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Remnant cholesterol as a cause of ischemic heart disease: Evidence, definition, measurement, atherogenicity, high risk patients, and present and future treatment

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ABSTRACT

This review focuses on remnant cholesterol as a causal risk factor for ischemic heart disease (IHD), on its definition, measurement, atherogenicity, and levels in high risk patient groups; in addition, present and future pharmacological approaches to lowering remnant cholesterol levels are considered.
Observational studies show association between elevated levels of remnant cholesterol and increased risk of cardiovascular disease, even when remnant cholesterol levels are defined, measured, or calculated in different ways. In-vitro and animal studies also support the contention that elevated levels of remnant cholesterol may cause atherosclerosis same way as elevated levels of low-density lipoprotein (LDL) cholesterol, by cholesterol accumulation in the arterial wall. Genetic studies of variants associated with elevated remnant cholesterol levels show that an increment of 1 mmol/L (39 mg/dL) in levels of nonfasting remnant cholesterol associates with a 2.8-fold increased risk of IHD, independently of high-density lipoprotein cholesterol levels. Results from genetic studies also show that elevated levels of remnant cholesterol are causally associated with both low-grade inflammation and IHD. However, elevated levels of LDL cholesterol are associated with IHD, but not with low-grade inflammation. Such results indicate that elevated LDL cholesterol levels cause atherosclerosis without a major inflammatory component, whereas an inflammatory component of atherosclerosis is driven by elevated remnant cholesterol levels.
Post-hoc subgroup analyses of randomized trials using fibrates in individuals with elevated triglyceride levels, elevated remnant cholesterol levels, show a benefit of lowering triglycerides or remnant cholesterol levels; however, large randomized trials with the primary target of lowering remnant cholesterol levels are still missing.

Contents

Abbreviations: HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; IHD, ischemic heart disease; LDL, low-density lipoprotein; LRP, LDL receptor-related protein; VLDL, very-low-density lipoprotein.

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1. Introduction including definition

Ischemic heart disease (IHD) is a leading cause of morbidity and mortality worldwide. An important risk factor for IHD is elevated levels of low-density lipoprotein (LDL) cholesterol, but even after lowering of LDL cholesterol to recommended levels, there is a considerable residual risk of IHD. Some of this residual risk may be explained by elevated remnant cholesterol levels (Chapman et al., 2011).

Lipoproteins transport water-insoluble triglycerides and cholesterol between tissues and organs in the body (Havel & Kane, 2001). They consist of a core of hydrophobic cholesterol esters and triglycerides surrounded by a hydrophilic mono-layer of phospholipids, free cholesterol, and apolipoproteins. The different classes of lipoproteins, i.e. chylomicrons, chylomicron remnants, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, lipoprotein(a) and high-density lipoprotein (HDL), are different in density as a result of the amount of triglycerides and cholesterol they contain, and have different apolipoproteins on their surface. Chylomicrons, chylomicron remnants, VLDL, and IDL are rich in both triglycerides and cholesterol and have relatively low densities, whereas LDL, lipoprotein(a), and HDL mainly contain cholesterol and have a higher density. Lipoproteins are produced by two pathways: 1) the endogenous pathway in which VLDL are assembled in hepatocytes and are converted to IDL and LDL by triglyceride lipolysis in plasma (on the luminal surface of endothelial cells lining the capillaries or in the lumen of vessels) and by exchange of lipids and apolipoproteins with other lipoproteins, and 2) the exogenous pathway in which chylomicrons are produced by enterocytes, and are converted to chylomicron remnants by triglyceride lipolysis in plasma (Havel & Kane, 2001). Finally, lipoprotein(a) is an LDL particle with an additional apolipoprotein(a) attached, and levels of this lipoprotein is mainly genetically determined (Nordestgaard et al., 2010; Kronenberg & Utermann, 2013).

After secretion of chylomicrons from the intestine and VLDL from the liver, both types of lipoproteins are enriched in apolipoprotein E during their degradation to remnants. Apolipoprotein E is important for the uptake of remnants by the liver, because it, like apolipoprotein B, functions as a ligand for hepatic receptors, i.e. the LDL and LRP (LDL receptor-related protein) receptors (Havel, 2010; Ramasamy, 2013). Consequently, mutations in the *APOE* gene can cause increased plasma levels of cholesterol and triglycerides (Frikke-Schmidt et al., 2000). However, hepatic uptake of remnants is complex and not fully understood, and mechanisms other than uptake via the LDL and LRP receptors have been proposed, such as internalization after binding to heparan sulfate proteoglycans (MacArthur et al., 2007).

