 229-34.
10. Shimano, H., et al., *Proposed guidelines for hypertriglyceridemia in Japan with non-HDL cholesterol as the second target.* J Atheroscler Thromb, 2008. **15**(3): p. 116-21.
11. Peters, A.L., *Clinical Relevance of Non-HDL Cholesterol in Patients With Diabetes.* Clinical Diabetes, 2008. **26**(1): p. 3-7.
12. Wu, L. and K.G. Parhofer, *Diabetic dyslipidemia.* Metabolism, 2014. **63**(12): p. 1469-79.
13. Stahel, P., et al., *The Atherogenic Dyslipidemia Complex and Novel Approaches to Cardiovascular Disease Prevention in Diabetes.* Can J Cardiol, 2018. **34**(5): p. 595-604.
14. Chehade, J.M., M. Gladysz, and A.D. Mooradian, *Dyslipidemia in type 2 diabetes: prevalence, pathophysiology, and management.* Drugs, 2013. **73**(4): p. 327-39.
15. Jialal, I., W. Amess, and M. Kaur, *Management of hypertriglyceridemia in the diabetic patient.* Curr Diab Rep, 2010. **10**(4): p. 316-20.
16. Goldberg, I.J., R.H. Eckel, and R. McPherson, *Triglycerides and heart disease: still a hypothesis?* Arterioscler Thromb Vasc Biol, 2011. **31**(8): p. 1716-25.
17. Eleftheriadou, I., et al., *The effects of medications used for the management of diabetes and obesity on postprandial lipid metabolism.* Curr Diabetes Rev, 2008. **4**(4): p. 340-56.
18. Watts, G.F. and F. Karpe, *Triglycerides and atherogenic dyslipidaemia: extending treatment beyond statins in the high-risk cardiovascular patient.* Heart, 2011. **97**(5): p. 350-6.

19. Schaefer, E.J., et al., *Elevated remnant-like particle cholesterol and triglyceride levels in diabetic men and women in the Framingham Offspring Study.* Diabetes Care, 2002. **25**(6): p. 989-94.
20. Varbo, A., et al., *Remnant cholesterol as a causal risk factor for ischemic heart disease.* J Am Coll Cardiol, 2013. **61**(4): p. 427-436.
21. Jorgensen, A.B., et al., *Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction.* Eur Heart J, 2013. **34**(24): p. 1826-33.
22. Varbo, A. and B.G. Nordestgaard, *Remnant cholesterol and ischemic heart disease.* Curr Opin Lipidol, 2014. **25**(4): p. 266-73.
23. Hong, L.F., et al., *Predictive value of non-fasting remnant cholesterol for short-term outcome of diabetics with new-onset stable coronary artery disease.* Lipids Health Dis, 2017. **16**(1): p. 7.
24. Marais, D., *Dysbetalipoproteinemia: an extreme disorder of remnant metabolism.* Curr Opin Lipidol, 2015. **26**(4): p. 292-7.
25. Varbo, A., et al., *Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation.* Circulation, 2013. **128**(12): p. 1298-309.
26. O'Brien, T., T.T. Nguyen, and B.R. Zimmerman, *Hyperlipidemia and diabetes mellitus.* Mayo Clin Proc, 1998. **73**(10): p. 969-76.
27. Lopes-Virella, M.F., et al., *Effect of metabolic control on lipid, lipoprotein, and apolipoprotein levels in 55 insulin-dependent diabetic patients. A longitudinal study.* Diabetes, 1983. **32**(1): p. 20-5.
28. Pfeifer, M.A., et al., *The response of plasma triglyceride, cholesterol, and lipoprotein lipase to treatment in non-insulin-dependent diabetic subjects without familial hypertriglyceridemia.* Diabetes, 1983. **32**(6): p. 525-31.
29. Agardh, C.D., P. Nilsson-Ehle, and B. Schersten, *Improvement of the plasma lipoprotein pattern after institution of insulin treatment in diabetes mellitus.* Diabetes Care, 1982. **5**(3): p. 322-5.
30. Dluhy, R.G. and G.T. McMahon, *Intensive glycemic control in the ACCORD and ADVANCE trials.* N Engl J Med, 2008. **358**(24): p. 2630-3.
31. Diabetes, C., I. Complications Trial /Epidemiology of Diabetes, and G. Complications Study Research, *Intensive Diabetes Treatment and Cardiovascular Outcomes in Type 1 Diabetes: The DCCT/EDIC Study 30-Year Follow-up.* Diabetes Care, 2016. **39**(5): p. 686-93.
32. Holman, R.R., et al., *10-year follow-up of intensive glucose control in type 2 diabetes.* N Engl J Med, 2008. **359**(15): p. 1577-89.
33. Cholesterol Treatment Trialists, C., et al., *Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis.* Lancet, 2008. **371**(9607): p. 117-25.
34. Murphy, S.A., et al., *Reduction in Total Cardiovascular Events With Ezetimibe/Simvastatin Post-Acute Coronary Syndrome: The IMPROVE-IT Trial.* J Am Coll Cardiol, 2016. **67**(4): p. 353-361.
35. Schwartz, G.G., et al., *Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome.* N Engl J Med, 2018. **379**(22): p. 2097-2107.
36. Wierzbicki, A.S., *FIELDS of dreams, fields of tears: a perspective on the fibrate trials.* Int J Clin Pract, 2006. **60**(4): p. 442-9.

