

Introduction

Coronary artery disease (CAD) is a major global health problem which results in significant morbidity and mortality, and an increasing burden of disease to health care systems. The risk of developing coronary artery disease is multifactorial with numerous studies identifying certain predisposing risk factors.

Hypercholesterolaemia is one such risk factor which, if identified early, can be managed either by lifestyle or medical means¹. There is however a small percentage of individuals in a population who have specific genetic mutations resulting in their hypercholesterolaemia being refractory to conventional treatment. These familial hypercholesterolaemia (FH) patients pose a challenge in controlling their lipid profile and therefore experience a higher incidence of premature CAD^{1, 2}.

Homozygous Familial Hypercholesterolaemia (HoFH)

Familial hypercholesterolaemia was first described in 1938 by Carl Muller, who described high cholesterol associated with physical signs and dominant inheritance amongst families³. In the 1970s, *Goldstein and Brown* described the genetic basis of FH³. Familial hypercholesterolaemia (FH) is an autosomal dominant Mendelian disorder usually caused by mutations in the low-density lipoprotein (LDL) receptor gene (LDLR)^{3, 4, 5, 6}. Heterozygous FH (HeFH) has an estimated prevalence of 1 in 250 to 500 people in Western nations³, with an even higher prevalence seen amongst migrant and settler populations³. In these populations the increased prevalence is due to the founder effect as shown in the South African Dutch (Afrikaner) and French Canadian communities where the prevalence of FH is as high as 1 in 72⁷. FH (HoFH) differs in that the prevalence is lower at 1 in 500 000 to

1000000, however, complications such as CAD and aortic stenosis tend to be more common and often occurs at an early age^{1, 8}. In order for HoFH to occur two unrelated HeFH individuals have to marry which is chanced as 1 in 250 000, then the chance of having a child with HoFH is 1 in 4 , which is 1 in 1 000 000⁹. However with the higher estimated prevalence of HeFH, the prevalence of HoFH may be higher. HoFH subjects who have the same mutation on both LDL receptor alleles of the same gene are known as true homozygous, while those who have 2 different mutations are termed compound heterozygous¹⁰. However the clinical phenotype of true homozygous or compound heterozygous FH is very similar. Recent studies have shown that mutations in two or more alleles of other genes can result in a phenotype indistinguishable from HoFH⁹. These genes are gain-of-function proprotein convertase subtilisin/kexin type 9 (PCSK9), LDL receptor adapter protein1 (LDLRAP1) and apolipoprotein B (APOB)⁹. LDLRAP1, unlike HoFH due to LDL receptor mutations, has a recessive inheritance but is extremely rare⁹.

To date, over 1600 LDLR mutations have been described with specific mutations being seen more commonly within certain population groups¹⁰. In the South African Afrikaner population 90% of FH patients are found to carry one of 3 LDLR mutations: the Afrikaner 1 (FH1)/ 681C > G, The Afrikaner 2 (FH2)/ 1285G> A and the Afrikaner 3 (FH3) / 523G>A mutations^{7, 11}. Furthermore other population groups within South Africa have been found to have founder mutations resulting in FH. The Jewish community, which are mostly of Lithuanian decent, have an increased prevalence of FH, mainly due to the FH-Piscataway/ 654-656-del mutation^{12, 13}. The Indian community in South Africa carry the FH Gujarat / P664L or less commonly the FH Durban 1 and FH Durban 2 mutations¹¹. It follows that within South Africa there are

multiple ethnic groups which harbour mutations resulting in South Africa having one of the highest prevalences of FH world-wide.

In HoFH, mutations in the LDLR gene which is located on the short arm of chromosome 19, can result in LDLR being either absent (<2% receptor activity) or defective (2-25% receptor activity)⁹. The severity of an individual's phenotype is dependent on the residual LDLR activity⁹. Individuals who have no or minimal receptor activity (receptor negative) have higher LDL-C levels and suffer more premature CAD and aortic stenosis^{4, 9}.

Mutations in the LDLR result in problems with LDL-C uptake by LDLR, failed synthesis, failed transport, defective LDLR for LDL binding, defective internalization and defective recycling^{10, 11}.

To date 6 classes of mutations have been documented¹¹.

Class 1: Synthesis of receptor or precursor is absent

Class 2: absent or impaired formation of receptor protein

Class 3: abnormal LDL binding (affects binding of LDL to LDLR)

Class 4: Defect in internalization of receptor complex (affects concentration of LDLR in clathrin pits)

Class 5: Defective LDLR recycling and rapidly degraded

Class 6: Receptors fail to be targeted to the basolateral membrane

As CAD and supra-aortic stenosis are common causes for morbidity and mortality at an early age in HoFH, the need for early detection and management is vital. In

families known with HeFH or HoFH, early screening of their children is recommended soon after birth^{1,9}. In individuals who present with markedly elevated LDL-C levels with no secondary causes or positive family history further work up might be needed. A family history of acute coronary event at a very early age should raise suspicion of possible FH. In individuals suspected of having FH, symptoms of ischaemic heart disease (IHD) or peripheral vascular symptoms should be asked. Other symptoms which should be gathered from history are tendonitis, arthralgia and unusual skin lesions (which could be indicative of cutaneous xanthomas)⁹.

On clinical examination, the skin and tendons, particularly the Achilles tendons, should be examined for features of planar xanthomas, tuberous xanthomas and tendon xanthomas, and the eyes for corneal arcus or xanthelasma which may be present in FH individuals. When examining the cardiovascular system, it is important to note all peripheral pulses, as well as any features of aortic stenosis⁹.

Individuals with HoFH usually have cholesterol levels greater than 13mmol/L⁹.

Diagnosis

If an individual is suspected of having HoFH, there are 2 recognized ways to confirm the diagnosis. Firstly genetic testing can be done to confirm LDLR mutation^{1,2,9}, however in some cases this might prove unhelpful as the causative gene or genes may not be identified⁹. The other way to make the diagnosis is based on clinical as well as laboratory investigations. An individual is likely to have HoFH if untreated LDL-C level is > 13 mmol/l or a treated LDL-C levels of >8 mmol/l with the presence of cutaneous or tendon xanthomas before the age of 10 years⁹. In addition, both parents should have untreated LDL-C levels consistent with HeFH^{1,9}.

The major problem associated with HoFH is that of cardiovascular complications. As these individuals have severely elevated LDL-C levels resulting in accelerated atherosclerosis, acute coronary syndromes incidence is drastically increased within HoFH individuals with most suffering from an acute coronary event during their adolescent years⁹. Accelerated atherosclerosis also causes plaque build-up and occlusive disease in other vessels such as the aorta, ileo-femoral arteries and renal arteries^{1,9}. An additional complication seen in HoFH individuals is early onset supra-aortic stenosis and aortic valve disease^{1,8,9}. Once aortic stenosis is established, even the optimal control of LDL-C levels does not slow the progression of worsening stenosis, with patients eventually developing left ventricular dysfunction and cardiac failure if left untreated^{1,8,9}. Because of the above mentioned cardiovascular complications, Cuchel *et al* recommends all individuals to have comprehensive cardiovascular work up done at the time of diagnosis⁹. This includes an echocardiogram and ischaemic heart disease work up with possible computed tomography coronary angiography every 5 years⁹.

Cholesterol

Cholesterol is an essential component of the human body, although in excess it can be detrimental. Cholesterol plays a vital role in cell membrane integrity and function; it is the precursor for certain steroid hormones, vitamin D and bile acids^{15, 16}.

Cholesterol being a lipid behaves as such and is hydrophobic. For cholesterol to be transported in plasma cholesterol needs to be bound to lipoproteins which are hydrophilic structures¹⁷. Lipoproteins are a micelle-like amalgamation of numerous proteins and lipids, with different lipoproteins being found based on certain properties such as size and lipid to protein ratio (density)¹⁸. The plasma level of certain

lipoproteins has clinical significance, whereby high levels of very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) increases the process of atherosclerosis and hence cardiovascular complications^{4, 17, 18}. Other lipoproteins such as high-density lipoproteins (HDL) may be beneficial as they may play a role in reverse cholesterol transport (RCT) and remove excess cholesterol from tissues and circulation, returning them back to the liver for processing^{17, 18}. Lipoproteins contain apolipoproteins which are proteins that bind to lipids and therefore play a role in the structure of lipoproteins^{15, 16}. Apolipoproteins also serve as a ligand, giving them the ability to bind to cell membranes and be taken up by tissues. Lastly apolipoproteins function as co-factors for enzymes¹⁶. Apolipoproteins B are present in low-density lipoprotein particles, which bind irreversibly to lipid droplets¹⁸. Other apolipoproteins form HDL particles which bind to lipid droplets reversibly. Apolipoproteins are synthesized mainly in the liver and intestines. The synthesis in the intestines is dependent on the amount of fat in an individual's diet¹⁷. Apolipoprotein synthesis in the liver is controlled by a host of factors, including dietary composition, hormones (insulin, glucagon, thyroxine, oestrogens, and androgens), alcohol intake, and various drugs (statins)^{15, 16, 18}.

Lipid metabolism

Lipids including cholesterol can be obtained via 2 pathways, these being the endogenous and exogenous pathways.

Exogenous pathway (figure 1)

Lipids in this pathway are obtained from an individual's diet. It has been shown that 95% of dietary lipids are triglycerides¹⁶, with the remainder being cholesterol

(esterified); free fatty acids (FFA), phospholipids and fat soluble vitamins¹⁶. Lipid digestion starts in the oral cavity and stomach to form emulsions¹⁸. These emulsions are then delivered to the intestines where they are mixed with bile salts micelles. It has been shown that a correlation exists between the amount of bile salts micelles and cholesterol absorption¹⁸. Cholesterol absorption occurs at the level of the jejunum, where the cholesterol enters the enterocyte. Studies have shown the Niemann-Pick C1-like1 (NPC1L1) protein to be responsible for the transport of cholesterol into the enterocyte^{19, 20}, with the drug ezetimibe inhibiting this protein and hence decreasing exogenous cholesterol absorption^{19, 20}.

