Using Mendelian randomisation to investigate the causal relationships between lipid profiles and diseases, lifestyle, and medication.

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ABSTRACT:

Background:

Maintaining balanced lipid levels of Low-density lipoproteins (LDL), High-density lipoproteins (HDL), and Triglycerides (TG) are crucial for the healthy functioning of the body. Disruptions in these lipid profiles have been strongly associated with a plethora of diseases, including atherosclerosis disorders, obesity, diabetes, and autoimmune diseases. Elevated lipid profiles are also frequently linked to suboptimal lifestyle choices, such as an unhealthy diet, and lack of exercise which can lead to obesity. The objective of this study was to understand the causal relationship between these lipids, and lifestyle factors, diseases, and medications. By exploring and analysing these complex interactions highlighted, we hope to identify effective strategies for maintaining optimal lipid levels, and enhance our understanding to further improve patient outcomes.

Methods:

This observational study was conducted using two-sample Mendelian Randomisation (MR) applied to Genome-Wide Association Studies (GWAS) summary statistics from the UK BioBank. The EpiGraphDB resource was initially used to identify potential causal relationships between lipids and diseases, medication, and lifestyle factors before a more extensive MR analysis was conducted to examine these causal associations in further detail.

Results:

EpiGraphDB analysis revealed several causal associations between lipids and factors such as medication, biomarkers, diseases, body measurements, and dietary modifications. Some of these associations included diseases such as chronic ischaemic heart disease, coronary atherosclerosis, and cholesterol-lowering medications, as well as dietary habits such as increased alcohol consumption and skimmed milk. When examining some of these causal relationships in detail across all five MR tests, I found that high levels of LDL consistently showed positive associations with cholesterol-lowering medication (Minimum p-value: 4.44E-14), and dietary modifications such as increased consumption of skimmed milk (Minimum p-value: 2.32E-16). There was also a significant causal association observed between increased LDL levels and the risk of major coronary heart disease events (Minimum p-value: 5.31E-12). Moreover, this study identified strong causal relationships between increased TG levels and myocardial infarction (Minimum p-value: 1.03E-09) and found that the increased risk of diabetes was causal for higher levels of TG (Minimum p-value: 2.45E-04). Increasing levels of TG had a positive causal relationship with an increased number of medications taken (Minimum p-value: 2.37E-19). Along with this, increased levels of HDL had evidenced a lower risk of vascular or heart-related complications (Minimum p-value: 3.86E-11).

Conclusion:

This study presents a comprehensive documentation of the causal relationships between LDL, HDL, TG, and diseases, medication, and lifestyle modifications using a two-sample MR analysis. Overall, these findings are important as they deepen our understanding of lipid-related diseases, and examine how existing cholesterol-lowering treatments designed to reduce lipid-related disorders can be used to improve public health outcomes.

DECLARATION

I declare that the dissertation titled ‘Using Mendelian randomisation to investigate the causal relationships between lipid profiles and diseases, lifestyle, and medication’ has been composed solely by myself, under the guidance of my supervisor Andrew Morris. No portion of this work referred to in the dissertation has been submitted in support of another degree or qualification of this or any other university or other institute of learning.

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1.0 INTRODUCTION:

Lipids are a group of diverse compounds with essential roles in the physiology and pathology of cells, (Naudi et al, 2015). They facilitate a range of biological functions such as cell regulation and proliferation, energy storage, and reshaping of the mechanism of cells by converting glycolysis to lipids, (Guo et al, 2020), (Beloribi-Djefaflia et al, 2016). Lipids also serve as essential structural components of cell membranes and play a crucial role in mediating cell signalling transmission as signalling molecules in cellular response pathways, (Ahmed et al, 2021), (Finkelstein et al, 2014). Dysregulation or imbalance of lipid levels is highly associated with disorders such as obesity, diabetes, and autoimmunity, (Finkelstein et al, 2014), (Klop et al, 2013). It can also lead to contracting atherosclerosis disorders such as heart attack, and stroke, and result in persistent cellular and tissue damage in both the peripheral nervous system and the brain, (Insull, 2009), (Nelson, 2013), (Solomon et al, 2017).

Various types of lipid fats, such as sterols, triglycerides, and other waxes are essential to maintain the healthy functioning of the human body, (Ahmed et al, 2018), (Ahmed et al, 2021). A cluster of disorders touched upon previously was caused by abnormal concentrations of these different lipid types, so it's essential to understand their functions and processes to examine their causal relationships with diseases, (Bitzur et al, 2009). Cholesterol, a type of sterol is an essential lipid that allows the body to manage energy storage and synthesise the structural and functional elements of the body including its hormones and membranes, (Reiner et al, 2014), (Jesús Millánn Núñez-Cortés et al, 2019). Cholesterol is transported primarily through the plasma, via Low-density lipoproteins (LDL) which are labelled as bad cholesterol, and High-density lipoproteins (HDL) noted as healthy cholesterol, (Arnold et al, 2003), (Scirica et al, 2005). Triglycerides (TG) are an important dietary lipid, composed of glycerol and three fatty acid molecules, and serve as the primary form of lipid storage and energy in the human body, (Lichtenstein, 2013), (Engelking, 2015), (Viecili et al, 2017).

An imbalance in the concentrations of these lipids beyond their healthy levels is known as dyslipidemia, (Mosca et al, 2022). This is when the levels of TG are exceedingly high, beyond the normal range which is less than 150mg/dl, and is often referred to as hypertriglyceridemia, (Yuan et al, 2007). Severe hypertriglyceridemia is observed when the levels of TG range between 200-500 mg/dl, (Yuan et al, 2007), (Criqui et al, 1993). Dyslipidemia encompasses elevated levels of LDL, beyond the optimal range which is noted as less than 100 mg/dl, and an exceptionally high LDL level greater than 190 mg/dl, along with an exceedingly reduced HDL level of less than 40 mg/dl, (Lee et al, 2023). Observational studies indicate that an estimated 70% of adults have imbalanced lipid levels outside the healthy concentration, (Gao et al, 2016), (Steinhagen-Thiessen et al, 2008), (Matey-Hernandez et al, 2018). These imbalances in HDL, LDL, and TG profiles reflect the abnormal lipid metabolism in dyslipidemia and increase the risk of vascular diseases such as atherosclerosis, and type 2 diabetes, (de Ferranti et al, 2020). Genetic and environmental factors can both influence the imbalance of the lipid profiles, (Patel et al, 2012).

Studies have revealed that genetics accounts for over 50% of the variance of lipid levels, with the reported heritability for HDL, LDL, and TG being at 0.3, 0.25, and 0.75, (Kathiresan et al, 2007), (Chen et al, 1990), (Pirruccello et al, 2010), (Pila et al, 2006), (Cadby et al, 2020), (Zaitlen et al, 2013). These include inherited disorders referred to as monogenic hyperlipidemia which can lead to elevated concentrations of LDL, and TG, with low concentrations of HDL, (Krasi et al, 2019). An example of these disorders is familial hypercholesterolemia (FH), an inherited disorder that is autosomal dominant, (Pejic, 2014). It's characterised by a high LDL profile, and is caused by mutations in the following genes; LDL receptor (*LDLR*), apolipoprotein B-100 (*APOB*), and the proprotein convertase

subtilisin/kexin type 9 (*PCSK9*), (Vrablik et al, 2020), (Krasi et al, 2019), (Austin, 2004). *PCSK9* plays a crucial role in cholesterol homeostasis, by binding to LDLR to promote its degradation and reduce LDL uptake, which leads to increased LDL levels, (Spolitu et al, 2019), (Austin, 2004), (Seidah et al, 2014). FH can also be recessively inherited and expressed due to variants in the LDLR adapter protein (*LDLRAP*) gene, (Vrablik et al, 2020). Hereditary disorders that lower HDL levels are commonly caused by Tangier disease, inherited through an autosomal recessive manner and homozygous mutations in proteins apolipoprotein A-1 or lecithin-cholesterol acyltransferase, (Hegele, 2009). Increased TG levels can be caused by a genetic disorder called Primary hypertriglyceridemia due to defects in TG synthesis, metabolism, and mutation known as p.Gln38Lys in the gene *APOC3*, (Sundaram et al, 2017). Examples of these disorders include familial chylomicronaemia syndrome (FSC), an autosomal recessive disease associated with mutations in the *LPL* or *APOC2* gene, and severe hypertriglyceridemia caused by mutations in genes *APOA5*, *LMF1*, or GPIHBP1 protein, (D'Erasmo et al., 2019), (Krasi et al, 2019).

The most effective way to understand the genetic variability and heritability of the blood lipid levels LDL, HDL, and TG includes the use of Genome-Wide Association Studies (GWAS), (Kathiresan et al, 2007). GWAS is a powerful study design and analysis tool that examines these lipid profiles in-depth by mapping all genetic loci associated with these phenotypes, (Kathiresan et al, 2007), (Bush, 2019), (García-Giustiniani et al, 2016), (Pirruccello et al, 2010). GWAS was conducted using 1.65 million study participants and captured genetic variation using single nucleotide polymorphisms (SNPs), which were statistically analysed by identifying causal variants, (Bush, 2019), (Leung et al, 2019). GWAS has identified 1,750 variants at 923 loci, associated with altered HDL, LDL, and TG levels and explains the role of genetic variation and heritability, (Graham et al, 2021), (Vries et al, 2019). GWAS has established that a considerable overlap of these loci are associated with both increased lipid levels and coronary artery disease (CAD), and are heritable, (Zimoń et al, 2021).