Appendix B: Data Capture Sheet

Patient variables at first presentation:
Demographic Data

1. Participant ID: _____ (auto generated)

2. GSH Folder number: _____ (text)

3. Date of presentation: _____ (date field)

4. Date of birth: _____ (date field)

5. Age at presentation: _____ (auto calculated)

6. Gender:
   1. Male
   2. Female

8. Ethnicity:

8.1 Documented
   1. Yes
   2. No

8.2 If yes,
   1. African
   2. Caucasian
   3. Mixed ancestry
   4. Indian

9. Height (cm): _____ (number)

10. Weight (kg): _____ (number)

11. BMI (kg/m^2): _____ (auto calculated)

12. Waist circumference (cm): _____ (number)

13. Hip circumference (cm): _____ (number)

Social habits

14. Alcohol consumption:
   1. Yes/No
   2. If yes, _____(grams/week)

15. Smoking
   a. Current _____(Yes/No)

If yes
   i. Age started _____(number)

ii. Cigarettes per day _____ (number)

b. Previous, _____ (Yes/No)

If yes, how many years stopped _____ (number)

c. Never _____

Medication at presentation
Lipid-lowering drugs:

16. Statins: 1. Atorvastatin _____(Yes/No)

Dose/mg _____(number)

2. Simvastatin _____(Yes/No)

Dose/mg _____(number)

3. Other statins _____(text)

Dose/mg _____(number)

17. Fibrates

1. Bezafibrate /mg _____

2. Fenofibrate /mg _____

18. Diabetic medication:

1. Metformin _____(Yes/No)
dose/mg _____

2. Sulphonylurea _____(Yes/No)
dose/mg _____

3. Insulin _____(Yes/No)
Units per day _____

4. Other/mg _____

Laboratory investigations

19. Baseline lipid profile (mmol/L):

Total Cholesterol _____
Total HDL-C _____
Total LDL-C _____

Total Trigs _____

Derived Remnant Cholesterol (mmol/L) _____ (automatic calculation)

20. Baseline apolipoproteins (mmol/L) Apo B _____

Apo A1_____

Lp(a) level (mg/dL) _____

Lipid electrophoresis Yes/No

Result:

Agarose Gel: _____

Acrylamide gradient gel electrophoresis: _____

21. Baseline fasting glucose / mmol/L _____

22. HbA1c (%): _____

23. Chronic kidney disease _____(Yes/No)

If yes, eGFR at presentation _____(number)

24. Thyroid function (TSH)

Elevated _____(Yes/No)

Level _____(mIU/L)

Follow Up:

These questionnaires will be done for each of the six-monthly visits, including the visits at which the triglycerides were the lowest.