Once inside enterocytes, exogenous cholesterol are packed into chylomicrons. Chylomicrons are made up of triglycerides, cholesterol and its esters, and it is these chylomicrons which are responsible for transporting dietary lipids to the liver and peripheral tissues^{17, 18}. Chylomicrons also contain apolipoproteins which function as ligands to the cell membranes of the liver as well as other tissues. Apolipoprotein B-48 is one such important apolipoprotein, which is amalgamated with cholesterol esters, triglycerides and phospholipids by microsomal triglyceride transfer protein (MTP)¹⁸. MTP role is not solely in the enterocytes in chylomicrons formation, but also plays an important step in VLDL synthesis¹⁸. Once packing of dietary lipids is complete the chylomicron leaves the enterocytes, passes through the thoracic duct and bloodstream. It is in the bloodstream that the chylomicrons accept apolipoprotein C2 (ApoC2) and apolipoprotein (ApoE) from HDL^{15, 18}. The addition of these apolipoproteins renders the chylomicron mature, with the newly bound ApoC2 interacting with lipoprotein lipase (LPL) in the walls of vessels found in muscle and adipose tissue. The role of LPL is such that it catalyses the hydrolysis of

triglycerides in the chylomicrons, with the resultant distribution of fatty acids to tissue and remodels the chylomicron into a remnant^{15, 17, 18}. As more triglycerides are hydrolysed the more ApoC2 is lost with the final result being decreased LPL activity and only ApoE remaining.

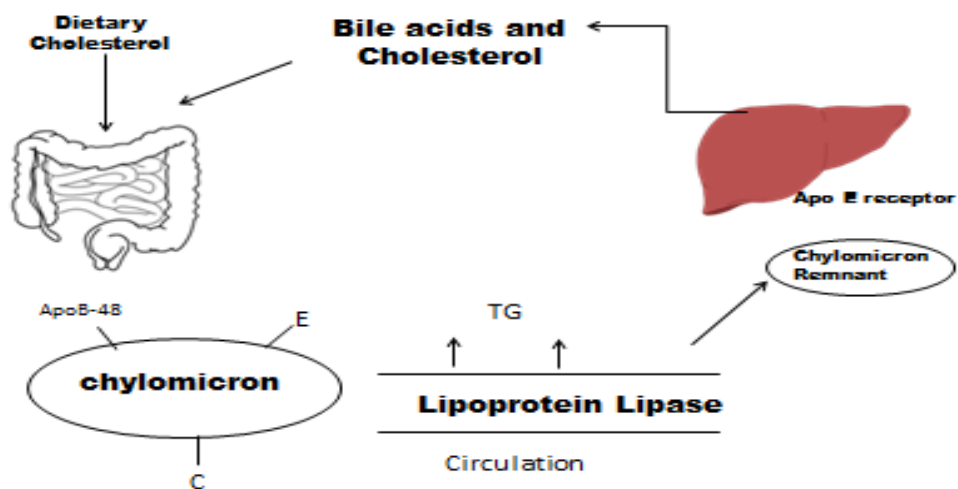


Figure1. Exogenous pathway. TG-triglycerides. Apo-E 48- Apolipoprotein E 48.

ApoE is vital for the uptake of the chylomicron remnant into hepatocytes, its interaction with hepatocyte surface molecules result in endocytosis mediated by LDLR and low density lipoprotein related 1 protein¹⁸. Once inside the hepatocytes further hydrolysis occurs with the production of free fatty acids and free cholesterol which is destined for the synthesis of VLDL¹⁸.

Endogenous pathway (figure 2)

The endogenous pathway starts in the liver. VLDL is continually synthesized by the liver and contains most of the body's endogenous triglycerides and to a lesser extent cholesterol. Apolipoprotein B-100 is an essential component of VLDL, with MTP contributing to the formation of the VLDL molecule^{15, 18}. As with chylomicron, once the VLDL is in circulation HDL exchanges ApoC2 and ApoE with the VLDL molecule allowing it to transfer free fatty acids to adipose tissue and muscles expressing the LPL. As the VLDL molecule circulates and the continual lipase activity removes triglycerides and ApoC2, it results in a molecule known as intermediate-density lipoprotein (IDL)¹⁸. IDL molecules still have ApoB-100 and ApoE, and it is the ApoE that allows the liver's LDLR to remove IDL from circulation. IDL which is not removed from circulation has continued lipase activity and becomes LDL. Individuals who have a genetic abnormality of the ApoE expression develop significantly higher plasma levels of cholesterol¹⁸.

The LDL particle has a high density of cholesterol which it carries and delivers to the peripheral tissues and liver, via the binding to LDLR and endocytosis. Apoprotein B100 is the principle ligand on the LDL surface which binds to LDLR of cell's^{15, 18}. Once bound the process of invagination and endocytosis occurs, with the cells liposomes destroying the LDL^{15, 18}. It follows that the number of hepatic LDLR determines the amount of LDL in circulation. Hepatic LDLR are down-regulated by increased dietary saturated fats and upregulated by decreased dietary fats and cholesterol. There is however another synthetic cholesterol pathway, with

hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase being the rate limiting step in *de novo* synthesis^{15, 18}. Expression levels of HMG-CoA and LDLR are negatively controlled by intracellular cholesterol levels. Sterol regulatory element binding protein 1 and 2 (SREBP1 and SREBP2) in the endoplasmic reticulum sense the intracellular cholesterol and migrate to the Golgi apparatus where it is cleaved. Once cleaved the SREBP migrates to the nucleus where it acts on the sterol regulatory element which stimulates the transcription of several genes¹⁸. These genes regulate LDLR and HMG-CoA reductase levels and activity respectively and aim to increase cellular cholesterol levels. LDLR function is also regulated by Proprotein Convertase Subtilisin Kexin type 9 (PCSK9)^{18, 21}.

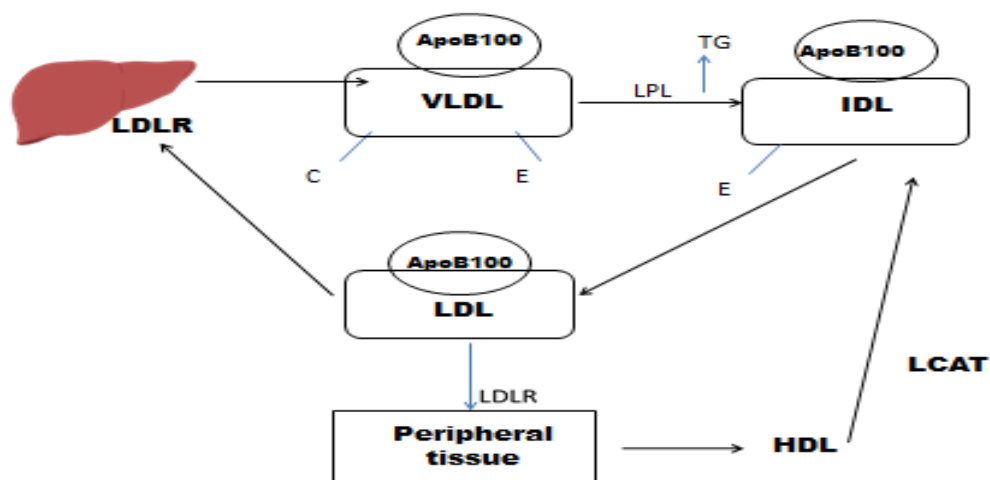


Figure 2. Endogenous pathway. LDLR-Low density lipoprotein receptor. LDL-low density lipoprotein. IDL-Intermediate density lipoprotein. HDL-High density lipoprotein. LCAT-Lecithin-cholesterol acyltransferase.

HDL is produced by the liver and intestines and is initially cholesterol-free¹⁶. Its main component is apolipoprotein A1, which not only forms its structure but in addition serves as the recognition molecule for proteins that interact with HDL^{15, 16}. HDL matures in the circulation with the procurement of phospholipids and apoprotein E and C from chylomicrons and VLDL. It is involved in a process known as reverse cholesterol transport (figure 3), whereby it takes up excess cholesterol in peripheral cells aided by the cholesterol-efflux regulatory protein and transports it directly to the liver where it is converted into bile acids and excreted¹⁸. The rate limiting step of reverse cholesterol transport is cholesterol efflux, which is regulated by ATP binding cassette A1 (ABCA1) gene product¹⁸. Free cholesterol in discoidal HDL is converted to Cholesteryl esters by Lecithin-cholesterol acyltransferase (LCAT), thus leading to the formation of mature HDL²². Phospholipids are required by LCAT and these phospholipids are made available by phospholipid transfer protein (PLTP)²², which mediates the transfer of phospholipids from VLDL to HDL (figure 3). Finally the HDL cholesterol ester and free cholesterol are transferred to hepatocytes, where scavenger receptor B1 (SR-B1) mediates uptake²². Once in the liver cholesterol can be recycled back into ABCA1 pathway or secreted into bile. Bile acid cycles from the liver to the gall bladder and duodenum. Once in the intestines it travels to the ileum where it is absorbed and transported back to liver via the portal vein. Around 95% of bile acid is reabsorbed from the intestines with 5 % being lost in the faeces^{17, 18}.

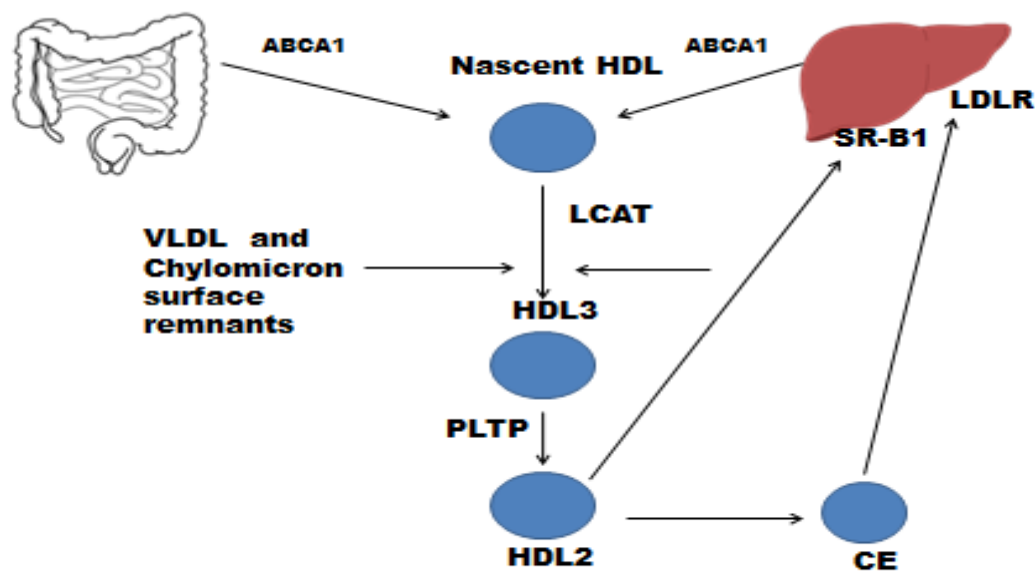


Figure 3. Reverse cholesterol Transport. HDL-High density lipoprotein. LCAT- Lecithin-cholesterol acyltransferase. CE- Cholesteryl Esters. PLTP- Phospholipid transfer protein. SR-B1-Scavenger receptor B1

As the risk of CAD is increased in FH, particularly HoFH, it is essential to obtain good LDL-cholesterol control. Statins which are HMG-CoA reductase inhibitors have been the main mode of treatment for HeFH ^{2, 23}. HoFH treatment has proven more difficult, with statin treatment alone not being able to obtain target LDL-C levels in the past. If available and affordable LDL apheresis can be done to lower LDL-C levels further²³. In 2002 Claude Gagne reported that in combination with a statin, ezetimibe, a drug which reduces the absorption of dietary and biliary cholesterol, can further reduce LDL-C levels in HoFH subjects ⁶. Similarly, Yamamoto *et al* showed a reduction in LDL-C in HoFH patients receiving LDL apheresis in combination with statin and ezetimibe therapy²⁴. More recently lomitapide, a microsomal triglyceride transfer protein (MTP) inhibitor, and mipomersen, an antisense oligonucleotide

targeting apolipoprotein B synthesis have also been shown to be partially effective in the treatment HoFH and has only recently be approved for use ^{5, 9, 25} . However, lomitapide and mipomersen are not affordable or available to many patients with HoFH worldwide and therefore drugs like ezetimibe will still have a role.