Environmental factors such as lifestyle factors, and exposure to pollutants also play a significant role in influencing lipid profiles, (Patel et al, 2012). Numerous studies have well-documented the correlation between lifestyle choices such as exercise, diet, smoking, and alcohol consumption and changes in lipid levels, (Kris-Etherton et al, 1988), (Schaefer, 2002), (Varady et al, 2005), (Patel et al, 2012). Cross-sectional studies have evidenced that regular physical exercise, a healthy diet, and non-smoking and alcohol consumption were strongly associated with a more balanced and healthy lipid profile, (Zhao et al, 2020). Previous research has also evidenced an adverse association between dyslipidemia, cardiovascular disorders, type 2 diabetes, and metabolic syndrome as a result of specific pollutants, such as dioxins, organochlorinated pesticides, polychlorinated biphenyls (PCBs), (Goncharov et al, 2008), (Lee et al, 2007), (Lee et al, 2010). In addition to this, air pollution is tangibly suspected to influence lipid levels, due to its association with cardiovascular disease, (Brook et al, 2010), (Patel et al, 2012).

Analysis of lipid profiles using GWAS, along with in-depth research at both molecular and cellular levels has led to profound implications for understanding the diseases related to TG, LDL, and HDL, (Wu et al, 2014). It supports the development of new therapies to treat such disorders and identifies populations most at risk of these disorders, (Wu et al, 2014), (Bush et al, 2012), (Viecili et al, 2017). Therapies and treatments designed to lower unhealthy LDL and treat cardiovascular disorders include a powerful class of medications such as statins, (Cannon et al, 2004). Other drugs assigned for this treatment include niacin and fibrates which raise HDL and ezetimibe which lowers LDL, often used in combination with each other to lower cholesterol absorption in the liver, (Scirica et al, 2005).

Despite such advancement, cholesterol-lowering therapies are still widely underused due to fear of side effects, cost, and the reluctance of patients to take medication, (Scirica et al, 2005).

Nonpharmacological approaches include increased exercise, weight loss, limited alcohol intake, and a balanced and healthy diet to improve cholesterol levels by increasing HDL and reducing TG levels, (Lichtenstein, 2003), (Silverman et al, 2016). However, the effectiveness of this approach varies significantly between patients, and it's increasingly difficult to raise healthy HDL, compared to lowering LDL and TG without pharmacological interventions, (Lichtenstein, 2003). While there are agreed benefits to using both nonpharmaceutical and drug therapies to maintain healthy lipid levels, it's still undecided as to what the best approach to do so is, (Silverman et al, 2016), (Grundy et al, 2004), (Stone et al, 2014), (Lloyd-Jones et al, 2016). This is because there is still limited knowledge on the effectiveness of non-statin approaches such as exercise and diet as they are less definitive in comparison, (Hegele et al, 2015).

Whilst hundreds of associated loci were identified through GWAS and numerous observational studies have investigated the effects of nutrition and environmental exposure, the association between genetic variation, lifestyle factors, environmental factors, diseases, and lipid abnormalities has yet to be fully understood, (Hu et al, 2020), (Muga et al, 2019), (Kim et al, 2022). This is because the correlative effects of these factors on lipids are not clearly evidenced in existing literature, due to limited study designs and analytical strategies utilised within this research area, (Muga et al, 2019). Furthermore, observational research has conflicting results, due to unobserved factors known as confounding variables influencing the association between exposures and their outcomes, reverse causality, and survival bias, (Davey Smith et al, 2014), (Daghlas et al, 2021). However, recent advancements in Mendelian randomisation (MR), an analytical method that utilises large-scale GWAS has overcome these limitations and investigated the causal relationships between risk factors and disease, health, and lifestyle outcomes, (Lawlor et al, 2008), (Smith et al, 2003). MR uses multiple genetic variants in the form of SNPs as instrumental variables (IVs) to estimate causal effects between an exposure and outcome, (Davey Smith et al, 2003).

The overall objective of this study was to investigate causal relationships between lipid profiles (LDL, HDL, and TG) and lifestyle factors, medications, and diseases. These relationships will be investigated through the application of MR analysis to GWAS from the UK Biobank, (Richmond et al, 2022). The findings of this study could allow us to further understand diseases associated with lipids at a more practical level and examine the effectiveness of current cholesterol-lowering interventions to decrease lipid-associated disorders and improve global health, (Rosenthal et al, 2000).

2.0 METHODS:

2.1 Mendelian Randomisation:

I examined the causal relationships between modifiable risk factors of lipids and factors such as disease, biomarkers, medication, and lifestyle factors, (Richmond et al, 2022), (Davies et al, 2018). To do this I used MR analysis which uses IVs such as genetic variants, to detect the causal relationships between the risk factor and the outcome, (Richmond et al, 2022), (Davies et al, 2018), (Evans et al, 2015), (Daghlas et al, 2021). This analytical method uses SNPs to overcome confounding variables that may affect the relationship between both the risk factor and outcomes, (Davies et al, 2018), (Lawlor et al, 2008). However certain assumptions and criteria must be met to validate the genetic variants, (Scosyrev et al, 2013). These assumptions are that (i) the genetic variant must be associated with the exposure; (ii) the genetic variant is independent of any confounding variables as they affect the casual relationship seen; (iii) the variant affects the outcome only through the risk factors, (Lee, 2020), (Boef et al, 2015), (Scosyrev et al, 2013). If unsatisfied, these assumptions lead to biased and highly misleading causal inferences, (Bound et al, 1993).

In this study, I specifically applied two-sample MR analysis to investigate causal relationships of lipid profiles, (Burgess et al, 2015). As reviewed by the current MR framework, this two-sample method uses summary statistics from GWAS, which has the advantage of not requiring access to individual-level genotype and phenotype data, (Gilbody et al, 2022). This method is also less likely to cause inherent bias and has higher sensitivity when evaluating potential mediators, (Relton et al, 2012). This is because it uses multiple genetic variants such as SNPs to explain the risk factor variation as they have higher statistical power compared to a single variant, (Lee, 2020), (Martens et al, 2006), (Burgess et al, 2013). Single variants can only explain very little of the phenotypic variations, and lead to weak instruments, hence the preference for using multiple variants, (Lee, 2020), (Martens et al, 2006), (Burgess et al, 2013).

2.2 EpiGraphDB for MR analysis:

I used EpiGraphDB (https://epigraphdb.org/), a data mining platform that implements a range of MR methods to investigate the causal relationships between exposures and outcomes, (Liu et al, 2021). EpiGraphDB reported numerous MR methods used which included Weighted median, FE IVW, and MR-Egger, (Lee, 2020). To do this, EpiGraphDB integrates UK biobank and genome-wide summary statistics data from the IEU GWAS database required for two-sample MR analysis, (Darrous et al, 2021), (Lee, 2020).

There is a substantial challenge regarding understanding diseases caused by environmental, and genomic factors, and how they all integrate. UK Biobank was a prospective study implemented to access all novel risk factors for a wide range of diseases and conditions, along with their adverse and favourable effects on health outcomes, (Sudlow et al, 2015), (Zhou et al, 2019). It's a large-scale, population-based database used to transform the understanding of diseases and their determinants by studying 502,000 volunteer patients aged 40–69 years over a decade, (Sudlow et al, 2015), (Conroy et al, 2022), (Ollier, 2005). This was a longitudinal study that collected various medical and demographic information, including extensive phenotypic and genotypic measurements of all patients, (Zhou et al, 2019), (Sudlow et al, 2015). Data from physical measurements, sample assays, questionnaires, and genome-wide genotyping were also collected, followed by closely examined follow-ups to note the range of health outcomes, (Sudlow et al, 2015).

Current UK BioBank studies obtain direct LDL, HDL, and TG measurements from blood samples collected at baseline, using biochemical assays known as direct homogenous Beckman assay, (Mora et al, 2019), (Fang et al, 2021). Advantages to the use of this particular assay include not requiring ultracentrifugation or precipitation, (Mora et al, 2019). The lipid profiles are analysed using different techniques, with HDL analysed using the enzyme immune-inhibition method, LDL requiring an enzymatic selective protection method, and TG using an enzymatic method for analysis, (Mora et al, 2019).

I examined the causal relationships of diseases, lifestyle, and other factors with the profiles of each lipid using the MR causal estimate feature within EpiGraphDB, (Liu et al, 2021). To access the data from EpiGraphDB, I used RStudio (https://posit.co/download/rstudio-desktop/), a development environment, along with R 4.3.1+ (https://cran.rstudio.com/) to use the R programming language, (Hackenberger, 2020), (Grömping, 2015).