We define remnant cholesterol as the cholesterol content of a subset of the triglyceride-rich lipoproteins called remnants, i.e. chylomicron remnants, VLDL, and IDL in the nonfasting state, and VLDL and IDL in the fasting state; in most individuals chylomicrons are not present in plasma as these particles are degraded to chylomicron remnants very fast due to rapid triglyceride hydrolysis by lipoprotein lipase. Remnant cholesterol levels are therefore highly correlated with triglyceride levels (Varbo et al., 2013a). In plasma, triglycerides and cholesterol are exchanged between HDL and remnants, and levels of HDL cholesterol and remnant cholesterol are inversely correlated. This has previously made it difficult to determine if it is low levels of HDL cholesterol per se or the concurrent high levels of remnant cholesterol and triglycerides per se that is the cause of the increased risk of IHD found in observational studies.

The aim of this review is first to summarize current evidence of elevated levels of remnant cholesterol as a causal risk factor for IHD including results from observational studies, experimental studies, genetic studies, and randomized clinical intervention trials. Second, we describe measurement of remnants, the pathophysiology behind the atherogenicity of remnants, and presence of elevated remnant cholesterol levels in high risk patient groups. Finally, we review present and future pharmacological approaches to lowering remnant cholesterol levels.

2. Remnant cholesterol levels and observational risk of ischemic heart disease

Large observational studies, and meta-analyses thereof, have shown that elevated triglycerides are associated with increased risk of cardiovascular disease (Austin, 1991; Hokanson & Austin, 1996; Nordestgaard et al., 2007; Freiberg et al., 2008; Di Angelantonio et al., 2009; Chapman et al., 2011; Varbo et al., 2011b); however, the association of elevated remnant cholesterol levels with cardiovascular disease risk is not as thoroughly studied. Although there has been many studies of the association of remnant cholesterol levels with cardiovascular disease, most have been relatively small case–control studies (Devaraj et al., 1998; Sakata et al., 1998; Kugiyama et al., 1999; Takeichi et al., 1999; Masuoka et al., 1998, 2000a, 2000b; Song et al., 2000; Higashi et al., 2001; Karpe et al., 2001; Inoue et al., 2004; Miwa et al., 2004; Oi et al., 2004; Hopkins et al., 2005; Lamon-Fava et al., 2008; Hiki et al., 2009) or observational studies (McNamara et al., 2001; Fukushima et al., 2001, 2004; Imke et al., 2005; Nakamura et al., 2005) using different assays (and thereby definitions) for determining remnant cholesterol levels. Most of the studies measured remnant cholesterol in the fasting state; however, studies of triglyceride levels, which are highly correlated with remnant cholesterol levels (Fig. 1), have shown that elevated nonfasting levels of triglycerides are consistently associated with increased risk of cardiovascular disease, and are possibly more useful for predicting risk of cardiovascular disease than fasting levels of triglycerides (Bansal et al., 2007; Freiberg et al., 2008; Mora et al., 2008; Stalenhoef & de Graaf, 2008; Nordestgaard et al., 2007, 2009; Kolovou et al., 2011; Langsted et al., 2011; Nordestgaard & Freiberg, 2011; Varbo et al., 2011b).

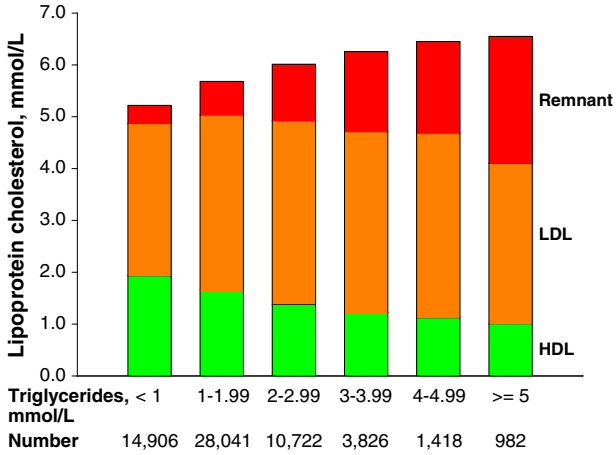

Fig. 1. Lipoprotein cholesterol as a function of increasing levels of nonfasting triglycerides. Data are from the Copenhagen General Population Study. $R^2 = 0.96$ for the correlation of remnant cholesterol levels with triglyceride levels. HDL = high-density lipoprotein, LDL = low-density lipoprotein.
Modified from Varbo et al. J Am Coll Cardiol 2013;61:427–436.