The Consensus Statement by the European Atherosclerosis Society for treatment of FH, starts with intensive education with the backbone being lifestyle management^{1, 9}. Diet, physical activity and cessation of smoking are imperative with multidisciplinary teams being involved. Once a diagnosis of FH has been made, immediate cholesterol lowering drugs should be initiated. The European Atherosclerosis society recommends initiating drug therapy in HeFH children from the age of 8 years ^{1, 9}. In HoFH children drug therapy should be initiated at diagnosis with the addition of lipoprotein apheresis, if available, from the age of 5 years and no later than 8 years⁹.

There are multiple treatment options available for familial hypercholesterolaemia.

Drug Therapy:

Statins

Statins inhibit HMG-CoA and therefore deplete the hepatocyte of cholesterol which in turn upregulates LDL receptors²³. Statins have been used in FH as mono or adjuvant therapy ⁵. Unlike HeFH, it has been shown that there is little dose response in HoFH patients ⁵. This might be explained by HoFH having no or defective LDLR and therefore lower hepatocyte cholesterol levels do not result in up regulation of LDLR. Statins however have been shown to decrease LDL levels between 10-25% by possible inhibition of cholesterol biosynthesis and by increasing cholesterol excretion

in intestines^{9, 23}. In addition, statins have been shown to reduce cardiovascular complications and improve survival in HoFH⁹.

Ezetimibe

Ezetimibe is a selective cholesterol absorption inhibitor which inhibits the absorption and uptake of cholesterol in the small intestine¹⁹. Ezetimibe was found to inhibit the protein known as Niemann-Pick C1-like 1(NPC1L1) which normally functions in the transportation of cholesterol from the intestinal lumen into the enterocytes¹⁹. As the delivery of cholesterol to the liver is decreased, LDLR are up regulated which results in more LDL being removed from circulation.

As the result of ezetimibe lowering the absorption of cholesterol, the composition of chylomicron alters and this is thought to change the metabolism of VLDL and IDL and therefore decrease its conversion into LDL²³. Ezetimibe has been shown to adequately decrease LDL levels when administered with statin in patients with HeFH^{19, 20} and autosomal recessive hypercholesterolaemia²⁶, however very few studies have tested its effect in HoFH. *Gagne et al* reported a 21% reduction amongst HoFH patients using ezetimibe in combination with a high dose statin, however the patients recruited in the study were also receiving LDL apheresis⁶. *Yamamoto* reported a 19% reduction in 6 patients with HoFH also receiving LDL apheresis²⁴.

Lomitapide

Lomitapide is a microsomal triglyceride transfer protein (MTP) inhibitor²⁷. As MTP is compulsory for the formation of VLDL and chylomicron, its inhibition in hepatocytes

and enterocytes respectively results in decreased VLDL and chylomicron levels and hence LDL^{23, 27}. Recent studies have shown up to a 50% reduction in patients on a combination of lomitapide and LDL apheresis after 26 weeks of treatment²⁷, with the main side effect being gastro-intestinal and deranged liver functions in keeping with liver fat deposition^{9, 23}.

Mipomersen

This second generation antisense oligonucleotide inhibitor, which targets messenger ribonucleic acid (mRNA) of apoB (the main apolipoprotein of LDL)^{5, 9, 23}, results in less VLDL being synthesised by the liver resulting in less LDL formation. LDL-C levels in HoFH have been shown to decrease by 25% after 26 weeks. It is administered weekly via intra-muscular injections with the main side effect being reported as injection site reactions as well as hepatosteatosis.

PCSK9 Inhibition

Proprotein convertase subtilisin/kexin type 9 (PCSK9) are proteins produced by hepatocytes, which target LDL receptors in clathrin coated pits to be degraded by lysosomal proteolysis^{21, 23}. It follows that inhibition of PCSK9, will result in more LDLR and therefore lower LDL levels in the blood. *Raal et al*²¹ showed that with the use of Evolocumab a monoclonal antibody against PCSK9, there was a 31% reduction in LDL level amongst patients with HoFH²¹.

Non-drug therapy:

LDL Apheresis

This technique has been approved and recommended for the treatment of HoFH, usually being initiated during early childhood. It has shown to improve life-expectancy and decrease cardiovascular disease significantly, by decreasing LDL levels on average around 48 %²³. Cutaneous xanthomas usually regress with long term use as an added benefit. It has few severe side effects and new studies have shown its safety even during pregnancy^{9, 23}. The disadvantage of this technique being its cost, availability and frequency needed to manage HoFH patients.

Liver transplantation can be used to correct the molecular defect; however the cost, and complications during and post-surgery make this option impractical in most situations.

The target for LDL in adults is set at <2.5 mmol/l, and <3.5 mmol/l in children^{1, 9}. If a patient is known to have established coronary artery disease, a lower LDL of < 1.8 is recommended⁹. The targets in HoFH patients should be the same but these are very difficult to achieve.

Rationale for the study

There has only been one previous report on the use of ezetimibe in combination with a statin in HoFH, yet in 2006 the American Heart Association recommended its use in the management of FH⁴. As South Africa has a large population of FH it is ideal to review the use of ezetimibe in HoFH patients.

Therefore the aims of this retrospective study are:

Study Objectives

1. To assess whether the addition of ezetimibe to statin therapy can result in further reduction in LDL-C in subjects with HoFH as compared to previous literature.
2. To assess whether LDL-C reduction (response rate) is affected by:
 - a. Different LDLR mutations in subjects with HoFH
 - b. Gender
 - c. Body Mass Index (BMI)
3. To compare patients which showed greater reduction in LDL-C, defined as a reduction in LDL-C of 20% or greater (responders) to those who responded poorly with < 20% reduction in LDL-C (non-responders), and to determine if factors such as FH genotype, gender or BMI play a role in predicting the LDL-C reduction with ezetimibe.

Methods

Study design

This was a retrospective review and interpretation of the existing clinical data obtained from the Lipid Clinic at the Charlotte Maxeke Johannesburg Academic Hospital.

Study population

A total of 48 patients known to the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) lipid clinic with confirmed HoFH, who received ezetimibe at a fixed

dose of 10mg daily in combination with a statin for at least a period of 3 months were included in study.

Inclusion criteria

1. HoFH diagnosed either by genetic analysis or on clinical criteria.
2. Subjects needed to have been on a stable dose of statin therapy for at least a 6 month period prior to starting ezetimibe.
3. All HoFH patients who received ezetimibe in combination with statin for at least a 3 month period, regardless of age, BMI or gender.

Exclusion criteria

1. Any HoFH patient who did not have a baseline lipogram just prior to initiating ezetimibe.
2. Any HoFH patient not already on a statin.
3. Any patient receiving LDL apheresis, mipomersen or lomitapide.

Ethics

The University of the Witwatersrand Ethics Committee granted ethics approval on 30/01/2015 (clearance certificate number M150107, Appendix A).

Confidentiality was maintained throughout the data collection process. A separate coding system known only to the primary investigator was used.

Data collection and analysis

The files of lipid clinic HoFH subjects fulfilling the inclusion and exclusion criteria were analysed. The lipid clinic has individual patient files with data including demographic data such as age, gender and BMI, full lipid profiles at various intervals since first diagnosis, different treatment regimens recorded over time, genetic study results, family histories, cardiovascular complications, smoking history and any other co-morbidity, as well as all other additional investigations.

Pre-treatment lipograms on statin therapy (baseline), as well as follow-up lipograms at 3 and 6 months were recorded.

The response to the addition of ezetimibe was assessed over the mentioned time frames, comparing percentage decrease in total cholesterol (TC) and LDL-C between the different time intervals as described by Gange *et al*⁶. In addition groups were divided by gender, BMI (normal vs overweight) and mutation types in order to compare response within and between groups.

Finally, patients were divided into two groups, those with a clinically significant reduction in LDL-C with the addition of ezetimibe (“responders” - defined as a LDL-C reduction of $\geq 20\%$) and those with $< 20\%$ reduction in LDL-C (“non-responders”).

As BMI ranges vary with age, the classification of “overweight” for patients under 20 years of age was defined using Centre for Disease Control (CDC) growth charts. Adults (>20 years of age), were divided into either normal or overweight categories, using the standard BMI classification.

All of the blood investigations, including lipograms, were analysed by the same laboratory (NHLS) used by the Charlotte Maxeke Johannesburg Academic Hospital.

All captured data was then transferred onto an Excel spreadsheet for data analysis.

Statistics

Descriptive and analytical statistics was used to assess data.

Descriptive statistics:

All data that was normally distributed was expressed as mean and standard deviation (SD). These included age, BMI, LDL-C, HDL-C and total cholesterol.

Non parametric descriptive tests were used for triglycerides (TG), and expressed as median and interquartile ranges.

Statistics used for objective 1: “To assess whether the addition of ezetimibe to statin therapy resulted in further reduction in LDL-C in subjects with HoFH”.

- An Ordinary one-way ANOVA was used to compare Baseline, 3 months and 6 months LDL-C, HDL-C and TC data. As certain patients missed follow up appointments for either 3 or 6 months, repeated ANOVA could not be used.
- A Mann-Whitney test was used to determine statistical difference in triglyceride levels between baseline, 3 and 6 months.
- The Graphpad Instat (Graphpad.com) program was used to analyse statistics

Statistics used for objective 2 :“To assess whether there was a difference in LDL-C reduction between and within groups”:

- For all comparisons including different FH genotypes (LDLR mutations), gender and BMI groups, an unpaired student t test was used to compare LDL-C at baseline and 3 months. (The Graphpad Instat program was used to analyse statistics)

Statistics used for objective 3: “Compare patients which showed higher responses (responders) to those who did not respond (non-responders).”