First, I considered the lipids as exposures and set the exposure traits tab to the lipids 'HDL Cholesterol', 'LDL Direct', and 'Triglycerides' individually. Along with this, I had set all parameters to default values. I selected a p-value threshold of P<10^-5 for a significant causal relationship to allow for the testing of multiple outcomes. A lower p-value is significant as it signifies a stronger

relationship between two variables, (Wu et al, 2022), (Mcleod, 2019), (Nahm, 2017). Therefore, the smaller the p-value, the stronger the evidence that the exposure affects the outcome, (Dahiru, 2008). I selected the 'Data Table' option, to view the results presented according to the exposure trait searched. EpiGraphDB runs a range of different MR methods, using different combinations of SNPs as IVs, and considers the most significant causal relationship from these different combinations applied. The best test conducted, and instruments selected are an essential part of the results that are presented. The results presented each outcome for which there was a significant causal relationship with the lipid as an exposure. The results varied, ranging from lifestyle factors such as diet, to diseases such as cardiovascular disorders, and diabetes, medications used, and other factors such as family history. The results contained essential information required to analyse the causal relationship such as p-value, and b-value which conveyed the direction of the causal relationship.

To process and view these results in RStudio and filter out unnecessary rows, I selected the 'Query' tab and clicked the 'R EpiGraphDB' option listed in the API Call. To access EpiGraphDB I installed its corresponding package 'epigraphdb' using the 'Tools' and 'Install Packages' options and applied the code in RStudio, (Liu et al, 2022), (Becker et al, 1988). This allowed access to a large-scale database containing all disorders, medications, and factors associated with lipids.

UK Biobank had two different measures for each lipid; raw values and inverse rank normalised values. Using the 'dplyr' package, which was installed and ran using the library function, I filtered the results to only retrieve those relationships containing the inverse rank normalised values, (Wickham et al, 2023), (Mcgowan et al, 2020), (Singh et al, 2019). This was because the GWAS results for inverse normalised traits are less prone to outliers, as a result of individuals with extreme lipid values. This data was exported into an Excel format to view in a more organised and clear way using the command function write.xlsx and saved into the directory where the folder labelled 'Exposure' was located. Three Excel datasets were exported for HDL, LDL, and TG showing all disease, medication, lifestyle, Body Mass Index (BMI) and impedance measurements, biomarkers, family history, and other outcomes associated with these lipids.

Next, I considered the lipids as the outcomes and set the outcome traits tab as the lipids 'HDL Cholesterol', 'LDL Direct', and 'Triglycerides' using the same process discussed previously. This was to investigate the causal relationship in the opposite direction and see if any of the exposures had a more significant effect on these lipids. I then exported these datasets into three Excel files into the folder labelled 'Outcome', for a more detailed comparison between the data in the 'Exposure' folder and 'Outcome' folder.

2.3 Exposures selected for more detailed investigation:

I analysed the datasets for LDL, HDL, and TG in more detail to select the exposure and outcome data that evidenced the strongest causal relationship between the lipids and lifestyle, medication, disease, and biomarker factors. I then identified lifestyle factors, medications, diseases, and biomarkers where the EpiGraphDB analysis showed a significant causal relationship as both an exposure and outcome with HDL, LDL, or TG. After this, I selected a moderate number of the strongest causal relationships, based on their significantly low p-values and varying directions expressed from their b-values, (Dahiru, 2008).

2.4 Detailed MR analysis:

For each causal relationship that I investigated in more detail, I applied five complementary methods of two-sample MR analysis, such as MR-Egger, weighted median, inverse variance weighted (IVW),

simple mode, and weighted mode, (Bowden et al, 2016), (Burgess et al, 2015). IVW is the most widely used approach, utilising GWAS summary statistics to infer the existence of the causal effect between exposures and outcomes, (Mounier et al, 2023). MR-Egger regression method uses summary statistics to test for directional pleiotropy where the same SNP associates directly with multiple traits, and estimates for causal effects, (Burgess et al, 2017). Where MR-Egger allows for pleiotropy, IVW assumes no pleiotropy. Median-based methods such as the weighted median test analyses whether the weighted majority of genetic variants are associated with the outcome, (Burgess et al, 2017). It's much more likely to give a valid causal estimate than IVW or MR-Egger, as it's more consistent with true causal effects, (Wootton et al, 2018), (Bowden et al, 2016). The weighted mode estimation assumes that the most common causal effects are consistent with true causal effects, (Hartwig et al, 2017), (Wootton et al, 2018). The simple mode MR estimation clusters the SNPs into groups based on the similarity of the causal estimates, which itself is based on the cluster which has the highest number of SNPs, (Hartwig et al, 2017), (Hwang et al, 2019). If the five MR methods applied (IVW, MR-Egger, weighted median, simple, and weighted mode estimators), which hold different assumptions regarding the IVs, all show a consistent direction of a causal relationship, then this determines a true causal relationship.

I carried out the two-sample MR analysis by installing the package 'TwoSampleMR', which is linked to the IEU GWAS database, and applied it to the Rstudio console to run the MR analysis, (Hemani et al, 2017). The list of outcomes and exposures of choice were imputed into the 'exposure_dat' and 'outcome_dat' functions, which later harmonised this data together and performed an MR analysis, (Hemani et al, 2017). The exposure and outcome were switched around after the initial analysis, to view the causal effects in the opposite direction and determine if the exposure and outcome support the predicted causal relationship in both directions. These graphs showed multiple curves from the different statistical methods estimated within MR. These graphs were downloaded and exported using the 'Export' tab and saved into the 'MR-Analysis' folder.

3.0 RESULTS:

3.1 EpiGraphDB causal relationships identified:

I started by assessing the causal associations between each lipid and diseases, medications, biomarkers, diet, and other factors using EpiGraphDB. Each lipid was first examined as an exposure, and then as an outcome. Next, I identified any factors that could have a causal effect on the lipid as both an exposure and outcome.

3.11 High-Density lipoprotein cholesterol:

When investigating HDL as the exposure variable, I observed significant causal effects between HDL and a range of factors, including 14 diseases, 14 medications, 11 biomarkers, 8 dietary factors, and 15 other factors such as family history, and body measurements (impedance measures, BMI). Among diseases, I discovered that HDL has the strongest causal associations with major coronary heart disease (CHD) events (p-value: 2.59E-06), vascular or heart complications (p-value: 4.25E-21), and ischaemic heart disease (p-value: 6.1E-07). Regarding biomarkers, I found a particularly strong association between HDL and apolipoprotein A (p-value: 2.89E-136), and for dietary modifications, cheese intake (p-value: 7.63E-07), and weekly alcohol consumption (p-value: 4.89E-08) showed the strongest causal effects.

When exploring HDL as the outcome variable, I discovered several causal relationships involving 5 biomarkers, 13 diseases, 10 dietary modifications, and other factors such as 47 body measurements

(impedance measures, BMI, and weight). Among diseases, the most notable causal associations were observed with vascular or heart problems (p-value: 4.72E-10), and rheumatoid arthritis (p-value: 7.6E-10). For dietary modifications, this study revealed that the absence of vitamins, and mineral supplements (p-value: 5.55E-237), and the consumption of skimmed milk (p-value: 5.59E-09) had the strongest causal effects on HDL levels. I also found that the most significant causal effect observed in biomarkers was with sex hormone-binding globulin (p-value: 1.04E-31).

Furthermore, when examining causal relationships in both directions, only vascular or heart problems were consistently observed as the most significant causal effect. As a result, further investigation was conducted to delve deeper into this relationship (Section 3.21).

3.12 Low-Density lipoprotein cholesterol:

When investigating LDL as the exposure, I discovered several causal effects between LDL and a range of factors, including 38 diseases, 24 medications, 10 dietary habits, and several other outcomes involving family and medication history. When examining diseases, this study observed significant associations between LDL and diseases such as major CHD events (p-value: 2.63E-20), ischaemic heart disease (p-value: 3.74E-23), and myocardial infarction (MI) (p-value: 1.59E-12). Regarding medications, the strongest causal effect observed was with cholesterol-lowering medication taken (p-value: 8.96E-54), and no medication taken for cholesterol (p-value: 1.39E-19).

When examining LDL as the outcome variable, I found significant causal effects related to 16 diseases, 7 medications, 2 dietary habits, 3 biomarkers, and 7 other factors including family history and leisure activities. Among diseases, the strongest causal relationships were observed with digestive system diseases (p-value: 1.66E-120), age-diagnosed diabetes (p-value: 2.48E-20), and major CHD (p-value: 2.72E-10). When examining medication, the most notable associations were found with medication for blood pressure (p-value: 9.04E-07) and no cholesterol-lowering medication taken (p-value: 9.05E-06). Regarding dietary habits, the most significant causal association observed was with skimmed milk (p-value: 1.54E-12).

In this study, I identified consistent causal associations between LDL and factors such as major CHD events and no cholesterol-lowering medication taken in both directions. I examined these relationships in further detail (Sections 3.24 and 3.32).

3.13 Triglycerides:

I observed multiple causal associations with TG as the exposure, which included 56 diseases, 28 biomarkers, 54 medications, 10 dietary habits, and 26 other factors such as family history and health measurements (pulse rate measures, health rating, and impedance measures). Among the diseases observed, the strongest causal associations were found with MI (p-value: 1.41E-23), major CHD events (p-value: 2.04E-21), and diabetes (p-value: 5.33E-12). Regarding medications, the strongest causal associations were with cholesterol-lowering medication (p-value: 5.7E-139), and the number of cholesterol-lowering medications taken (p-value: 6.13E-28). Within the biomarkers examined, the most substantial causal relationship was found with mean haemoglobin concentration (p-value: 7.83E-67). Additionally, the strongest association with TG among the dietary habits examined was alcohol intake frequency (p-value: 1.98E-61).