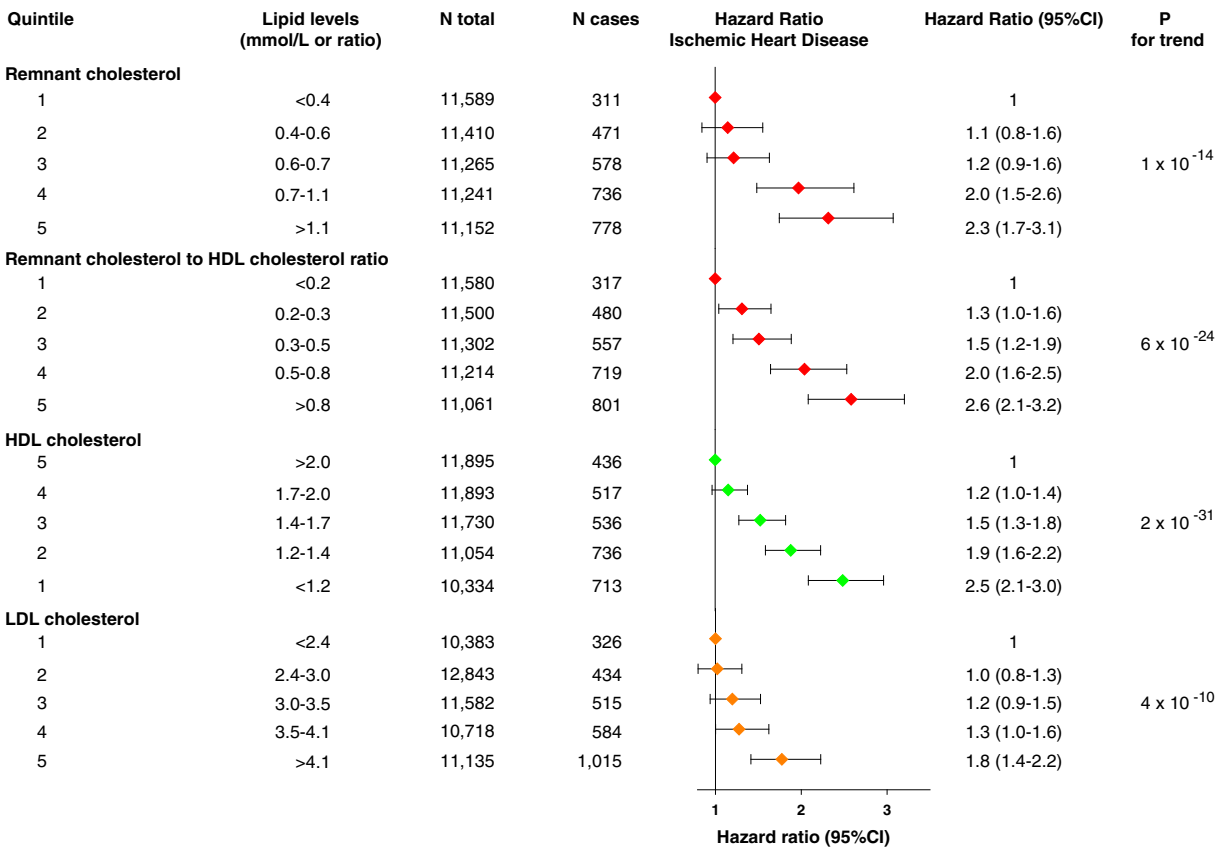

Fig. 2. Lipoprotein levels in quintiles and risk of ischemic heart disease in the general population. Risk of ischemic heart disease as a function of lipoprotein levels in quintiles were estimated in the Copenhagen General Population Study and the Copenhagen City Heart Study combined. Hazard ratios were estimated by Cox proportional hazards regression models, adjusted for age (as time scale), sex, smoking, hypertension, time since last meal, time of day for blood sampling, and lipid lowering therapy. P for trend was by Cuzicks extension of the Wilcoxon rank sum test. CI = confidence interval, HDL = high-density lipoprotein, LDL = low-density lipoprotein.
Modified from Varbo et al. J Am Coll Cardiol 2013;61:427–436.

Most people are in the nonfasting state most of the day, since fasting implies not eating for at least 8 h, and for most people in affluent countries this is only the case early in the morning. This argues for examining association of remnant cholesterol levels with cardiovascular disease in the nonfasting state. We previously reported an association of elevated nonfasting remnant cholesterol levels, calculated as nonfasting total cholesterol minus HDL cholesterol minus LDL cholesterol, with increased risk of IHD in a large sample of the general population (Varbo et al., 2013a). Individuals with nonfasting remnant cholesterol levels in the upper fifth quintile, that is, remnant cholesterol levels >1.1 mmol/L (>43 mg/dL), had a hazard ratio for IHD of 2.3(95% confidence interval: 1.7–3.1) compared to individuals with nonfasting remnant cholesterol in the lowest quintile, that is, <0.4 mmol/L (<16 mg/dL) (Fig. 2, top panel). Similarly, the ratio of nonfasting remnant cholesterol to HDL cholesterol was also significantly associated with increased risk of IHD, with a hazard ratio of 2.6(2.1–3.2) for a ratio >0.8 compared to a ratio <0.2 (Fig. 2, upper middle panel). In that study, elevated levels of nonfasting remnant cholesterol and increased ratio of nonfasting remnant cholesterol to HDL cholesterol associated with increased risk of IHD in a magnitude similar to those seen for elevated LDL cholesterol and reduced HDL cholesterol levels (Fig. 2, lower and lower middle panel), both of which are well-known markers of increased risk of cardiovascular disease.