- A Fisher’s exact test was used to compare Gender and mutation differences between responders and non-responders group
- A Student unpaired t test was used to compare BMI between responders and non-responders

Limitations: The Retrospective study design does not allow for control of variables that could confound the results. Other variables such as compliance to lipid lowering therapy and adherence to diet which can impact on the degree of hypercholesterolaemia were not considered.

Results

A total of 48 patients with confirmed HoFH and meeting the inclusion criteria were entered into the study. The majority of patients in the study were on maximum doses of either Atorvastatin or Rosuvastatin prior to ezetimibe initiation and fasting lipograms pre-ezetimibe was recorded as baseline. The demographics for the patients are shown in table 1, with gender, age, ethnicity, BMI, smoking status,

cardiovascular disease (CVD) complications and statin therapy used represented.

Table 1 further subdivides demographics amongst males and females.

	MALE n=24	FEMALE n=24	ALL n=48
Age mean (SD) years	23 (15.2)	26 (13.8)	25 (14.4)
Age range (years)	3-48	7-48	3-48
BMI kg/m²			
Total (SD)	22.7 (6.9)	24 (7.14)	23.4 (7.1)
> 20 years old mean (SD)	27.7 (7.8)	28.3 (6.5)	28 (7.8)
Overweight n (%)	13 (45%)	10 (34%)	23
<20 years (SD)	14.3 (7.8)	17.9 (6.1)	16.2 (7.0)
Overweight n (%)	0	0	0
Ethnicity			
White	20 (42%)	21 (88%)	41 (86%)
Indian	4 (8%)	3 (6%)	7 (14%)
Smoking Status			
Ex	4 (17%)	0	4 (8%)
Current	4 (17%)	2 (8%)	6 (13%)
Never smoked	16 (67%)	22 (92%)	38 (79%)
CVD complications			
Both IHD and Aortic stenosis	6 (25%)	5 (21%)	10 (21%)
Only IHD	7 (29%)	4 (17%)	11 (23%)
Only Aortic stenosis	2 (8%)	8 (33%)	10 (21%)
No documented CVD	9 (38%)	7 (29%)	16 (33%)
Total IHD			44%
Total Aortic stenosis			42%
Statin			
Atorvastatin 40mg	4 (17%)	1 (4%)	5 (10%)
Atorvastatin 80mg	18 (75%)	23 (96%)	41 (86%)
Rosuvastatin 30mg	1 (4%)	0	1 (2%)
Rosuvastatin 40mg	1 (4%)	0	1 (2%)
	SMOKER	NON SMOKER	EX
IHD	4	13	4

Table 1: Illustrating the demographics of patients in study. Age and BMI are shown as means, while ethnicity, smoking status, CVS complications and statins given as actual numbers (%).

A total of 24 males and 24 females with HoFH were included in the study. Ages ranged from 3 years of age up to 48 years amongst the total group. The average age amongst males was 23 ± 15.2 years and females 26 ± 13.8 years. Females had a higher BMI in total $24 \pm 7.1 \text{ kg/m}^2$ compared to males $22.7 \pm 6.9 \text{ kg/m}^2$ (figure 4)

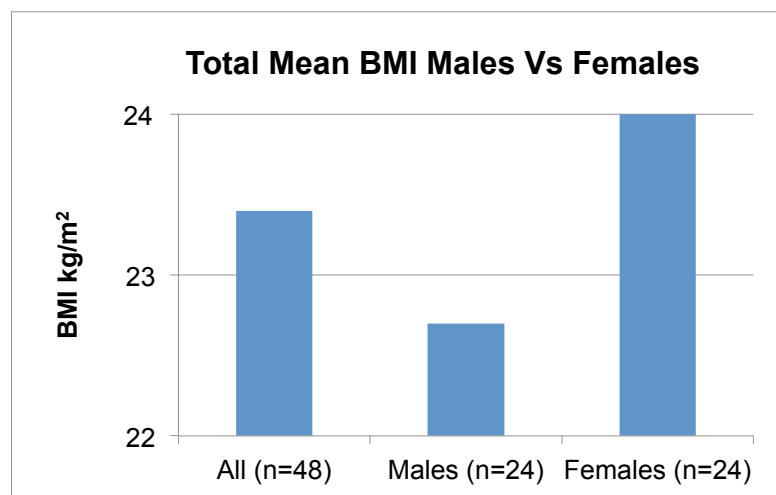


Figure 4. Total mean BMI of males and females

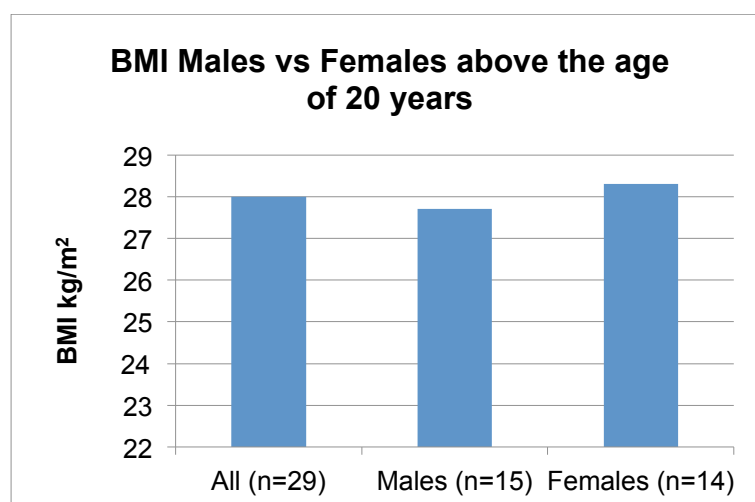


Figure 5: Mean BMI (body mass index) amongst males and females above the age of 20 years

When considering BMI in individuals above the age of 20, males had an average BMI ($27.7 \pm 7.8 \text{ kg/m}^2$) similar to that of females ($28.3 \pm 6.5 \text{ kg/m}^2$) (figure 5). Within the above 20 years of age population group, 79% of patients were overweight, with males making up 45% and females 34%. In patients below 20 years of age, males had a mean BMI of $14.3 \pm 7.8 \text{ kg/m}^2$, and females $17.9 \pm 6.1 \text{ kg/m}^2$ (figure 6). There were no patients below 20 years of age who were overweight according to their BMI.

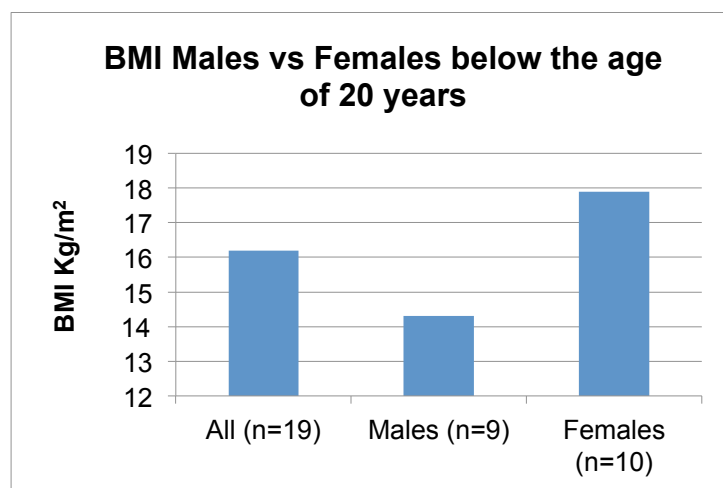


Figure 6: Mean BMI (body mass index), between males and females below and equal to 20 years of age.

From an ethnicity view, 86% of individuals were of white ethnicity with the remaining 14% being of Indian decent (table 1). With regards to smoking the majority of patients did not smoke (77%), some individuals were previous smokers and a small percentage still currently smoked (13%, figure 7). To note, 62% of patients who had

IHD were not smokers, with only 19% of IHD patients either being ex-smokers or current smokers (table 1).

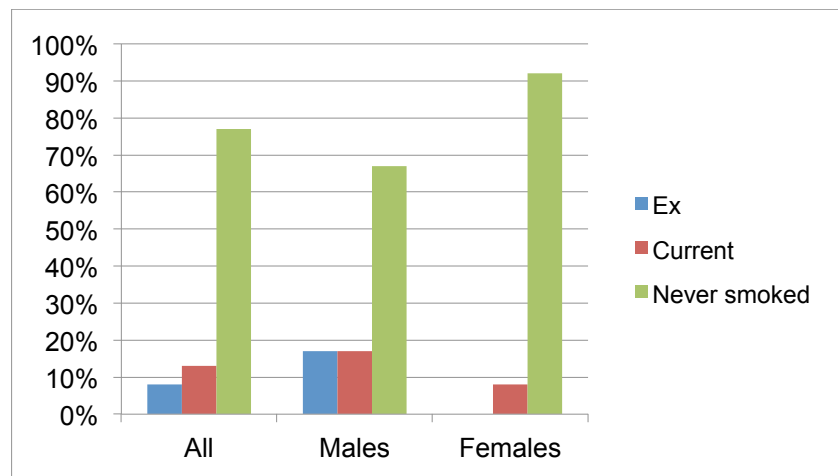


Figure 7: Percentage of smoking statuses amongst different gender groups

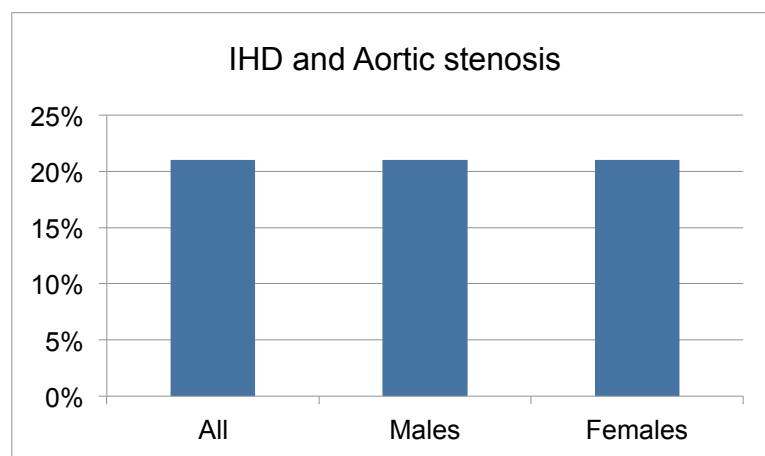


Figure 8: Percentage of patients with both Aortic stenosis and IHD

Cardiovascular complications within this population group were documented as having IHD (history of myocardial infarction or having coronary artery bypass surgery), aortic stenosis or no disease. Within the population 21% of individuals had

both IHD and aortic stenosis (figure 8). Males showed to have a higher percentage of IHD (50%), as compared to 38 % seen amongst females (figure 9)