1. Date of follow up visit: _____
2. Random glucose (mmol/L) _____
3. HBA1C (%) _____
4. Lipids (mmol/L)
   - Total Cholesterol _____
   - Total HDL-C _____
   - Total LDL-C _____
   - Total Triglycerides _____
   - Calculated remnant chol _____ (automatic calculation)
5. Smoking
   1) Current _____(Yes/No)
      If yes
      iii. Age started _____(number)
      iv. Cigarettes per day _____(number)
   b) Previous, _____(Yes/No)
      If yes, state how many years stopped _____(number)

c) Never _____

2) Alcohol consumption: Yes/No _____
If yes, _____(grams/week)

7. Statins:
1. Atorvastatin _____(Yes/No)
Dose/mg _____(number)

2. Simvastatin _____(Yes/No)
Dose/mg _____(number)

3. Other statins _____(text)
Dose/mg _____(number)

8. Fibrates:
1. Bezafibrate /mg _____
2. Fenofibrate /mg_____

9. Diabetic medication:
1. Metformin _____(Yes/No)
Dose/mg _____

2. Sulphonylurea _____ (Yes/No0
Dose/mg _____

3. Insulin _____(Yes/No)
Units per day _____

4. Other/mg _____

Appendix C: Ethics Approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

24 February 2020

HREC REF: 077/2020

A/Prof D Blom
Division of Lipidology
6th floor, Chris Barnard Building
FHS

Dear A/Prof Blom

PROJECT TITLE: A DESCRIPTIVE STUDY OF THE TYPE 2 DIABETIC POPULATION WITH HYPERTRIGLYCERIDAEMIA OF MORE THAN 2,5 MMOL PER LITRE AT PRESENTATION WAS SUBSEQUENTLY ANALYSIS OF THE BASELINE AND FOLLOW-UP VARIABLES (MMED DEGREE - DR BARBARA VERMOOTEN)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 28 February 2021

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: - Dr Barbara Vermooten will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

HREC 077/2020sa

HUMAN RESEARCH ETHICS COMMITTEE
0 8 JUN 2022
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.06.2023
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	8/6/2022

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC	
	Thank you for your Study Deviation HREC Chair Signature Date: 8/6/22

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	07/06/2022		
HREC REF Number	077/2020	Current Ethics Approval was granted until	28 FEB 2021
Protocol title	A descriptive study of the type 2 diabetic population with hypertriglyceridemia of more than 2.5mmol/L at presentation with subsequent analysis of their baseline and follow up variables.		
Protocol number (if applicable)	NA		
Are there any sub-studies linked to this study?		☐ Yes	☐ No X
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Professor Dirk Blom		
Department / Office Internal Mail Address	Department of Medicine, University of Cape Town Dirk.Blom@UCT.ac.za		

1.1 Does this protocol receive US Federal funding?	☐ Yes	☑ No

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-submission@uct.ac.za)	☐ Yes	☐ No

If yes in 1.2 please complete section 1.3 below for invoicing purposes

1.3 Ethics Renewal Fee

Please (tick ✓)appropriate box for billing purposes:

Submission Type	Description	New fee (Vat incl.)	tick ✓
Research funded solely from UCT departmental/ divisional/group budget	Annual evaluation of research progress report for re-certification	**R0,00**	☑
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R7000,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Expedited review	**R3 710.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National grant funded research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R6000.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National Grant funded research for Annual evaluation of research progress report for re-certification for Expedited review	**R1 500,00**	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for invoicing, either complete section 1 or 2 :

1. Invoice billing – Directly to Sponsor

Sponsor's name	
Billing Address of Sponsor:	
Vat Number:	
Contact person	
Telephone number	
Email Address	

2. **Internal Journal Billing:**	
Fund Number:	
Cost Centre Number:	
Account Holder Name:	
Division of Account Holder:	

2. List of documentation for approval

No documentation submitted.