3. Remnant cholesterol, genetic variation, and causal risk of ischemic heart disease

Plasma levels of remnant cholesterol (and likewise triglycerides) are partly genetically determined by common and rare genetic variants (Yuan et al., 2007; Hegele et al., 2009; Johansen et al., 2011a,2011b), and partly determined by lifestyle factors such as diet, obesity, alcohol intake, and physical activity (Chapman et al., 2011). Several rare genetic variants are known to influence levels of triglycerides, and thus remnant cholesterol levels, such as deleterious mutations in the *LPL*, *APOC2*, *APOA5*, *LMF1*, and *GPIHBP1* genes that in homozygote or compound heterozygote forms can cause severe chylomicronemia (Brunzell & Deeb, 2001; Johansen & Hegele, 2011). However, there are also numerous common genetic variants that influence levels of remnant cholesterol, but with a smaller effect size than the rare genetic variants. Many of the common variants have been indentified in genome wide association studies (GWAS) aimed at identifying genetic variants associated with levels of triglycerides (Kathiresan et al., 2008; Willer et al., 2008; Teslovich et al., 2010). Associations of some of these common genetic variants with lipoprotein levels were replicated in large general population studies by us (Varbo et al., 2011a; Jorgensen et al., 2013), which showed that variants in *TRIB1* (rs2954029), *GCKR* (rs1260326), and three variants in *APOA5* (rs651821, rs3135506, and rs619054) were associated with higher levels of nonfasting triglycerides, remnant cholesterol, and LDL cholesterol, and with lower levels of HDL cholesterol, and also that the *TRIB1* and *APOA5* variants were associated with increased risk of IHD and myocardial infarction; however, the *GCKR* variant was not associated with risk of IHD or myocardial infarction. *TRIB1* encodes tribble-1 which is a protein with a regulatory effect on mitogen-activated protein kinase (Kiss-Toth et al., 2004), and *TRIB1* is an example of a gene identified in GWAS that was previously not known to be involved in atherosclerosis development.

In the metabolism of lipoproteins, the cholesteryl ester transfer protein (CETP) exchange triglycerides for cholesterol esters between