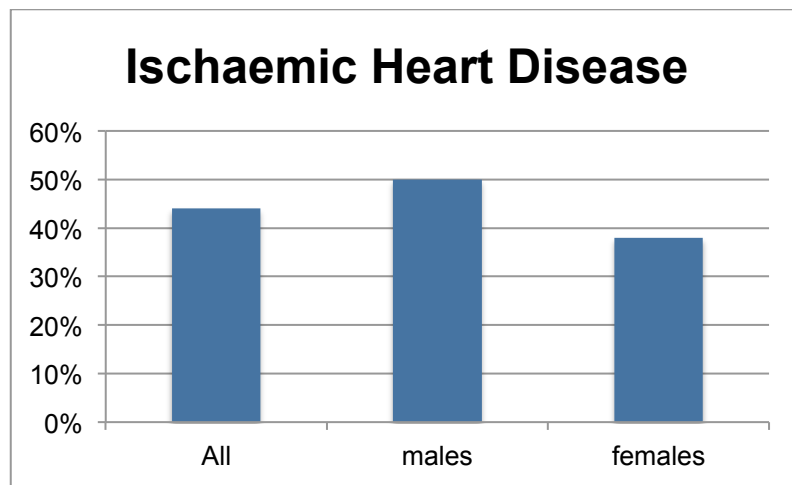


Figure 9: Percentage of patients with only Ischaemic heart disease (IHD)

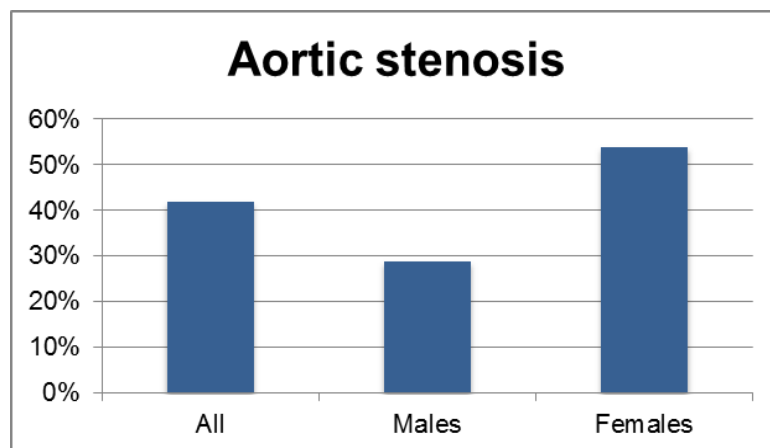


Figure10: Percentage of patients with only aortic stenosis

Females on the other hand had a higher incidence of aortic stenosis with 54% as compared to 29% of males (figure 10). A total 33% of individuals had no recorded cardio-vascular disease. In total 44% of patients had IHD and 42% had aortic stenosis.

At baseline, 85% of individuals were on 80mg of Atorvastatin, with only a small percentage being on atorvastatin 40mg (10%), or Rosuvastatin 30mg and 40mg daily 2% each (figure 11).

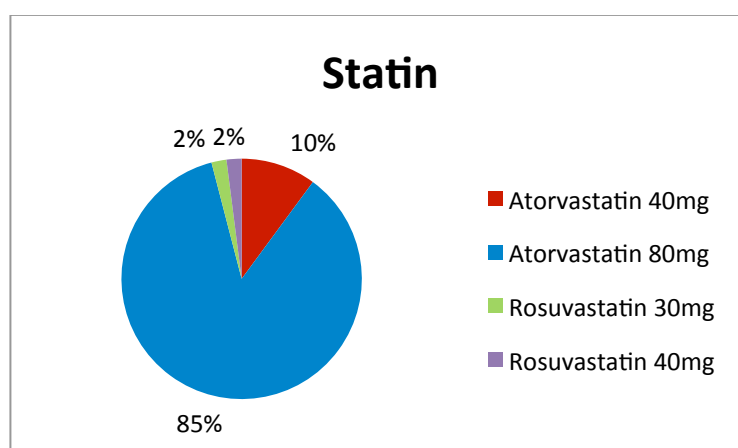


Figure 11: Percentage break down of the types of statins and dose that patients were on prior to study.

	Baseline	3 months	p value	6 months	p value	ANOVA
TC mmol/L	n=48	n=39	post hoc	n=44	post hoc	
mean $\pm$ sd	13.9 $\pm$ 3.9	11.8 $\pm$ 3.4	<0.01	12 $\pm$ 3.3	<0.01	P=0.0009
% change from baseline		-16.30%		-16.70%		
LDL mmol/L						
mean $\pm$ sd	12.1 $\pm$ 3.3	10.0 $\pm$ 3.4	<0.01	10.4 $\pm$ 3.3	<0.05	P=0.0018
% change from baseline		-18.90%		-17.60%		
HDL mmol/L						
mean $\pm$ sd	1.1 $\pm$ 0.3	1.1 $\pm$ 0.3	>0.05	1.1 $\pm$ 0.3	>0.05	P=0.8491
% change from baseline		0.60%		3.50%		
TG mmol/L						
median (IQR)	1 (0.7, 1.5)	1 (0.7, 1.8)	>0.05	0.9 (0.7, 1.7)	<0.05	P=0.8936
% change from baseline		0%		-16.70%		

Table 2: Mean $\pm$ Standard deviation (SD) of total cholesterol (TC), LDL-C, HDL-C and Median

triglycerides (TG) at baseline, 3 months and 6 months in total study group. IQR: inter-quartile range.

Table 2 shows a LDL-C of 12.1 ± 3.3 mmol/L at baseline, decreasing to 10 ± 3.4 mmol/L at 3 months (CI 0.7720 to 4.363, $p = < 0.01$) and 10.4 ± 3.3 mmol/L at 6 months (CI 0.2747 to 3.748, $p < 0.05$) after initiation of ezetimibe treatment. The percentage drop in LDL-C from baseline to 3 months was -18.9% and from baseline to 6 months of -17.6% (figure 12). Similarly there was a decrease of TC from baseline (13.9 ± 3.9 mmol/L) to 3 months (11.8 ± 3.4 mmol/L, CI 0.8617 to 4.46 $p < 0.01$), and baseline to 6 months (12 ± 3.3 mmol/L, CI 0.5065 to 4/00 $p < 0.01$). The mean percentage change from baseline of total cholesterol was -16.3% and -16.7% at 3 and 6 months respectively (figure 13). There was no significant change in HDL-C, (baseline 1.1 ± 0.3 mmol/L to 3 months 1.1 ± 0.3 mmol/L , with a percentage change of 0.6% ($p > 0.05$)). Similarly, there was no change from baseline to 6 months 1.1 ± 0.3 mmol/L, with percentage change of 3.5% ($p > 0.05$). Changes in triglyceride levels were also not significant, the change from baseline (0.7, 1.5 IQ) mmol/L to 3 months 1 (0.7, 1.8 IQ) had a mean percentage change of 0%. Triglycerides baseline to 6 months 0.9 (0.7, 1.7 IQ) had mean percentage change of -16.7 % ($p < 0.05$).

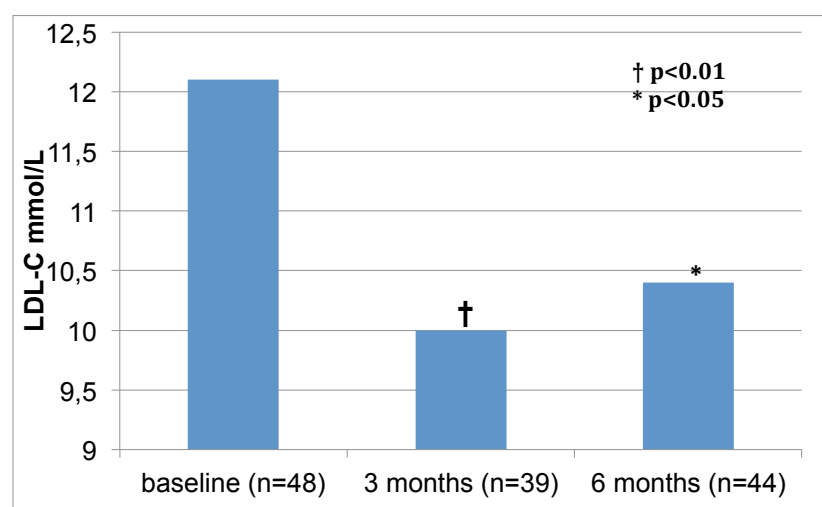


Figure 12: Mean LDL-C at baseline, 3 months and 6 months

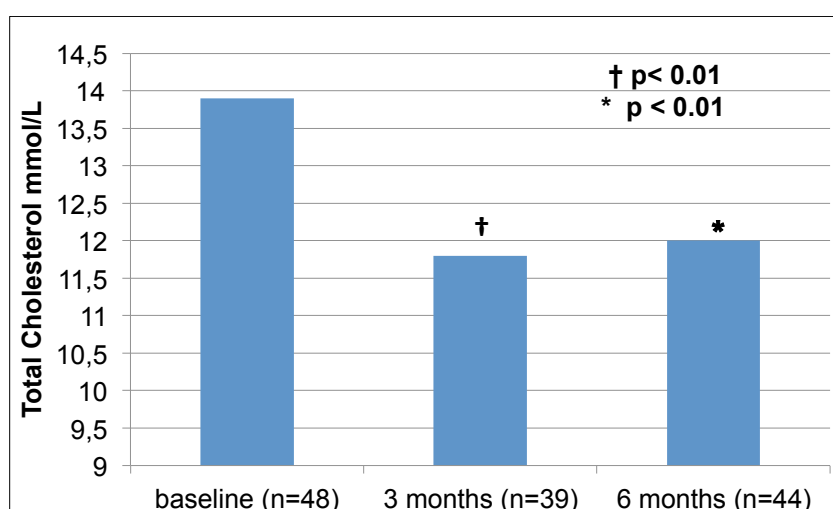


Figure 13: Mean total cholesterol at baseline, 3 months and 6 months

Table 3 illustrates the LDL-C change within the overweight and normal BMI population and compared LDL-C levels at the same time interval. In the overweight group baseline LDL-C was 11.4 ± 3.8 mmol/L vs 12.7 ± 3.2 mmol/L in the normal BMI group, $p=0.14$. After 3 months the mean LDL-C in the overweight group dropped to 8.2 ± 3.1 mmol/L vs 10.7 ± 3.5 mmol/L in the normal BMI group, $p=0.02$. The mean percentage change at 3 months was -20.5% in the overweight group, compared to -15.7% in the normal BMI group.