When investigating TG as the outcome, I discovered numerous causal relationships involving 17 biomarkers, 12 diseases, 4 dietary habits, 6 medications, and 60 other factors, including measurements (body measurements, impedance measures, BMI, and overall health ratings). Notably, among the diseases examined, the most significant associations were observed with

diabetes (p-value: 7.97E-13), and rheumatoid arthritis (p-value: 2.56E-11). In terms of dietary habits, the frequency of alcohol intake showed the most prominent causal relationship (p-value: 2.56E-133). When exploring medications, the number of cholesterol-lowering medications taken displayed the most substantial association (p-value: 5.41E-07).

In evaluating the most notable causal relationships observed bidirectionally, this study identified diabetes, MI, and the number of cholesterol-lowering medications taken for treatment as the strongest associations to investigate (Sections 3.22, 3.23, and 3.31).

3.2 Causal relationship between lipids and diseases:

I started examining the specific causal relationships between lipids and diseases selected from the EpiGraphDB analysis using MR analysis. Each lipid was studied as both the exposure and outcome variable. Then, I analysed the nature of these causal associations using the plotted MR graphs and tables that displayed the b-value and p-value across all five MR tests, which were necessary for this assessment.

3.21 Causal relationship between high-density lipoprotein, and vascular or heart problems:

The EpiGraphDB analysis identified causal relationships of HDL with multiple cardiovascular-related outcomes, including chronic ischaemic heart disease, coronary atherosclerosis, major CHD events, and vascular or heart problems. I studied the causal relationship with vascular or heart problems in more detail.

This study found a strong correlation between HDL and vascular or heart complications when using HDL as the exposure (Figure 1). The five different MR tests conducted observed a consistent positive causal relationship between HDL and no vascular or heart complications present, where higher levels of HDL are shown to lower the risk of contracting such complications (Figure 1a). The causal association was strongly supported by the IVW method (b-value: 0.0336, p-value: 3.86E-11) as seen in Table 1a. When investigating this causal relationship in the opposite direction (Figure 1b), the causal effects did not show a consistent direction across the five MR tests (Table 1b).

3.22 Causal relationship between triglycerides, and diabetes:

When examining TG, the EpiGraphDB analysis revealed several diseases, including ischaemic heart disease, atherosclerotic heart disease, and diabetes. This study specifically concentrated on analysing the causal association between TG and diabetes.

I found that across all five MR tests, there was an inconsistent direction of causal effects (Figure 1c). This is because some tests such as IVW observed a positive direction (b-value: 0.0114, p-value: 2.08E-06), whereas some tests such as MR-Egger saw a negative direction (b-value: -0.0089, p-value: 3.01E-05), as seen in table 1c. When investigating this causal relationship in the opposite direction as seen in Figure 1d, the results observed an increased chance of diabetes being causal for high levels of TG. This was supported by the consistent positive direction seen across all five MR tests (Table 1d), including the weighted median test (b-value: 0.5312, p-value: 2.45E-05).

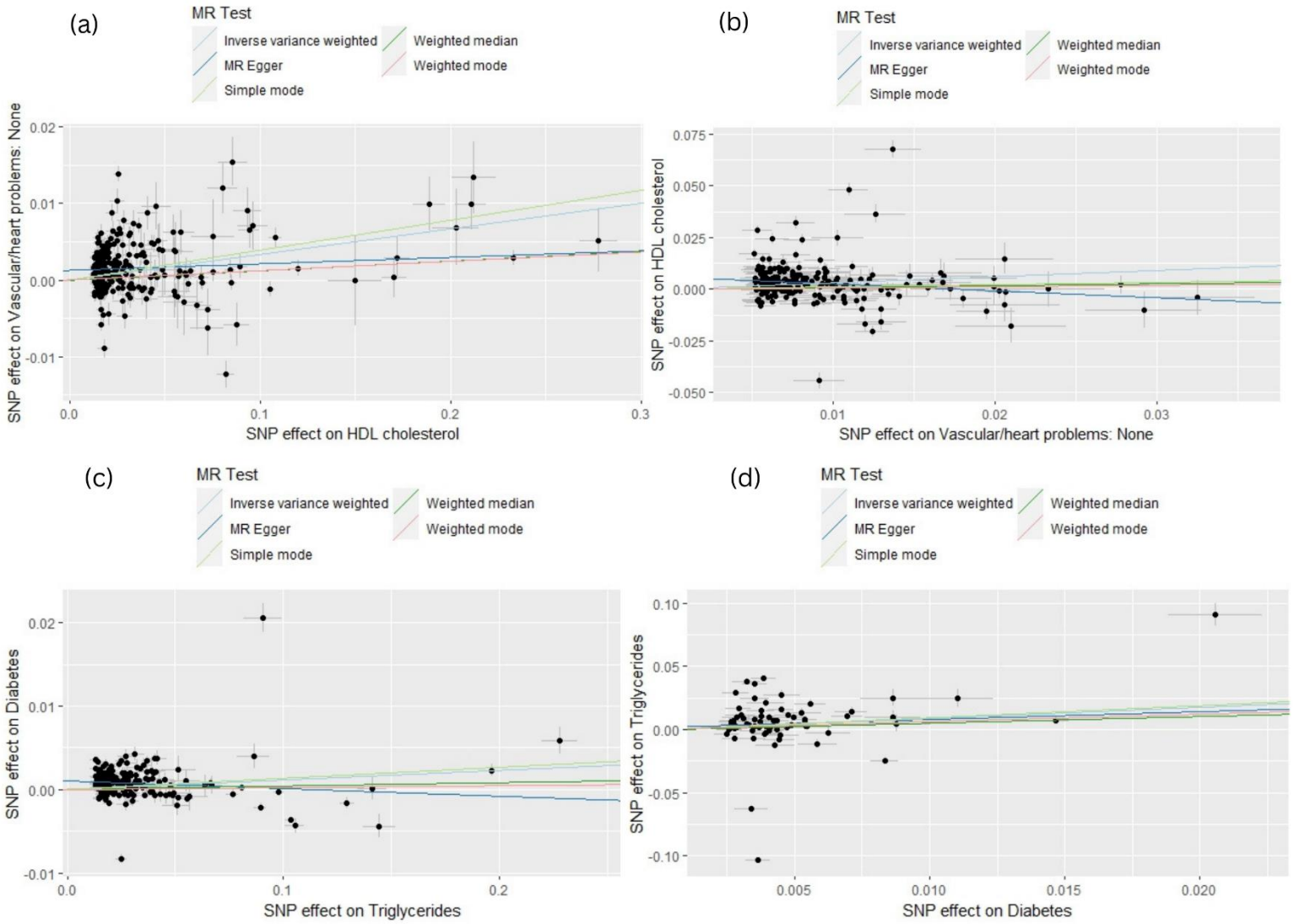

***Figure 1*. Causal relationship between HDL cholesterol, triglycerides, vascular/heart problems, and diabetes.** *The panel shows the Mendelian randomisation analysis used to determine the causal relationship between lipids HDL cholesterol as the (a) exposure and (b) outcome and heart or vascular problems. It also shows the causal relationship of triglycerides as the (a) exposure and (b) outcome for diabetes. Each point corresponds to a SNP, that is associated with the lipid trait, and each SNP is then plotted according to its effect size on the lipid exposure on the x-axis, and on the disease outcome on the y-axis. The grey bars indicate 95% confidence intervals, and the coloured lines represent fitted regressions of the causal relationship between exposure and outcome using the five MR tests.*

(a) HDL-Vascular/Heart problems					
outcome	**exposure**	**method**	**nsnp**	**b**	**pval**
Vascular/heart problems: None	HDL	MR Egger	252	0.0085	2.33E-01
Vascular/heart problems: None	HDL	Weighted median	252	0.0124	1.46E-03
Vascular/heart problems: None	HDL	IVW	252	0.0336	3.86E-11
Vascular/heart problems: None	HDL	Simple mode	252	0.0390	1.22E-03
Vascular/heart problems: None	HDL	Weighted mode	252	0.0121	2.97E-05
(b) Vascular/Heart problems-HDL					
outcome	**exposure**	**method**	**nsnp**	**b**	**pval**
HDL cholesterol	Vascular/heart problems: None	MR Egger	216	-0.3317	7.04E-02
HDL cholesterol	Vascular/heart problems: None	Weighted median	216	0.0843	2.41E-02
HDL cholesterol	Vascular/heart problems: None	IVW	216	0.2969	7.32E-06
HDL cholesterol	Vascular/heart problems: None	Simple mode	216	0.1115	2.65E-01
HDL cholesterol	Vascular/heart problems: None	Weighted mode	216	0.0669	2.69E-01
(c) Triglycerides-Diabetes					
outcome	**exposure**	**method**	**nsnp**	**b**	**pval**
Diabetes	Triglycerides	MR Egger	205	-0.0089	3.01E-05
Diabetes	Triglycerides	Weighted median	205	0.0041	7.60E-02
Diabetes	Triglycerides	IVW	205	0.0114	2.08E-06
Diabetes	Triglycerides	Simple mode	205	0.0132	1.32E-01
Diabetes	Triglycerides	Weighted mode	205	0.0023	2.90E-01
(d) Diabetes-Triglycerides					
outcome	**exposure**	**method**	**nsnp**	**b**	**pval**
Triglycerides	Diabetes	MR Egger	68	0.6259	6.27E-01
Triglycerides	Diabetes	Weighted median	68	0.5312	2.45E-05
Triglycerides	Diabetes	IVW	68	0.8977	1.04E-01
Triglycerides	Diabetes	Simple mode	68	0.9505	6.06E-03
Triglycerides	Diabetes	Weighted mode	68	0.6039	2.61E-04