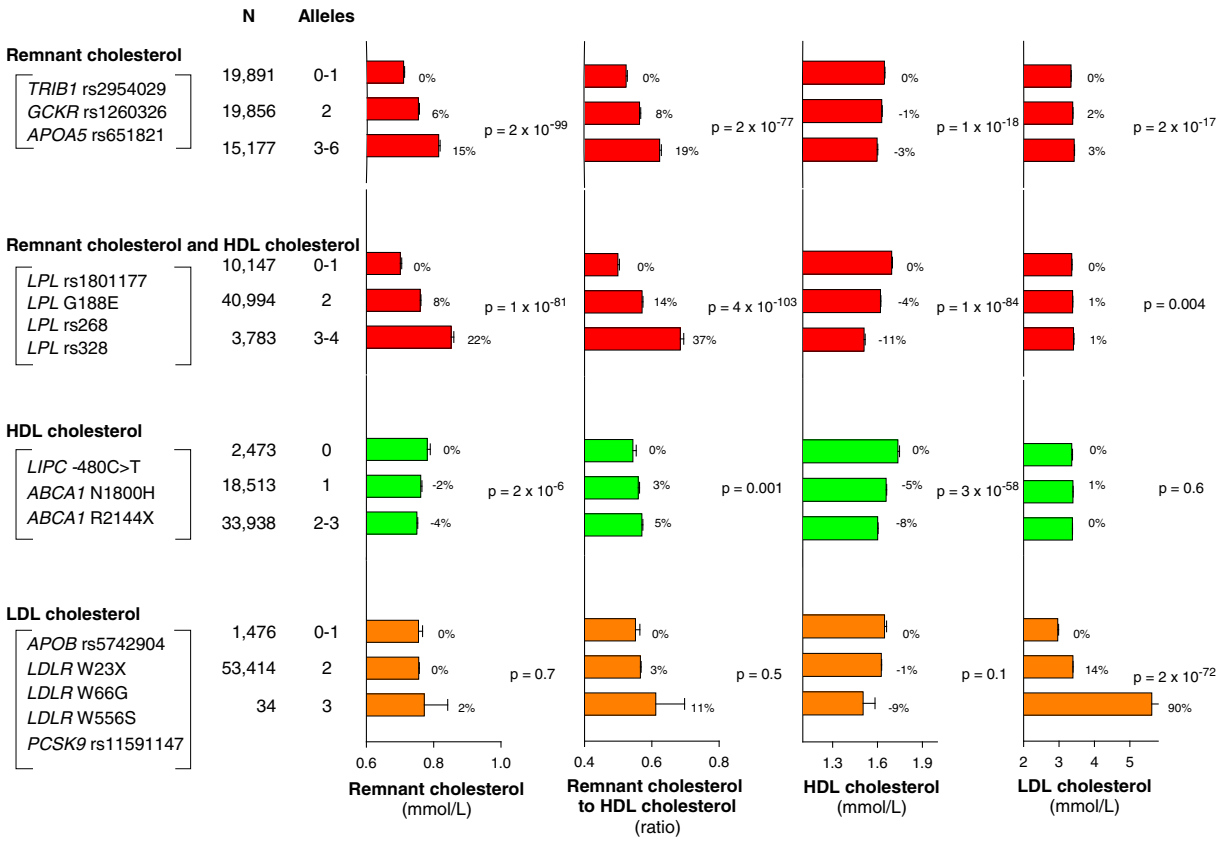

Fig. 3. Lipid levels as a function of genotypes in allele counts. Bars show mean lipid levels with standard error whiskers in participants from the Copenhagen General Population Study and the Copenhagen City Heart Study not using lipid lowering therapy. Genetic variants included in each group are shown in brackets. P-values for trend were estimated by Cuzicks extension of the Wilcoxon rank sum test. HDL = high-density lipoprotein, LDL = low-density lipoprotein.
Modified from Varbo et al. J Am Coll Cardiol 2013;61:427–436.

remnants and HDL particles, leading to inversely correlated levels of remnant cholesterol and HDL cholesterol (Fig. 1). Therefore, most genetic variants that are associated with elevated levels of remnant cholesterol are also associated with low levels of HDL cholesterol, and to some extent also with elevated LDL cholesterol levels. This has previously made it difficult to separately determine the causality of elevated levels of remnant cholesterol and low levels of HDL cholesterol on risk of IHD. To circumvent this, we conducted a Mendelian randomization study on 73,513 participants from three Danish studies (Varbo et al., 2013a), in which we used 15 genetic variants combined into four allele scores that affected levels of either 1) nonfasting remnant cholesterol alone, 2) remnant cholesterol and HDL cholesterol combined, 3) HDL cholesterol alone, and as a positive control 4) LDL cholesterol alone (Fig. 3). In that study, we found the group of genetic variants affecting only remnant cholesterol levels, and the group of genetic variants affecting both remnant cholesterol and HDL cholesterol levels, to be causally associated with IHD, with a causal odds ratio of 2.8(1.9–4.2) per 1 mmol/L (39 mg/dL) higher level of remnant cholesterol and a causal odds ratio of 2.9(1.9–4.6) for a 1 ratio increase of remnant cholesterol to HDL cholesterol, respectively (Fig. 4). However, the group of genetic variants that only affected levels of HDL cholesterol was not causally associated with risk of IHD. From this, we concluded that elevated levels of remnant cholesterol cause IHD, independently of HDL cholesterol levels.

4. Measurement of remnant cholesterol

Separation of lipoproteins into different classes was first done by ultracentrifugation in the 1940's (Gofman et al., 1956). Since then, assays for measuring remnants using different methods have been developed (Havel, 2000; Nakajima et al., 1993, 2011; Ooi & Nordestgaard, 2011; Hanada et al., 2012). However, as lipoprotein remnants are different both in composition of lipids and apolipoproteins as a result of different stages of triglyceride lipolysis and exchange of apolipoproteins and lipids with other lipoproteins, and also because remnants are formed by two different pathways, i.e. the endogenous and the exogenous pathways, it is difficult to create an assay that measures all remnants at the same time (Ooi & Nordestgaard, 2011). An alternative to directly measuring remnants is to calculate nonfasting remnant cholesterol levels as nonfasting total cholesterol minus HDL cholesterol minus LDL cholesterol, like it has been done in several studies of large Danish cohorts (Nordestgaard et al., 2007; Freiberg et al., 2008; Varbo et al., 2011a; Varbo et al., 2011b; Jorgensen et al., 2013; Varbo et al., 2013a; Varbo et al., 2013b). Although possibly not as precise as directly measuring remnants, the advantage of calculating remnant cholesterol is that it is inexpensive, and can be done from a standard lipid profile, as long as it has been taken in the nonfasting state, which makes it useful for clinicians anywhere.

5. Atherogenicity of elevated remnant cholesterol

Mechanistically, the explanation for a causal effect of elevated levels of nonfasting remnant cholesterol on increased risk of IHD likely is simple and straight forward, that is, that remnant cholesterol, like LDL cholesterol, enter and is trapped in the intima of the arterial wall (Shaikh et al., 1991; Nordestgaard et al., 1992; Nordestgaard et al., 1995), leading to accumulation of intimal cholesterol, atherosclerosis and ultimately IHD (Fig. 5).