	Baseline	3 months
Overweight		
Mean LDL-C (SD) mmol/L	11.4 (3.8)	8.2 (3.1)
% change from baseline		-20.50%
N	21	21
Normal BMI		
Mean LDL-C (SD) mmol/L	12.7 (3.2)	10.7 (3.5)
% change from baseline		-15.7%
N	27	27
P value	0.14	0.02

Table 3: Mean LDL-C and mean percentage change between normal and overweight groups at baseline and 3 months

When comparing the two groups, mean LDL-C was less at baseline and 3 months in the overweight group compared to the normal BMI group (figure 14), with a statistical difference being seen at 3 months between the groups. In addition, the response to ezetimibe was more evident in the overweight group with a higher mean percentage reduction (figure 15).

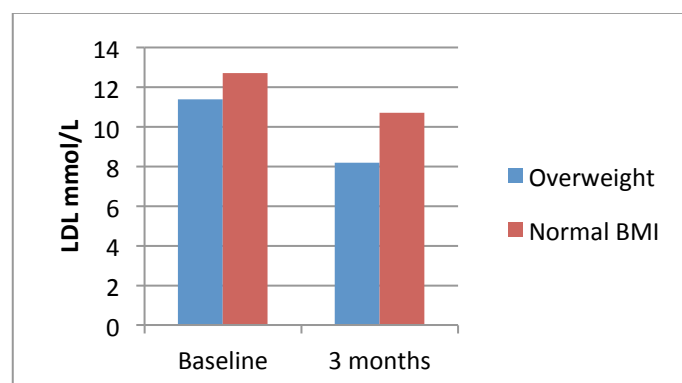


Figure 14: Graphically representation of mean LDL-C between normal and overweight population groups.

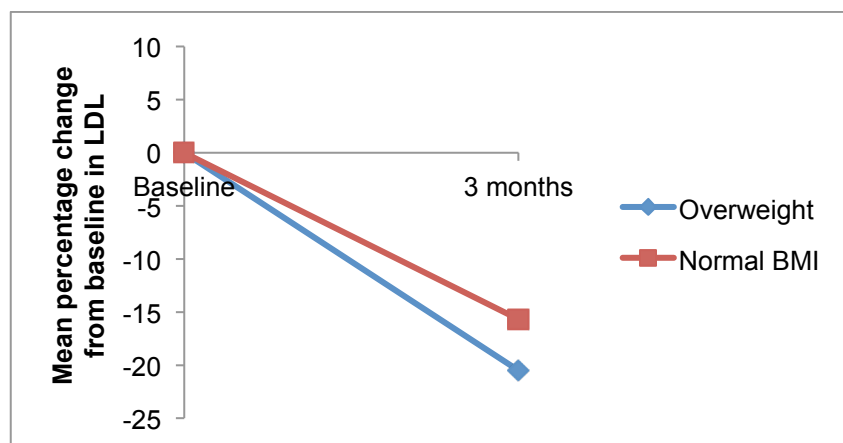


Figure 15: Mean percentage change of LDL-C between normal and overweight population groups over 3 months.

	Baseline	3 months
FH1/FH1		
Mean LDL-C (SD) mmol/L	12.7 (2.9)	10.3 (3)
% change from baseline		-17.00%
N	17	17
FH1/FH2		
Mean LDL-C (SD) mmol/l	14.9 (2.6)	12.8(2.5)
% change from baseline		-11.5%
N	11	11
P value	0.04	0.027

Table 4: Mean LDL-C and mean LDL-C percentage change in FH1/FH1 and FH1/FH2 groups from baseline to 3 months

Table 4 compares different FH genotype (LDL receptor mutation) responses during the study. In the FH1/FH1 group mean baseline LDL-C of 12.7 ± 2.9 mmol/L decreased to 10.3 ± 3 at 3 months with a mean percentage decline of -17%. In comparison, the FH1/FH2 group mean baseline LDL-C was higher at 14.9 ± 2.6 mmol/L, which decreased to 12.4 ± 2.5 mmol/L with a mean percentage decline of -11.5% at 3 months. Using an unpaired student t test, a significant difference between FH1/FH1 and FH2/FH1 was found at baseline $p=0.04$, and an even greater difference at 3 months $p=0.027$ (table4).

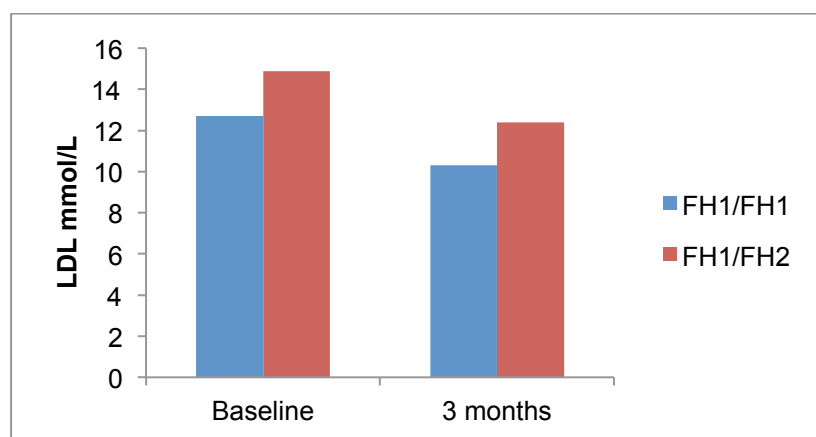


Figure 16: Mean LDL-C at baseline and 3 months amongst FH1/FH1 and FH1/FH2 population groups.

At baseline and 3 months, it can be seen that the average mean value for LDL-C in the FH1/FH1 group is lower compared to the FH1/FH2 group (figure 16). When looking at the percentage reduction graph (figure 17), the difference in response between the different mutations can be appreciated.

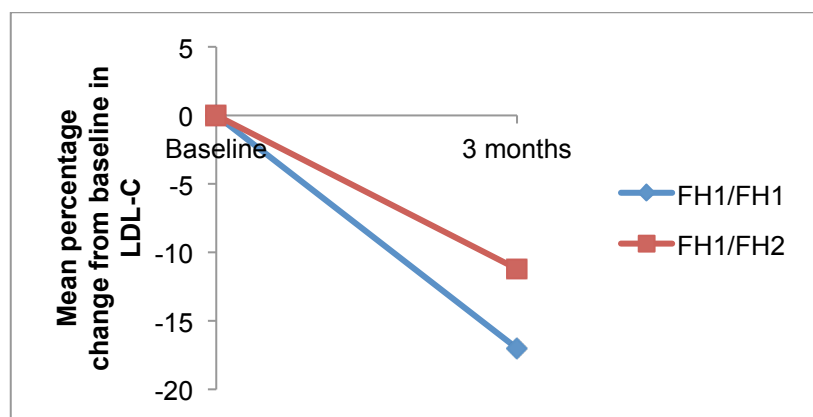


Figure 17: Mean percentage change from baseline of LDL-C between FH1/FH1 and FH1/FH2

	Baseline	3 months
Female		
Mean LDL-C (SD) mmol/L	12.4 (3.5)	10.2 (4.3)
% change from baseline		-20.7%
N	24	24
Male		
Mean LDL-C (SD) mmol/L	11.8 (3.4)	10.1 (2.9)
% change from baseline		-17%
N	24	24
P value	0.36	0.78

Table 5: Mean LDL-C percentage change and mean LDL-C at baseline and 3 months in males and females.

When comparing genders, females baseline 12.4 ± 3.5 mmol/L declined to 10.2 ± 4.3 mmol/L after 3 months with a percentage change of -20.7% (table 5). Males baseline 11.8 ± 3.4 mmol/L declined to 10.2 ± 2.9 mmol/L after 3 months with a mean percentage change of -17%. No statistical significance was shown between genders LDL-C at baseline and 3 months.

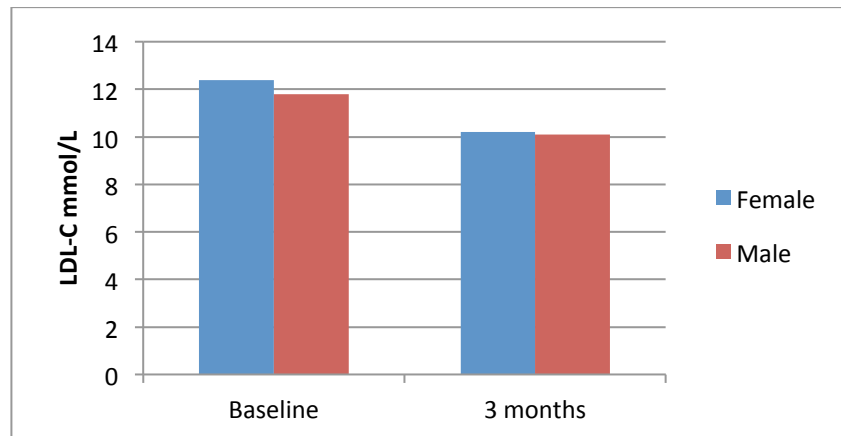


Figure 18: Mean LDL-C between males and females at baseline and 3 months

From figure 18, it can be seen that females have a slightly higher mean LDL-C level at baseline compared to males. After 3 months the difference margin is almost equal as females had a more pronounced percentage reduction. This larger percentage reduction in females at 3 months is illustrated in figure 19.

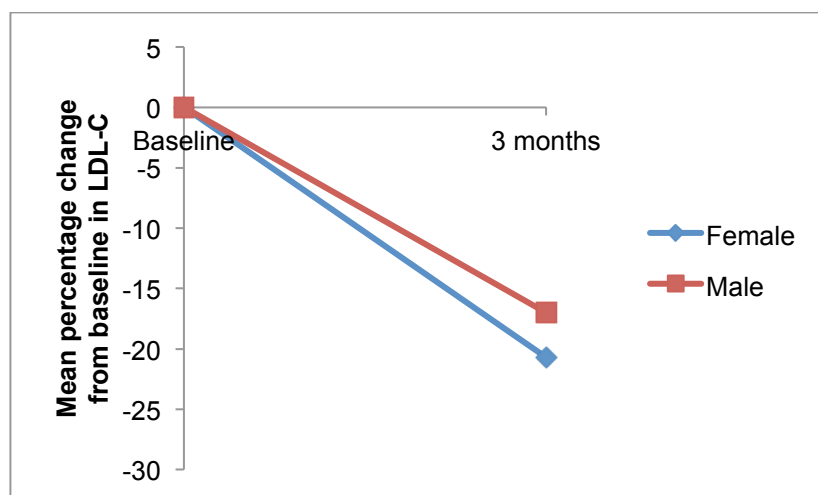


Figure 19: Mean percentage change from baseline in LDL-C between males and females

	RESPONDER n=22	NON- RESPONDER n=26	p value	ALL n=48
AGE (MEAN)	25.5	23.9		25
<18 Years (%)	8 (42%)	11 (58%)	>0.05	19
> 18 Years (%)	14 (48%)	15 (52%)		29
GENDER				
Males	8 (33%)	16 (67%)	>0.05	24
Females	14 (58%)	10 (42%)		24
BMI				
Overweight (%)	10 (43%)	13 (57%)	>0.05	23
Normal (%)	12 (48%)	13 (52%)		25
ETHNICITY				
White	17 (41%)	24 (59%)		41
Indian	5 (71%)	2 (29%)		7
MUTATION				
FH1/FH1 (%)	7 (41%)	10 (59%)	>0.05	17
FH1/FH2(%)	2 (18%)	9 (82%)		11
FH1/FH3(%)	1 (25%)	3 (75%)		4
FH3/FH3(%)	1 (50%)	1 (50%)		2
FH664/FH664(%)	5 (71%)	2 (29%)		7
5765C/5765C(%)	0 (0%)	1 (100%)		1
FH1/?(%)	1 (100%)	0		1
FH1/D961N(%)	2 (100%)	0		2
fh1/d2006				
(c662A>6/c681C>6)(%)	1 (100%)	0		1
C81C>6/C246C>A(%)	2 (100%)	0		2

Table 6: Demographics between responders and non-responders. Age represented as mean (%). Gender, BMI, ethnicity, mutation and age >18 and <18 years as actual number (%).