Table 1. Causal relationship between HDL cholesterol, triglycerides, vascular/heart problems, and diabetes across all five MR tests. *Different MR analysis methods were used to determine the causal relationship between HDL cholesterol and vascular/heart problems, with HDL as the (a) exposure and (b) outcome, and the causal relationship between triglycerides and diabetes with triglycerides as (c) exposure and (d) outcome. The nsnp values show the number of SNPs selected as the instrumental variables, with the b-value and p-value to show the direction and statistical significance of the causal relationships.*

3.23 Causal relationships between triglycerides and myocardial infarction:

The EpiGraphDB analysis revealed multiple causal associations between TG and diseases, as previously mentioned. This study specifically concentrated on the causal association between TG and MI. The findings indicated that the likelihood of contracting MI increases as the level of TG increases (Figure 2a). This was supported by the consistent positive causal relationship observed across all five MR tests (Table 2a). The most significant causal association was detected particularly by the IVW method (b-value: 0.0063, p-value: 1.03E-09). When exploring the causal relationship between TG and MI in the opposite direction, the causal effects did not exhibit a consistent direction across the various MR methods.

3.24 Causal relationships between low-density lipoprotein and major coronary heart disease event:

When investigating LDL, the EpiGraphDB analysis revealed several diseases, including diseases of the circulatory system, and other cardiovascular diseases such as ischaemic heart disease, atherosclerotic heart disease, and major CHD. I specifically studied the causal relationship with major CHD events in more detail.

This study found that as the level of LDL increases, the risk of contracting a major CHD event also increases (Figure 2b). This is strongly supported by all five MR tests, which observed a consistent positive causal relationship across all the tests (Table 2b), such as the IVW test (b-value: 0.0128, p-value: 9.88E-11) and weighted mode test (b-value: 0.0102, p-value: 5.31E-12). This causal relationship in the opposite direction from the EpiGraphDB analysis observed an inconsistent direction across all MR methods. These findings further strengthen the notion that LDL has a more significant causal effect on the risk of contracting major CHD events.

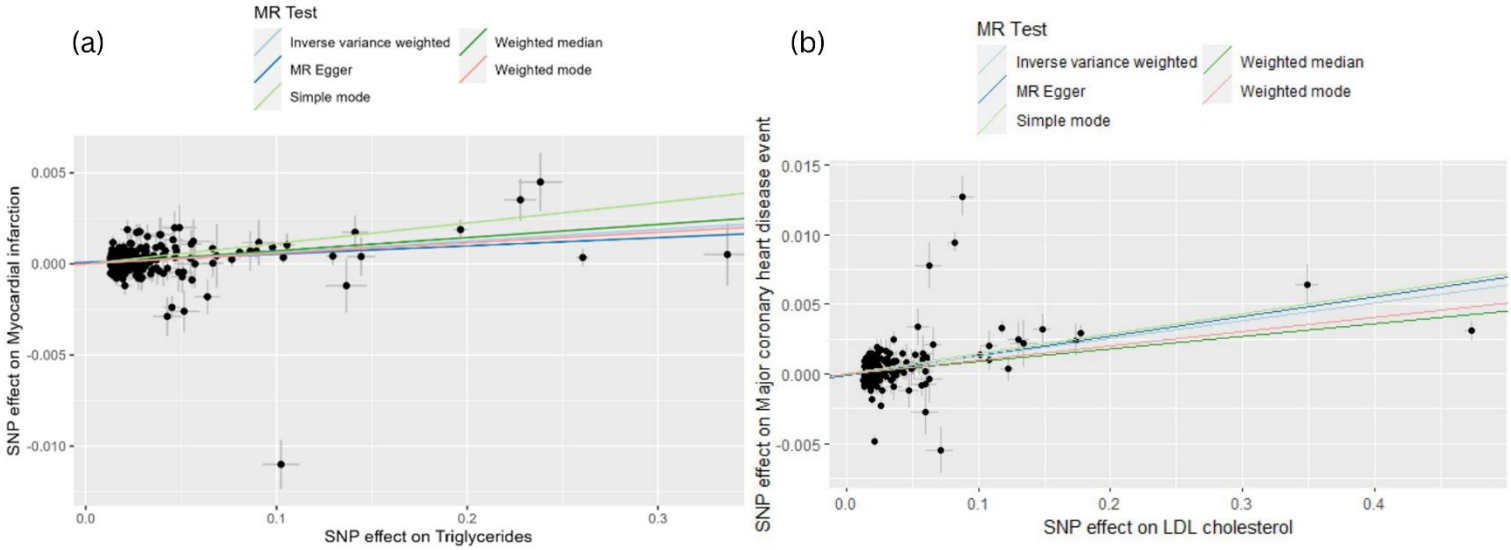

Figure 2. Causal relationship between triglycerides, LDL cholesterol, myocardial infarction, and major coronary heart disease. *The panel shows the Mendelian randomisation analysis used to determine the causal relationship between (a) triglycerides (exposure) and myocardial infarction (outcome), and (b) LDL cholesterol (exposure) and major coronary heart (outcome) disease. Each point corresponds to a SNP, that is associated with the lipid trait, and each SNP is then plotted according to its effect size on the lipid exposure on the x-axis, and on the disease outcome on the y-axis. The grey bars indicate 95% confidence intervals, and the coloured lines represent fitted regressions of the causal relationship between exposure and outcome using the five MR tests.*

(a) Triglycerides - Myocardial infarction (MI)					
outcome	**exposure**	**method**	**nsnp**	**b**	**pval**
Myocardial infarction	Triglycerides	MR Egger	234	0.0045	3.07E-03
Myocardial infarction	Triglycerides	Weighted median	234	0.0072	2.24E-07
Myocardial infarction	Triglycerides	IVW	234	0.0063	1.03E-09
Myocardial infarction	Triglycerides	Simple mode	234	0.0112	8.63E-04
Myocardial infarction	Triglycerides	Weighted mode	234	0.0058	5.47E-06
(b) LDL- Major coronary heart disease event					
outcome	**exposure**	**method**	**nsnp**	**b**	**pval**
Major coronary heart disease event	LDL	MR Egger	160	0.0141	3.08E-07
Major coronary heart disease event	LDL	Weighted median	160	0.0091	2.34E-09
Major coronary heart disease event	LDL	IVW	160	0.0128	9.88E-11
Major coronary heart disease event	LDL	Simple mode	160	0.0144	1.33E-05
Major coronary heart disease event	LDL	Weighted mode	160	0.0102	5.31E-12

Table 2. Causal relationship between triglycerides, LDL cholesterol, myocardial infarction, and major coronary heart disease event across all five MR tests. *Different MR analysis methods were used to determine the causal relationship between (a) triglycerides and risk of myocardial infarction and (b) LDL cholesterol and risk of major coronary heart disease events, with the lipids as the exposure, and diseases as the outcome. The nsnp values show the number of SNPs selected as the instrumental variables, and the b-value and p-value show the direction and statistical significance of the causal relationships.*

3.3 The causal relationship between lipids and medication:

I assessed the causal relationships between the lipids and medications similarly selected from EpiGraphDB as previously discussed. As before, each lipid selected was analysed as both the exposure and outcome variable, before investigating the causal effects of each lipid across all five MR tests.

3.31 Causal relationships between triglycerides and medication:

EpiGraphDB analysis revealed several associations between TG and medication such as cholesterol-lowering medication, and medications including simvastatin. I examined the causal relationship between TG and medication, to determine whether medication and imbalanced levels of TG had a strong association. This study specifically examined the causal relationship between TG and the number of medications taken in more detail.

The findings revealed that as the level of TG increased, the number of medications taken for treatment increased (Figure 3a). This was strongly supported by the consistent positive relationship observed across all five MR tests conducted, with the IVW MR test showing the strongest association (b-value: 0.0653, p-value: 2.37E-19) as seen in Table 3a. The EpiGraphDB analysis showed that there was no consistent causal relationship in the opposite direction, unlike what was seen in Figure 3a.

3.32 Causal relationships between low-density lipoprotein and medication:

EpiGraphDB analysis discovered several causal associations between LDL and medication, such as cholesterol-lowering medications, atorvastatin medication, and blood pressure medication. This study particularly investigated the causal association with no medication taken in greater detail to investigate whether LDL and medication have a strong correlation.