Different sizes of lipoproteins presumably play a role in their ability to enter the arterial wall and get trapped. Studies in rabbit and in

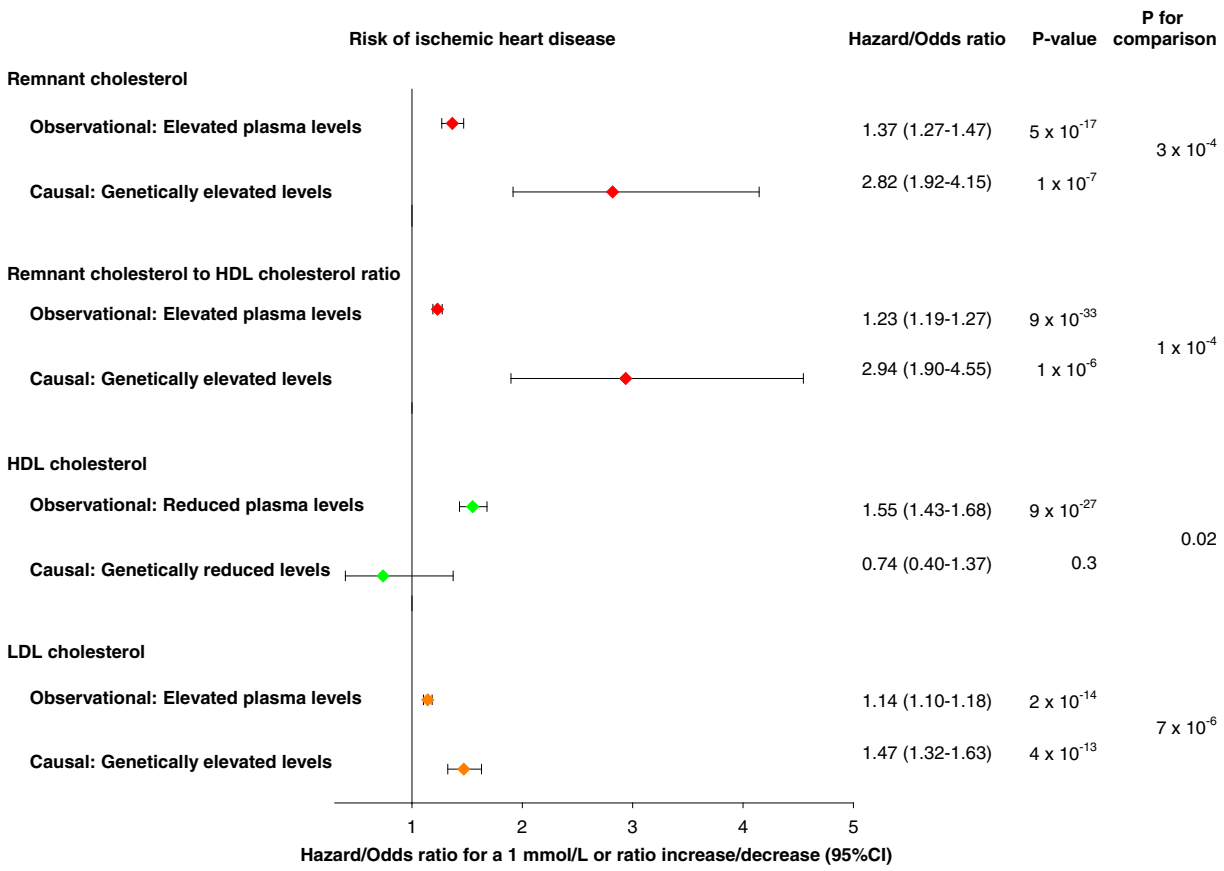

Fig. 4. Observational and causal risk estimates for ischemic heart disease. Observational risk estimates in hazard ratios for a 1 mmol/L or 1 ratio increase or decrease in plasma lipoprotein levels for 68,328 participants from the Copenhagen General Population Study and the Copenhagen City Heart Study combined, estimated by Cox proportional hazards regression models with adjustment for age (time scale), sex, smoking, hypertension, time since last meal, time of day for blood sampling, and lipid lowering therapy. Causal risk estimates were from 73,513 participants from the Copenhagen General Population Study, the Copenhagen City Heart Study, and the Copenhagen Ischemic Heart Disease Study combined, and estimated by instrumental variable analyses. CI = confidence interval, HDL = high-density lipoprotein, LDL = low-density lipoprotein.
Modified from Varbo et al. J Am Coll Cardiol 2013;61:427–436.

human atherosclerotic plaque tissue have shown that chylomicrons and large VLDL particles are too large to enter the arterial wall (Nordestgaard & Zilversmit, 1988; Nordestgaard et al., 1988), but that their remnants penetrate the arterial intima and get trapped in the connective tissue of the arterial wall and eventually accumulate (Shaikh et al., 1991; Nordestgaard et al., 1992; Rapp et al., 1994; Nordestgaard et al., 1995). In line with this, patients who are homozygous for rare deleterious mutations in the *LPL* gene, causing chylomicronemia with extremely high levels of large chylomicrons and VLDLs, have an increased risk of pancreatitis, but not an increased risk of IHD (Brunzell & Deeb, 2001). Also, in-vitro studies have shown that remnants, unlike LDL, may not need to be oxidized in order to be taken up by macrophages to cause foam cell formation and atherosclerosis (Nakajima et al., 2006).