A total number of 22 patients fell into the responder group and 26 in the non-responder group (table 6). Of the population, 58% of females were responders, compared to only 33% of males. When considering age, 42% of patients below 18 years were responders, with the difference decreasing in the above 18 year group (48% responders vs 52% non-responders). The FH1/FH1 (n=17) mutation group had 41 % responders versus 59% non-responders. The difference in distribution was more profound in the FH1/FH2 (n=11) mutation group with only 18% being responders compared to 82 % non-responders. Conversely, the FH664/FH664 mutation (n=7) had 71% responders compared to 29% non-responders. The remaining mutation groups had too few patients to draw on any observations. There was not much difference between the distribution of responders vs non-responders with regards to BMI (table 6). Overweight responders made up 43 %, with overweight non-responders 57%, there was an even smaller difference between normal BMI responders (48%), and non-responders (52%). When considering ethnicity, 41% of whites were responders, with a higher number of responders seen amongst Indians (71%). Fisher's Exact test showed no significant difference in the mutation, age and gender ($p>0.05$) between the responder and non-responder groups. In addition the student unpaired t test also failed to show significance between responders and non-responders with regard to their BMI.

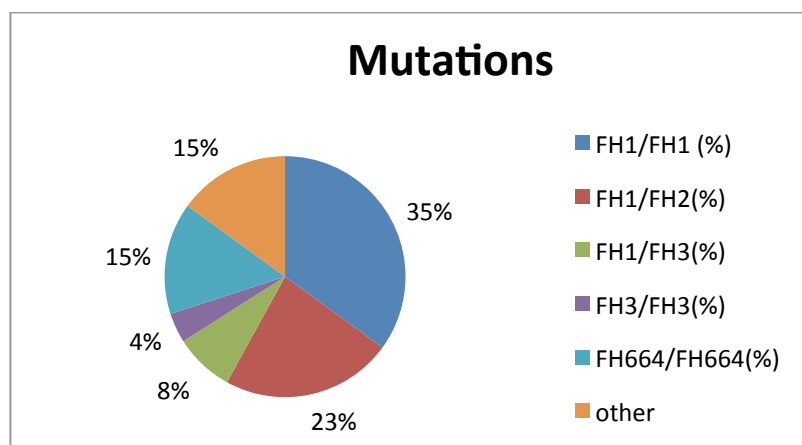


Figure 20: Pie chart illustrating percentage make up of each mutation group.

Figure 20, illustrates the percentage of different mutations in the total population. It can be seen that FH1/FH1 (35%) is the most common mutation found within the Charlotte Maxeke Johannesburg Academic Hospital HoFH population group, with FH1/FH2 (23%) and FH664/FH664 (15%) making up the other most common mutations.

	RESPONDERS (n=22)	NON- RESPONDERS (n=26)	ALL (N=48)
LDL CHOLESTEROL			
BASELINE	11.4 ± 3.6 (n=22)	12.4 ± 3.8 (n=26)	12.1 ± 3.3
3 MONTHS	8.7 ± 3.3 (n=20)	11.7 ± 3.9 (n=19)	10.0 ± 3.4
6 MONTHS	8.5 ± 2.5 (n=19)	12.1 ± 3.7 (n=25)	10.4 ± 3.3
(% CHANGE FROM BASELINE)			
3 MONTHS	-27.8%	-8.90%	-18.90%
6 MONTHS	-31.4%	-6.70%	-17.60%
OTHER LIPIDS (%CHANGE FROM BASELINE)			
HDL-C			
3 MONTHS	0.10%	0%	0.60%
6 MONTHS	0.10%	5.70%	3.50%
TRIGLYCERIDES			

3 MONTHS	-6.3 %	0%	0%
6 MONTHS	-17.6 %	-16.7%	-16.7%

Table 7: Mean $\pm$ SD of LDL-C between responders and non-responders at baseline, 3 months and 6 months. Mean percentage change of LDL-C, HDL-C and triglycerides from baseline at 3 month and 6 months.

Table 7 illustrates the mean LDL-C between responders and non-responders at baseline, 3 and 6 months. Non-responders had a mean LDL-C level higher than the responder group at baseline (difference of 1 mmol/L), 3 months (difference of 3 mmol/L) and 6 months (difference of 3.6 mmol/L). With regards to other lipids, HDL-C had minimal mean percentage change between responders (0.1% at 3 months and 0.1% at 6 months) and non-responders (0% at 3 months and 5.7% at 6 months). Triglyceride mean percentage change appeared to show a greater change at 6 months (-16.7%), compared to 3 months (0%). There was not much difference between TG mean percentage change between responders (-17.6%) and non-responders (-16.7%).

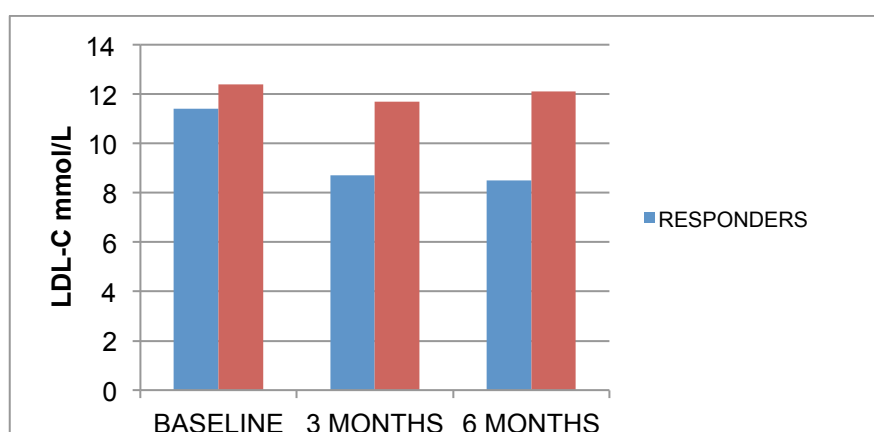


Figure 21: Mean LDL-C at baseline, 3 months and 6 months between responders and non-responder

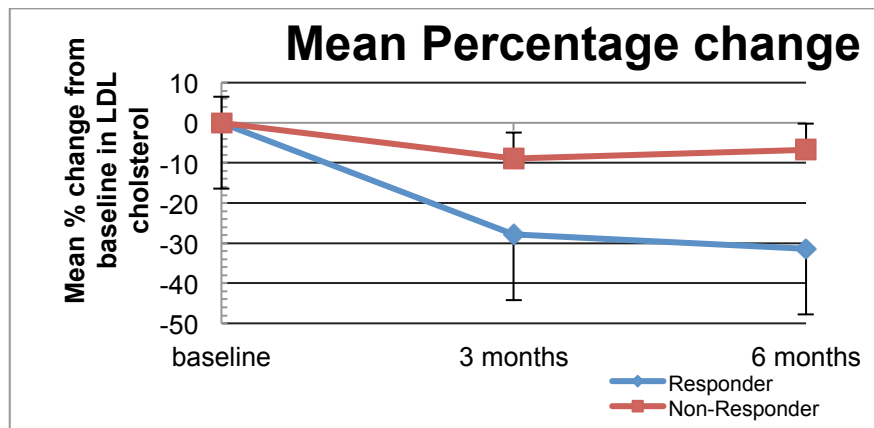


Figure 22: Mean percentage change of LDL-C between responders and non responders from baseline at 3 and 6 months

Figure 22 shows the mean percentage change from baseline between responders and non-responders over a 6 months period. As can be seen, both responders and non-responders mean LDL-C percentage change decreased at 3 months, with responders mean percentage change being -27.8% and non-responders being only -8.9%. After 6 months the non-responders mean percentage change dropped to -6.7%, while the responder group improved to -31.4%.

Discussion

HoFH is a very rare condition affecting a small percentage of the population. As such, only one study has reported the percentage drop in LDL-C in HoFH patients with the addition of ezetimibe to statin therapy⁶. One of the objectives of this study was to compare outcomes to that of Gagne *et al* and see if a similar decline in LDL-C was seen. The two main differences between the two studies were, firstly none of the participants in this study were receiving LDL apheresis, whereas several patients

were in the comparative study. Secondly, 86% of this study group was on 80mg of atorvastatin, compared to only 51.5% in the Gagne study.

In the study by Gagne *et al*, the average age of participants was 32 ± 3 years in a total of 33 participants who received a statin and ezetimibe⁶. In comparison this study had a total of 48 participants with an average age of 25 ± 14.4 years. The difference in age between the 2 studies can be explained by Gagne *et al* only including individuals who were 12 years and older and who had a weight of $\geq 40\text{kg}$ ⁶. Gagne *et al* had a total of 17 females and 16 males as compared to this study of 24 males and 24 females. In this study participants were made up of 86% whites and 14% Indians, whereas Gagne *et al* had only white participants. When comparing BMI, the population's mean was $23.4 \pm 7.1\text{kg/m}^2$ compared to Gagne $26 \pm 1\text{ kg/m}^2$. To note in this study, the average BMI in females was slightly higher in the above 20 year group (28.3 kg/m^2) compared to males (27.7 kg/m^2). This difference is much greater in the below 20 year group with females having a BMI of 17.9 kg/m^2 compared to males 14.3 kg/m^2 . This larger difference can possibly be explained by children and adolescents having different growth patterns and body fat percent during puberty²⁸.