The analysis indicated that as the level of LDL increases the likelihood of not requiring medication to be taken for cholesterol is lower (Figure 3b). This was supported by the five different MR tests

conducted which observed a consistent negative causal relationship between LDL and no cholesterol-lowering medication taken (Table 3b). The most significant causal association was detected using the tests IVW (b-value: - 0.0662, p-value: 4.44E-14), and weighted median (b-value: -0.08, p-value: 1.69E-26). The EpiGraphDB results strongly showed that there was an inconsistent causal association between LDL and no cholesterol-lowering medication in the opposite direction, with LDL as the outcome.

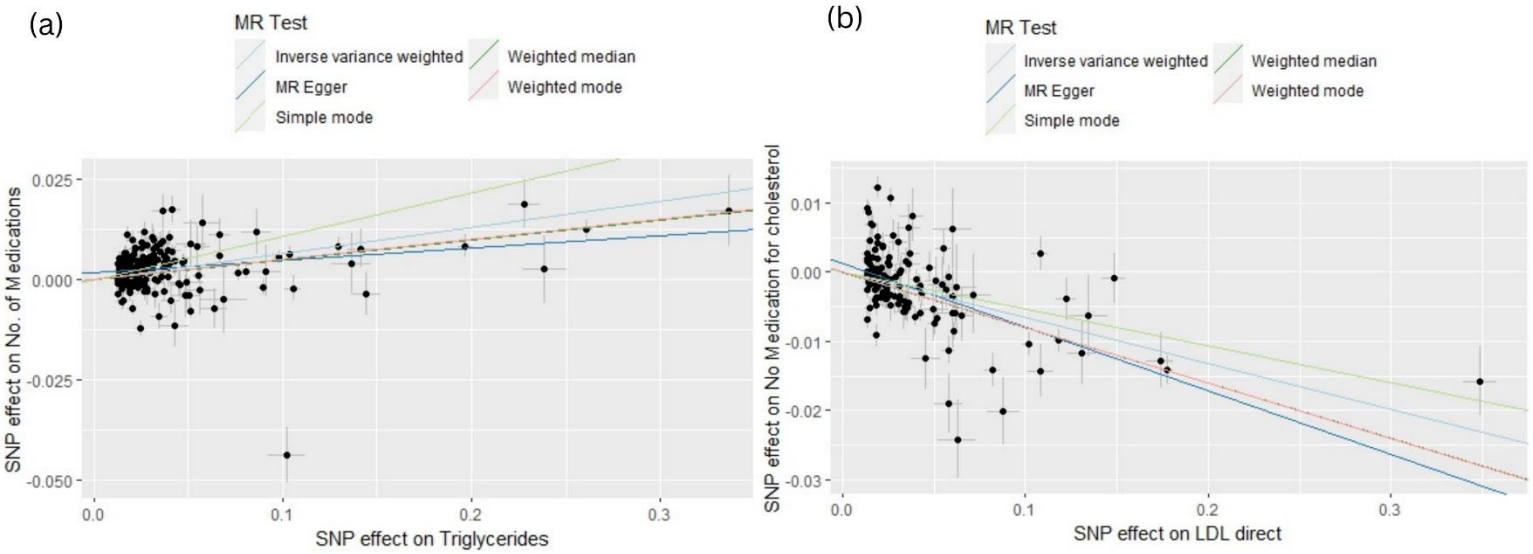

Figure 3. Causal relationship between triglycerides, LDL cholesterol, number of medications, and use of cholesterol-lowering medication. *The panel shows the Mendelian randomisation analysis used to determine the causal relationship between (a) triglycerides (exposure) and the number of cholesterol-lowering medications taken (outcome), and (b) LDL cholesterol (exposure) and no cholesterol-lowering medication taken (outcome). Each point corresponds to a SNP, that is associated with the lipid trait, and each SNP is then plotted according to its effect size on the lipid exposure on the x-axis, and on the disease outcome on the y-axis. The grey bars indicate 95% confidence intervals, and the coloured lines represent fitted regressions of the causal relationship between exposure and outcome using the five MR tests.*

(a) Triglycerides - Number of Medications					
outcome	**exposure**	**method**	**nsnp**	**b**	**pval**
No. of Medications	Triglycerides	MR Egger	211	0.0306	2.69E-03
No. of Medications	Triglycerides	Weighted median	211	0.0496	7.34E-13
No. of Medications	Triglycerides	IVW	211	0.0653	2.37E-19
No. of Medications	Triglycerides	Simple mode	211	0.1087	2.48E-05
No. of Medications	Triglycerides	Weighted mode	211	0.0499	4.6E-16
(b) LDL - No Medication taken for cholesterol					
outcome	**exposure**	**method**	**nsnp**	**b**	**pval**
No Medication for cholesterol	LDL direct	MR Egger	145	-0.0920	2.19E-10
No Medication for cholesterol	LDL direct	Weighted median	145	-0.0800	1.69E-26
No Medication for cholesterol	LDL direct	IVW	145	-0.0662	4.44E-14
No Medication for cholesterol	LDL direct	Simple mode	145	-0.0534	1.10E-02
No Medication for cholesterol	LDL direct	Weighted mode	145	-0.0804	8.88E-19

Table 3. Causal relationship between triglycerides, LDL cholesterol, number of medications, and use of cholesterol-lowering medication across all five MR tests. *Different MR analysis methods were used to determine the causal relationship between (a) triglycerides and number of medications taken and (b) LDL cholesterol and cholesterol-lowering medication, with lipids as the exposure, and medication as the outcome. The nsnp values show the number of SNPs selected as the instrumental variables, and the b-value and p-value show the direction and statistical significance of the causal relationships.*

3.4 The causal relationship between low-density lipoprotein and diet:

EpiGraphDB analysis uncovered numerous causal associations between LDL and diet such as oily fish, cheese, and salt intake, frequent alcohol consumption, and skimmed milk intake. Multiple MR tests were conducted in EpiGraphDB to investigate the causal relationships between LDL and dietary factors and determine whether there was a strong causal correlation. The specific relationship between LDL and skimmed milk, which is a healthier choice of milk containing lower percentages of fat, was studied in more detail to determine if this healthier choice can have a prominent effect on LDL levels.

When LDL was investigated as the exposure, this study found that higher levels of LDL are associated with increased skimmed milk consumption, (Figure 4a). This was supported strongly across all five MR tests, which observed a consistent positive causal relationship (Table 4a), with the IVW test particularly supporting this true causal relationship (b-value: 0.0213, p-value: 2.32E-16).

Contrastingly, when examining LDL as the outcome, the causal relationship observed shows that increased consumption of skimmed milk has the effect of lowering LDL levels, (Figure 4b). This consistent negative slope was seen across all five MR tests (Table 4b), with the IVW test strongly supporting this association (b-value: -1.3148, p-value: 2.42E-12).

When comparing these findings in both exposure and outcome analysis, there was an overall inconsistency in these two causal relationships. As LDL levels increased, there was an observed increase in skimmed milk consumption. However, when skimmed milk consumption increased, there was a decrease in LDL levels.

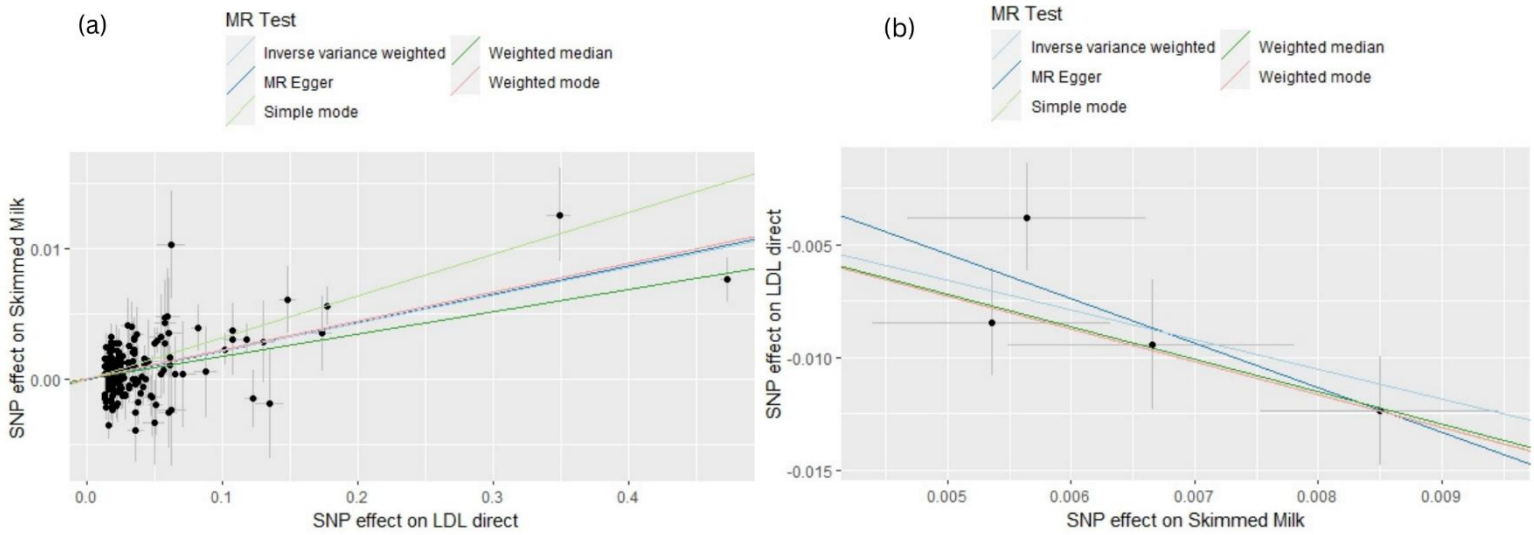

Figure 4*. *Causal relationship between LDL cholesterol and skimmed milk. *The panel shows the Mendelian randomisation analysis used to determine the causal relationship between LDL cholesterol as (a) exposure and (b) outcome for skimmed milk, which is a healthy dietary modification. Each point corresponds to an SNP, that is associated with the lipid trait, and each SNP is then plotted according to its effect size on the LDL exposure on the x-axis, and on the skimmed milk as the outcome on the y-axis, and vice-versa. The grey bars indicate 95% confidence intervals, and the coloured lines represent fitted regressions of the causal relationship between exposure and outcome using the five MR tests.*