Remnants may also be involved in the development of atherosclerosis and IHD by other pathways than by accumulation of cholesterol in the arterial wall. Experimental studies have found remnants to be associated with mechanisms underlying endothelial dysfunction, such as impaired vasodilation (Zheng & Liu, 2007) and enhanced inflammatory response (Giannattasio et al., 2005; Ting et al., 2007; Alipour et al., 2008; Wang et al., 2009; Zilversmit, 1979), and to play a role in plaque disruption and thrombus formation (Falk et al., 1995; Davies, 1996; Moyer et al., 1998; Kohler & Grant, 2000; Sambola et al., 2003; Grant, 2007). Also, our results from a large multi-directional Mendelian randomization study of 60,608 individuals with 10,668 cases of IHD indicated that elevated levels of remnant cholesterol may not cause atherosclerosis in exactly the same way as LDL cholesterol (Varbo et al., 2013b). We thus found that elevated levels of remnant cholesterol were causally associated with both low-grade inflammation and IHD; however, elevated levels of LDL cholesterol were associated causally with IHD, but not with low-grade inflammation (Fig. 6). These results were consistent even in participants without obesity and without diabetes mellitus. Such results indicate that elevated LDL cholesterol levels cause atherosclerosis without a major inflammatory component, whereas an inflammatory component of atherosclerosis is driven by elevated remnant cholesterol levels.

6. Remnant cholesterol levels in high risk patient groups

The most common cause of elevated remnant cholesterol and triglycerides is obesity (Table 1). Likewise, poorly controlled diabetes mellitus and excessive alcohol intake are common causes of elevated remnant cholesterol levels. In some women, high estrogen levels, either endogenous during pregnancy or from exogenous sources like oral contraceptives or hormone replacement therapy, can lead to high triglycerides, and possibly high remnant cholesterol levels. Kidney and liver diseases, and a number of different drugs, including oral glucocorticoids, can lead to elevated remnant cholesterol levels. Importantly, on top of these lifestyle and exogenous factors just mentioned, the genetic make-up of the individual person, including both rare and common genetic variants, determines the extent of elevated triglycerides and remnant cholesterol levels (Hegele et al., 2009; Johansen et al., 2011a; Johansen et al., 2011b).

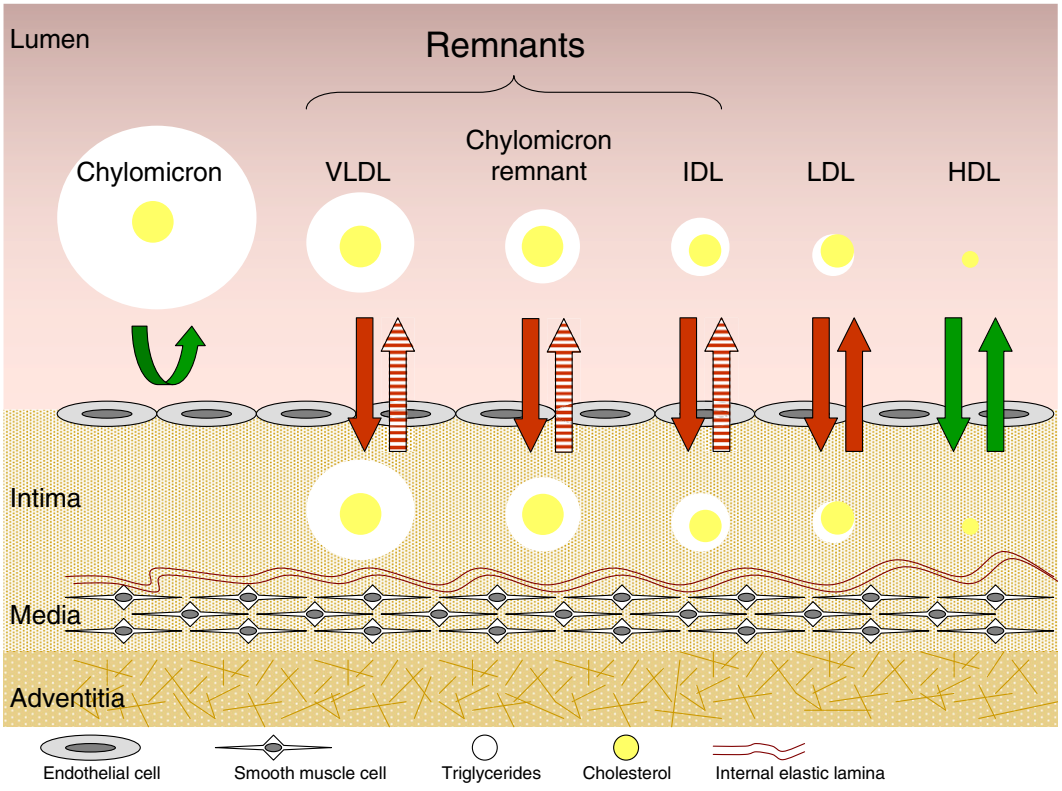

Fig. 5. Lipoproteins and the arterial wall. The figure shows the hypothesis of the mechanism by which remnants and LDL enter and are trapped in the arterial wall, and eventually cause atherosclerosis. HDL = high-density lipoprotein, IDL = intermediate-density lipoprotein, LDL = low-density lipoprotein, VLDL = very-low-density lipoprotein.