This is a noninterventional, record review study.

3. Protocol status (tick ✓)

☐	Open Enrolment
☐	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☑	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	100
Number of participants enrolled, since last HREC Progress report (continuing review)	0
Additional number of participants still required	0

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	0

6. Cumulative summary of participants

Total number of participants who provided consent	N/A

Number of participants determined to be ineligible (i.e. after screening)	**N/A**
Number of participants currently active on the study	**N/A**
Number of participants completed study (without events leading to withdrawal)	**N/A**
Number of participants withdrawn at participants' request (i.e. changed their mind)	**N/A**
Number of participants withdrawn by PI due to toxicity or adverse events	**N/A**
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	**N/A**
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	**N/A**
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	**N/A**

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
All data collection complete. Analysis and write up to be completed.

8. Protocol violations and exceptions (tick ✓ all that apply)

☑	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

☑	No Prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
N/A

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	☑ Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	☑ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	☑ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	☑ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
☑	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.
This is a noninterventional retrospective review. The study is considered minimal risk and there is no literature that would be relevant to this risk..

13. Insurance

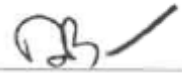
Please confirm that valid no fault insurance is still in place? (tick ✓)		
☐ Yes	☐ No	☑ Not Applicable – N/A
If yes, please complete the following:		
Insurer's name:		
Policy no.		*Coverage Period:
For UCT sponsored studies please liaise the insurance office via fhs.sponsorship@uct.ac.za regarding the required documentation and information required obtain a renewed UCT No-fault Insurance Certificate.		

14. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	☑ No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

15. Signature

My signature certifies that the above is complete and correct.

Signature of PI		Date	7 June 2022

UNIVERSITY OF CAPE TOWN

HUMAN RESEARCH ETHICS COMMITTEE
08 JUN 2022
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Form FHS011: Study deviation

HREC office use only (FWA00001637; IRB00001938)			
This serves as acknowledgement of a protocol deviation as described below.			
Chairperson of the HREC signature/ Designee		Date	8/6/2022

Note: Please note that incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.

Please clarify your plan for research-related activities during COVID-19 lockdown

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	2022/06/07
HREC REF Number	077/2020
Project Title	A descriptive study of the type 2 diabetic population with hypertriglyceridemia of more than 2.5mmol/L at presentation with subsequent analysis of their baseline and follow up variables.
Protocol number (if applicable)	N/A
Principal Investigator	Professor Dirk Blom
Department / Office Internal Mail Address	Department of Medicine, University of Cape Town Dirk.Blom@UCT.ac.za

2. Protocol deviation description

Please describe the deviation below, including the reason why the deviation occurred.
Late submission of annual reapproval. Data collection was complete with in the first year of approval, i.e., within the approved period. Unfortunately, the project could not be entirely completed within one year as planned as data analysis and write-up are currently still ongoing. Data analysis is about 95% complete and write-up is at approximately 90%. Due to human oversight the reapproval application was not submitted on time.

3. Follow-up actions

3.1 Please describe any follow-up action(s) taken or planned as a result of this deviation e.g. DSMB reporting, report to sponsor, informing participants.
Nil needed This delayed reapproval has no impact on any patients as this is an anonymized, retrospective folder review. There is no intervention and this is a minimal risk study.

3.2 Please describe what action(s) have or will be taken to prevent similar deviations in future.
Add student protocols to calendar that lists reapproval dates for clinical trials. Utilising this mechanism annual reapproval should be applied for timelessly going forward.

4. Principal Investigator's acknowledgement of responsibility

This signature indicates the PI has reviewed the deviation, taken appropriate follow-up action and implemented or plans to implement preventative steps where possible.			
Signature of PI		Date	7 June 2022