(a) LDL - Skimmed Milk					
outcome	**exposure**	**method**	**nsnp**	**b**	**pval**
Skimmed Milk	LDL direct	MR Egger	160	0.0218	2.99E-09
Skimmed Milk	LDL direct	Weighted median	160	0.0171	1.3E-06
Skimmed Milk	LDL direct	IVW	160	0.0213	2.32E-16
Skimmed Milk	LDL direct	Simple mode	160	0.0320	2.64E-04
Skimmed Milk	LDL direct	Weighted mode	160	0.0221	1.49E-12
(b) Skimmed Milk - LDL					
outcome	**exposure**	**method**	**nsnp**	**b**	**pval**
LDL direct	Skimmed Milk	MR Egger	4	-1.9807	2.09E-01
LDL direct	Skimmed Milk	Weighted median	4	-1.4384	6.56E-09
LDL direct	Skimmed Milk	IVW	4	-1.3148	2.42E-12
LDL direct	Skimmed Milk	Simple mode	4	-1.459	2.59E-02
LDL direct	Skimmed Milk	Weighted mode	4	-1.4563	2.12E-02

Table 4 Causal relationship between LDL cholesterol, and skimmed milk across all five MR tests. *Different MR analysis methods were used to determine the causal relationship between LDL cholesterol and Skimmed milk, with LDL cholesterol as the (a) exposure and (b) outcome. The nsnp values show the number of SNPs selected as the instrumental variables, and the b-value and p-value show the direction and statistical significance of the causal relationships.*

4.0 DISCUSSION:

Dyslipidemia is often classed as imbalanced levels of LDL, HDL, and TG, and can result in metabolic disorders including Gaucher's disease and Tay-Sachs disease, and is often associated with cardiovascular disorders such as ischaemic heart disease, (Natesan et al, 2021), (Moini et al, 2020). Dyslipidemia has a genetic and environmental component, influencing its prevalence, (Cole et al, 2015). In this study, I investigated the causal relationship between lipids and factors such as medication, diseases, and diet using two-sample MR analysis.

The findings of this research indicated a strong correlation between the abnormally high levels of LDL, HDL, and TG, and various diseases, medications, and dietary factors. Specifically, high levels of LDL have been observed to be associated with diseases such as major CHD. MR analysis revealed that higher levels of LDL are associated with an increased risk of contracting a major CHD event. This study has highlighted that elevated LDL levels beyond the healthy concentration range serve as a significant risk factor for CHD events. This is due to LDL's role in transporting cholesterol to the arteries, which in excess can result in the accumulation of cholesterol and lead to heart disorders such as CHD, (Mykkänen et al, 1999), (Castelli et al, 1992), (Pinal-Fernandez et al, 2018), (Borén et al., 2020). Smaller LDL particles can also undergo oxidation, which promotes the activation of endothelial cell inflammation within the blood cells, (Navab et al, 2004), (Poznyak et al, 2021). This contributes heavily to plaque formation, which, when ruptured can obstruct the blood vessels, and progress into CHD, (Navab et al, 2004), (Liang et al, 2020). The findings of this study strongly align with an existing study conducted by Burgess et al, which investigated the causal effects of blood lipids on CHD, and observed strong causal effects of increased LDL levels on CHD risk, (Burgess et al, 2016). While my findings are consistent with the results seen in Burgess et al, and utilised a similar range of MR tests, my study used more MR tests (MR-Egger, weighted median, inverse variance weighted (IVW), simple mode, and weighted mode), in addition to the few tests such as MR-Egger, standard MR, and weighted median that was used in the comparative study. The study of Burgess et al focused primarily on lipid profiles as the exposure, whereas my analysis centered on investigating the causal relationship between LDL and CHD events and determining the direction of this causal relationship.

EpiGraphDB analysis results noted a significant correlation between TG and disorders such as MI. When further investigated, I found that as the level of TG increases, the risk of experiencing an MI (heart attack) also rises. High levels of TG are strongly associated with cardiovascular-related disorders and significantly elevate the risk of heart attack or stroke, (Liang et al, 2022). This is because TG is transported in the plasma by specific triglyceride-rich lipoproteins, which deposit into the coronary arteries in a similar manner as LDL, (Do et al, 2013), (Meade-Kelly, 2013). This process results in the development of atherosclerosis, where the arterial walls harden and block blood-flow through the arteries, which elevates the risk of heart attacks, (Do et al, 2013), (Meade-Kelly, 2013), (Dron et al, 2020), (Zhang et al, 2022). A study conducted by Jørgensen et al yielded consistent findings with my research, applying a similar MR analysis approach to investigate elevated TG levels as a causal risk factor of MI, and observed that elevated levels of TG increased the risk of MI, (Jørgensen et al, 2013). Although my findings align with the results seen in the comparative study, it is important to note that my study utilised summary statistics from GWAS and the UK BioBank, which provided a substantially larger sample size compared to the smaller sample size of 5,705 MI cases and 54,408 control cases utilised in Jørgensen et al, (Jørgensen et al., 2013).

A comprehensive EpiGraphDB analysis revealed an interesting association between TG and diabetes. However, upon more detailed examination, this study found that when analysed across the five different MR tests, there was an inconsistent direction of the association. It was observed that diabetes had a significant causal effect on increased levels of TG. Notably, when TG was analysed as the outcome, the results saw that individuals with diabetes were more likely to have higher levels of TG. This could be attributed to confounding factors such as obesity, irregular physical activity, and unhealthy dietary habits which are consistently associated with diabetes, and elevated TG levels, often in conjunction with each other, (Kyrou et al, 2020), (Philippou et al, 2012), (Zou et al, 2020), (Neuenschwander et al, 2019), (Neuhouser et al, 2002). Obesity, often measured by an increased BMI of above 30.0, is associated with a higher risk of contracting diabetes, (Gray et al, 2015), (Kivimäki et al, 2022), (Gupta et al, 2021), (Aberra et al, 2020). Furthermore, overweight, and obese individuals tend to have an abnormally high level of TG, with research strongly indicating that obesity is linked to both elevated TG levels and diabetes, (Klop et al, 2013), (Al-Goblan et al, 2014). Lifestyle factors such as regular exercise are associated with preventing obesity, lowering TG levels by targeting the apoC3 levels associated with hypertriglyceridemia, and improving insulin resistance in diabetes, (Wang et al, 2019), (Colberg, et al 2016). Unhealthy diet has strong associations with increased levels of TG and diabetes, where a higher intake of saturated fat, carbohydrates, and red meat increases the levels of TG and contributes to the risk of insulin resistance in diabetes, (Packard et al, 2020), (Sami et al, 2017), (Panagiotakos et al, 2005).

I found that my results strongly align with the existing literature by De Silva et al, which performed an MR analysis using 10 common genetic variants for TG levels against type 2 diabetes, with 5,637 cases and 6,860 control subjects being assessed, (De Silva et al, 2011). De Silva et al.'s results were consistent with my findings, where there was no conclusive causal association between TG as the exposure and diabetes as the outcome. The methodology of this comparative study seemed stronger and focused specifically on type 2 diabetes, as they gathered genetic variants directly from the cases and control groups investigated. In comparison, I had only utilised summary data from GWAS and focused on diabetes as a broad disease subject, (Chen et al, 2021), (Beck et al, 2020). Despite these differences, my findings reported that there was a consistent causal relationship between diabetes as the exposure and TG as the outcome, which was not reported by findings in the research of De Silva et al.