When analysing the cardiovascular complications, it was noted that a significant proportion of patients had either IHD and aortic stenosis or both (21%), Males had the higher prevalence of IHD with 12 out of 24 males versus females 9 out of 24. Females however had a higher prevalence of aortic stenosis (13 out of 24). It is unclear whether gender plays a role in the occurrence of aortic stenosis, but the literature supports the observation that the risk of developing aortic stenosis is related to serum cholesterol level²⁹. As mentioned above, females had a higher mean LDL-C at baseline compared to males (table 5). Lastly, when comparing

studies, Gagne *et al* had patients who were also receiving LDL apheresis in addition to statin and ezetimibe.

1. Reduction in LDL-cholesterol, when combining ezetimibe with statin therapy compared to previous literature.

When comparing this study to that of Gagne *et al*, several similarities and differences in baseline and on treatment lipid profiles were found. Mean total cholesterol, LDL-C, Triglycerides and HDL-C between the 2 studies at baseline and 3 months in patients who received ezetimibe in addition to a statin are shown in table 8.

Baseline Lipogram mmol/L	Gagne et al	Current study
TC	9.8 ± 0.57	13.9±3.9
LDL-C	8.1 ± 0.57	12.1 ± 3.3
HDL-C	1.08 ± 0.05	1.1 ± 0.3
TG	1.2	1.0

Table 8: Comparison of mean total cholesterol (TC), LDL-C, HDL-C and triglycerides (TG) at baseline, between current and previous studies.

It can be seen that mean Total cholesterol and LDL-C at baseline were higher in this study when compared to Gagne *et al*. However, many patients in Gagne *et al* study were receiving LDL apheresis. There was no notable difference between triglycerides and HDL-C values at baseline between the two studies.

3 month Lipogram mmol/L	Gagne et al	% change	Current study	% change
TC	8.0	-18.7%	11.8	-16.3%

LDL-C	6.4	-20.7%	10	-18.9%
HDL-C	1.08	-2.8%	1.1	0.6%
TG	1.2	-10.8%	1.0	0%

Table 9: Comparison of mean percentage change of LDL-C, total cholesterol(TC), HDL-C and triglycerides (TG) at 3 months between current and previous study.

Table 9, compares mean total cholesterol, LDL-C, HDL-C and Triglycerides at 3 months, with their respective percentage change from baseline. With respect to table 9 it can be seen that although the mean total cholesterol and LDL-C values differed between the 2 groups, overall the percentage reduction at 3 months with the addition to ezetimibe was similar.

2. The effect of BMI, Gender and LDLR mutations in predicting response rate to statins and ezetimibe

When considering certain factors in predicting an individual's response with the addition of ezetimibe, BMI, gender and LDLR mutations were considered. BMI was divided as either being normal or overweight. The difference between the two groups at baseline was 1.3mmol/L which was not significant when using the student t test. However, after 3 months on ezetimibe the difference was 2.5 mmol/L, with a significant p value of $p=0.02$ and a greater mean percentage change (-20.5%) in the overweight group compared to normal BMI (-15.7%). A possible reason for overweight individuals responding better can be postulated by the mechanism of action of ezetimibe. Overweight individuals can be assumed to have their BMI by following poor dietary habits, thus by inhibiting cholesterol absorption in the

intestines, a greater reduction in their total cholesterol can be expected. Of interest, overweight individuals had a lower baseline LDL-C compared to normal BMI individuals which is contrary to what is expected based on current data and therefore difficult to explain³⁰.

When the 2 commonest mutations were analysed (FH1/FH1 and FH1/FH2), there was a significant difference between their LDL-C values at baseline (difference of 2.2 mmol/L, $p=0.04$), which increased at 3 months (difference 2.5mmol/L, $p=0.027$). In addition, the mean percentage reduction of the FH1/FH1 was much larger (-17%), compared to FH1/FH2 (-11.5%) after 3 months. The reason for the larger difference seen between the above mentioned mutations is possibly due to the effect of the mutation on the LDLR. FH1 mutations results in a defective LDLR, while FH2 results in a negative LDLR. Therefore, although the FH1 receptors are defective, they still have some functionality, whereas FH2 has none. This explains why the LDL-C at baseline has a lower mean value and why ezetimibe had a more pronounced effect in this group.

Lastly, when considering gender, females had a slightly higher mean LDL-C at baseline compared to males (difference of 0.6 mmol/L, $p=0.36$), with the difference gap almost negligible after 3 months (difference 0.1mmol/L, $p=0.78$). A possible reason for females having a higher mean LDL-C at baseline could be related to them having a higher BMI. The mean percentage reduction of LDL-C at 3 months was similar between the 2 groups, with females having a slightly higher reduction. This result is expected, as gender has not been shown to affect LDL-C/TC nor response to ezetimibe.

3. The effect of BMI, mutation, age and gender in determining patients who are more likely to respond to ezetimibe

Certain variables were analysed to determine if certain factors predict response to ezetimibe. The study group was divided up into responders and non-responders based on their percentage reduction and variables were assessed to determine a correlation. To note there was a -27.8 % reduction within the responder group (n=22), compared to -8.9% reduction amongst non-responders (n=26).

When considering age, the percentage distribution between those that fell into the responder and non-responder group was similar. Using Fisher's exact test no significance was found. No research has been done to determine if ezetimibe efficiency is different amongst different age groups. However, it might be postulated that since individuals are born with the LDLR mutation, with little chance of it being acquired during their lifetime, age would not be expected to play a role in their response to ezetimibe. Gender seemed to show a slight difference between distribution with females (58%) falling into the responder group versus males (33%), however there was no statistical significance. When considering BMI, there was no difference in the distribution between normal and overweight individuals between the 2 groups. However, as mentioned above, overweight patients did show a higher percentage reduction with the addition of ezetimibe (-20.5% vs -15.7%), which was statistically significant. Interestingly, females had an average BMI (24 kg/m^2) which was higher than the males (22.7 kg/m^2), which might explain why a greater percentage of females fell within the responder group compared to males. Supporting this idea of BMI determining the response to ezetimibe, is the study by

Gagne *et al*, whose population had an average BMI of 26kg/m² and an overall better mean percentage reduction of -20.7 %⁶.

The 2 most common mutations were FH1/FH1 and FH1/FH2, with the remaining being too few in numbers to analyse. When reviewing FH1/FH1, 41% fell into the responder group which was similar to the BMI and age distribution. However, when considering FH1/FH2 there is a notable difference in the distribution, with only 2 out of 11 (18%) falling within the responder group. There was however no statistical significance using contingency tables between the above two mutations, however the population size is small and it can be speculated that if a larger group was analysed, there may indeed be an trend for FH1/FH2 to show poorer predictive responses to ezetimibe. The possible explanation for the difference in response between the 2 mutations can be explained on molecular and genetic level. As mentioned above, LDLR mutations can result in either a defective receptor or a negative receptor. The FH1 mutation has been shown to result in a defective receptor, while the FH2 receptor results in a negative receptor. Therefore, the effect of ezetimibe increasing the number of LDLR in the liver by decreasing the amount of cholesterol absorbed in the intestines might have some affect in patients with FH1 (defective LDLR). It follows that since the FH2 results in a negative LDLR, a much less/no response is expected with the addition of ezetimibe. Supporting this speculation is seen above where FH1/FH1 mutation resulted in a -17% percentage reduction, compared to only -11.5% percentage reductions seen in the FH1/FH2 group.

Lastly, the FH664/FH664 mutation which is found in the Indian population was interestingly shown to result in 71% of patients with this mutation falling into the responder group. As this population group is only made up of a small percentage of

the study group (n=7), no conclusion can be drawn about the response in this mutation group, with additional studies needed.

When considering what is mentioned above with regard to the possibility of BMI and mutation playing role in predicting response the patient's individual data was analysed again. Firstly if indeed FH1/FH2 results in predicting a lower response to ezetimibe then what factors did the patients with FH1/FH2 have that resulted in them falling into the responder group. Interestingly, the 2 patients with FH1/FH2 that were responders were both overweight, whereas the FH1/FH2 patients that were non-responders were made up of 67% normal BMI. However, as the sample size was small no definitive conclusions can be drawn.

Limitations

As this was a retrospective study, certain factors such as dietary intake amongst others could not be controlled.

The sample size was small, however based on HoFH being a rare condition, a larger sample size would be difficult in a single-centre study.

The limit set to define responders vs non-responders was based on previous literature results in HoFH and general population. If a lower limit was used, it is possible that certain factors might be identified to predict an individual's response.

Conclusion

Overall this study supported previous data on HoFH, with a high percentage of coronary artery disease and aortic stenosis seen.

This study's findings are similar to that of Gagne *et al*, with the mean percentage reduction of LDL-C being -18.9% compared to Gagne *et al* -20.7%. This percentage reduction is only slightly less than that reported in non-FH patients where ezetimibe decreased LDL-C by 23-24%³¹. It therefore appears that the mechanism whereby ezetimibe inhibits cholesterol absorption is constant in most cases regardless of LDLR mutations.

This study also analysed the effect of BMI, Gender and LDLR mutation, to determine if it played a role in the ability of ezetimibe to lower LDL-C. There was no significant difference when comparing gender, however a notable difference was seen between overweight and normal BMI individuals, with a higher mean percentage reduction seen amongst overweight individuals. This affect could be explained by the action of ezetimibe and the likely poor dietary habits of patients in the overweight group. Furthermore, the type of LDLR mutation does seem to play a role in its ability to respond to ezetimibe, with the LDLR negative group showing a lower mean percentage reduction compared to the LDLR defective group.

When considering if certain factors such as BMI, age, LDLR mutation and gender could predict whether an individual would be classified as a responder or non-responder, no significant factors were identified. However, the sample sizes were small and the trends seemed to favour both LDLR and BMI in determining response.

In conclusion, HoFH a rare condition which results in marked elevated LDL-C levels and complicated by early onset cardiovascular disease, was seen amongst this population group. The addition of ezetimibe to statin therapy amongst HoFH individuals showed a similar mean percentage reduction as shown in previous

studies. As the addition of ezetimibe can significantly reduce LDL-C further in HoFH patients, it is recommended that all patients with HoFH be on ezetimibe in addition to statins. BMI and LDLR mutations contribute to differences in response to ezetimibe, with overweight patients and those with defective LDLR having better responses. There was no identifiable factors which would predict the individual response to ezetimibe, however if a larger sample size was analysed, certain factors might be shown to predict response. Overall, patients with HoFH carry an increased risk for cardiovascular complications, which is related to their markedly elevated LDL-C levels. The cornerstone to managing these patients is lowering the LDL-C, which has been a challenge as they are unable to attain optimal LDL-C levels on statin therapy alone. Although new medications are becoming available to overcome this problem (for example PCSK9-inhibitor therapy), in resource limited centres ezetimibe can be used in combination with statin therapy to decrease LDL-C further.

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APPENDIX A