This study also discovered a strong association between HDL (healthy cholesterol), and vascular or heart problems. Further MR analysis revealed that increasing levels of HDL were associated with a lower risk of vascular or heart problems. Elevated levels of HDL lead to decreased progression of atherosclerotic plaques, which, if allowed to progress, can eventually rupture, and obstruct the arterial walls in the heart, (Fieg et al, 2016), (Costopoulos et al, 2017), (Shah, 1997). HDL prevents this progression and helps to remove fat, within the artery walls, thus reducing plaque formation and lowering the risk of heart complications, (Kosmas et al, 2018). The presence of HDL prevents the displacement of endothelial nitric oxide synthase (eNOS) from caveolae and the impairment of nitric oxide (NO) production, which oxidised LDL causes, (Feig et al, 2016), (Kawashima et al, 2014). The presence of HDL also maintains the proper functioning of endothelial nitric oxide synthase (eNOS) in the caveolae, and prevents the disruption of nitric oxide (NO) production caused by oxidised LDL, (Feig et al, 2016), (Kawashima et al, 2014). This is crucial as dysregulated levels of NO caused by eNOS, are highly associated with accelerating atherosclerosis, and causing vascular or heart problems, (Feig et al, 2016), (Uittenbogaard et al, 2000), (Stary et al, 1995), (Kawashima et al, 2014).

In a thorough examination of the EpiGraphDB results, a strong correlation was observed between LDL and TG levels and administration of medication. Notably, when examining the MR analysis of LDL, I found a significant causal effect between LDL levels and the need for taking cholesterol-lowering medication. As the levels of LDL increased, the likelihood of not requiring cholesterol-lowering medication decreased substantially. This is because imbalanced lipid levels, specifically elevated LDL, have been extensively associated with a negative impact on cholesterol and overall health, as discussed previously, (Pinal-Fernandez et al, 2018). To address such concerns, cholesterol-lowering medications such as statins, are commonly prescribed as the primary treatment options, (Pinal-Fernandez et al, 2018). These medications decrease LDL levels predominantly by hindering the activity of HMG-CoA reductase, which upregulates LDL receptor expression, and accelerates the clearance of LDL, (Silverman et al, 2016), (Feingold, 2021).

Furthermore, the relationship between TG and medication usage was explored extensively, and a strong causal association was discovered. As the levels of TG increased, the number of medications taken also increased. Several types of medications are required for treating high levels of TG which include statins, and non-statins medications such as niacin, fibrates, and omega-3-fatty acids, (Ginsberg, 1998), (Feingold, 2021). High levels of TG are often carried by very-low-density lipoproteins (VLDLs), which can accumulate in the arteries and lead to health complications such as atherosclerosis, heart disease, and stroke, (Packard et al, 2020), (Ginsberg, 1998). Medications such as statins can clear this accumulation of TG, by promoting the removal of VLDLs through the LDL-receptor pathway, (Ginsberg, 1998). Statins can decrease VLDL levels, by as much as 35-45%, to reduce the level of TG entering the arteries, (Ginsberg, 1998). Niacin, for example, has been long used to treat lipid disorders and is capable of lowering TG levels by 20-50%, (McKenney, 2004), (Kamanna et al, 2008). Niacin does this by directly inhibiting hepatocyte diacylglycerol acyltransferase-2, an important enzyme in TG synthesis, (Kamanna et al, 2008). This process accelerates the intracellular degradation of apolipoprotein B, which contains the lipoproteins VLDL and LDL, to inhibit the transport of TG which can accumulate within the arteries, (Kamanna et al, 2008), (Djadjo et al, 2023). Fibrates such as fenofibrate, bezafibrate, and gemfibrozil, are highly effective first-line medications that efficiently reduce elevated levels of TG, achieving reductions of up to 30-50%, (Young et al, 2007). Fibrates exert their TG-lowering effects by inhibiting the hepatic extraction of free fatty acids, thereby reducing TG production, and enhancing their breakdown, (Young et al, 2007), (Kester et al, 2012), (Sharma et al, 2010), (Docherty et al, 2014). Omega-3 fatty acids (FAs), such as docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids are known to lower elevated levels of plasma TG by over 30%, (Skulas-Ray et al, 2019). They do this by increasing fatty

acid oxidation, which in turn suppresses hepatic lipogenesis and VLDL production, (Khan et al, 2021), (Bornfeldt, 2021), (Shearer et al, 2012), (Oscarsson et al, 2017). Because these different drugs effectively target various pathways to lower TG, an increased number of these statin and non-statin medications combined could be a beneficial way of more efficiently lowering TG levels, (Khaldoon Alaswad et al, 1999), (O'hare, 2020), (Young et al, 2007).

This study found that dietary modifications, such as incorporating healthy skimmed milk can have a significant impact on the level of LDL. When observing LDL as the exposure variable, the findings evidenced a positive correlation between increased skimmed milk consumption, and individuals with elevated LDL levels, where LDL is associated with higher skimmed milk intake. This is because research has observed that elevated levels of saturated fat have been shown to increase LDL levels, (Siri-Tarino et al, 2010). This is found to occur through the inhibition of hepatic LDL receptor activity, and the increased production of apoB-containing lipoprotein, which reduces the clearance of LDL particles, (Dietschy, 1988), (Siri-Tarino et al, 2010), (Chiu et al, 2017). Additionally, several studies noted that a higher intake of saturated fats increases the susceptibility of LDL particles to aggregate, leading to oxidative modifications and inflammation in the arteries, which can contribute to conditions like atherosclerosis, (Ruuth et al, 2021), (Ala-Korpela, 2000), (Öörni et al, 2015), (Haka et al, 2009). As a result, patients with high LDL levels are making healthier dietary choices, by choosing higher nutritional and lower saturated fat-containing options such as skimmed milk, (Oh et al, 2015), (DiNicolantonio et al, 2018). In contrast, when examining LDL as the outcome variable, a negative causal association was observed where an increased intake of skimmed milk leads to a decrease in LDL levels. This is likely due to the lower fat concentration in skimmed milk which could lower LDL levels, (Fried et al, 2012). However, additional research will be required to understand the complex causal relationship between LDL and skimmed milk intake.

This study utilised GWAS summary statistics from the UK Biobank, which is a large, prospective cohort study consisting of 500,000 participants aged 40-69 years, (Conroy et al, 2019). The summary data used within this study to determine the causal associations of lipids is of high quality. This is because GWAS data applied extensive collection methods including biological samples of blood, saliva, and urine, and physical measurements, such as blood pressure, spirometry, and anthropometry were employed, (Conroy et al, 2019). All of this reinforces the broad quality of the associations detected between common genetic variants and disease traits and therefore enhances the power to detect causal relationships, (Beck et al, 2020), (Uffelmann et al, 2021). This research demonstrates a notable strength in its implementation of the two-sample MR analysis. By integrating summary data from GWAS, this approach allows for a detailed assessment of all associations between risk factors and outcomes using multiple genetic markers, (Deng et al, 2020). The use of five MR tests further enhances the robustness of the findings, as they support the assessment of a true causal relationship if the direction of the causal relationships is consistent across all the MR tests employed. This establishes a reliable and accurate interpretation of the true causal relationships observed in this study, (Zhang et al, 2022).

While this study provides a detailed analysis of causal relationships between lipids and factors such as diseases, medications, and diet, it's important to consider some study design limitations. The use of UK Biobank and GWAS summary statistics, although providing broad and high-quality genetics data, with a large sample size of 500,000 participants, had 94.6% and 96% of participants identifying as being of white ethnicity, (Fry et al, 2017), (Niedzwiedz et al, 2020), (Conroy et al, 2019), (Popejoy et al, 2016). This lack of data from non-European populations considerably limits the applicability of GWAS and UK Biobank findings to certain ethnic groups, (Peterson et al, 2019). It also poses a significant barrier to the widespread use of GWAS in future clinical applications to all individuals,

irrespective of their ethnicity, (Haga, 2009), (Ioannidis, 2009). Another limitation to consider is the critical assumption of MR independence, which states that the IVs are solely associated with the risk factors and not with any confounding variables, (Davies et al, 2018), (Lee, 2020). However, this assumption may not always align with the complex nature of the causal relationships between lipids and diseases, as lipids can be influenced by various factors including genetics and environmental changes such as lifestyle and diet, (Muga et al, 2019). Consequently, it becomes challenging to disentangle the effects of these confounding variables, as they may impact the causal associations observed in this study.

Future works include integrating and validating the findings of this study into other larger-scale biobanks such as the Estonian Biobank, FinnGen, and Million Veterans Program, (Leitsalu et al, 2015), (Kurki et al, 2023), (Levey et al, 2021), (Hofmeister et al, 2022). This will further support future healthcare and research strategies involving lipid-related disorders, medications, and lifestyle factors, to improve global health, (Coppola et al, 2019). Because this study uses GWAS summary data collected primarily from European populations, these findings do not represent other ethnic populations, (Vassos et al, 2019). Therefore, it will be essential to validate the findings of this research in other ethnic groups, to further investigate any changes observed in the causal associations between lipid profiles and various factors including diseases, medications, and lifestyle modifications.

5.0 CONCLUSION:

This study's detailed findings shed light on the crucial necessity of managing lipids and the importance of cholesterol-lowering medications for addressing abnormal levels of LDL and TG, as well as dietary modifications to promote overall health and well-being. This research also underlines the importance of maintaining optimal levels of LDL, HDL, and TG to counteract the risk of developing disorders such as MI, diabetes, and heart or vascular complications. This study emphasises the need for applying appropriate management and treatment of lipid levels through lifestyle modifications, medications, and dietary interventions. Overall, these findings have important implications for public health, future treatment therapies, and research.

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