The chylomicronemia syndrome with typically triglycerides >25 mmol/L (>2200 mg/dL) is a special case with increased risk of pancreatitis and usually normal risk of IHD (Brunzell & Deeb, 2001), unless triglycerides are lowered substantially, when the risk of IHD in these patients may still be increased (Benlian et al., 1996); importantly, the patients in the later study displayed multiple risk factors for IHD. The simple explanation for this seemingly paradox is that at very high triglyceride levels, lipoproteins are too large to enter into the arterial intima to cause atherosclerosis (Nordestgaard & Zilversmit, 1988; Nordestgaard et al., 1988), while when triglycerides are lowered

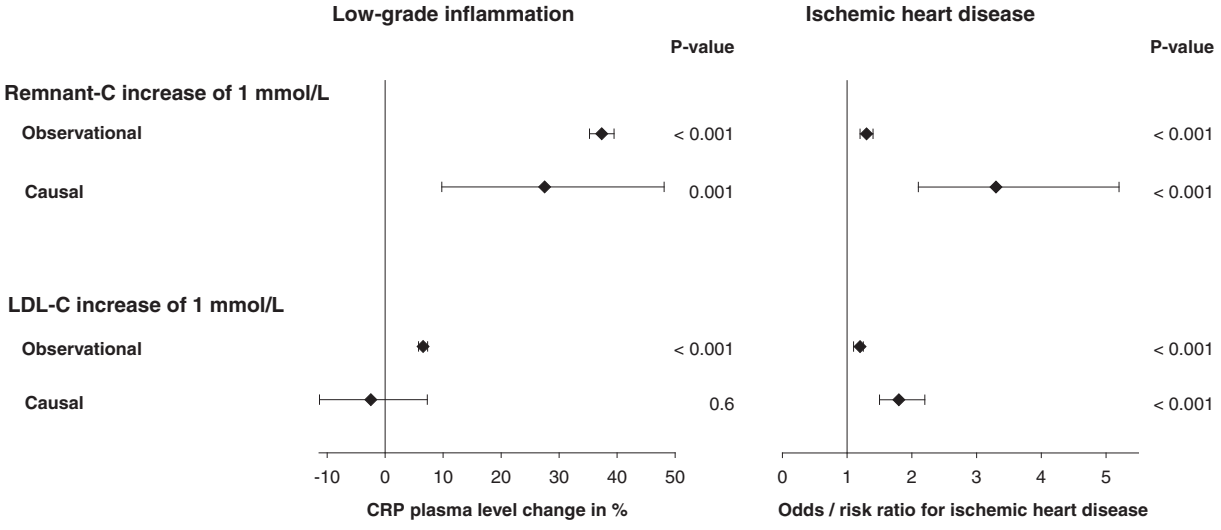

Fig. 6. Observational and causal associations between remnant cholesterol and low-density lipoprotein cholesterol with low-grade inflammation and ischemic heart disease. The left side of the figure shows observational and causal change in C-reactive protein levels in percent with 95% confidence intervals per 1 mmol/L (39 mg/dL) higher level of remnant cholesterol and per 1 mmol/L (39 mg/dL) higher level of low-density lipoprotein cholesterol in 48,250 participants from the Copenhagen General Population Study. Observational estimates were by linear regression and causal estimates by instrumental variable analyses. The right side of the figure shows observational odds ratios and causal risk ratios with 95% confidence intervals for ischemic heart disease per 1 mmol/L (39 mg/dL) higher level of remnant cholesterol and per 1 mmol/L (39 mg/dL) higher level of low-density lipoprotein cholesterol in 60,608 participants from the Copenhagen General Population Study, the Copenhagen City Heart Study, and the Copenhagen Ischemic Heart Disease Study combined. Observational odds ratios were estimated by logistic regression and causal risk ratios by instrumental variable analyses. C = cholesterol, CRP = C-reactive protein, LDL = low-density lipoprotein.
Modified from Varbo et al. Circulation 2013;128:1298–1309.

Table 1
Causes of elevated remnant cholesterol and triglycerides.

Obesity
Diabetes mellitus
Alcohol excess
Estrogen (incl. pregnancy)
Kidney disease
Liver disease
Some drugs
Other rare causes
Common genetic variants
Rare genetic variants

substantially, lipoproteins become smaller and are then able to penetrate into and get trapped in the arterial wall (Shaikh et al., 1991; Nordestgaard et al., 1992; Rapp et al., 1994; Nordestgaard et al., 1995).

The classic genetic forms of the chylomicronemia syndrome are caused by homozygosity (or compound heterozygosity) for deleterious mutations in the *LPL* gene encoding lipoprotein lipase, the enzyme degrading triglycerides in plasma, or mutations in genes encoding cofactors and other proteins necessary for lipoprotein lipase function like in *APOC2*, *APOA5*, *LMF1*, and *GPIHBP1* (Brunzell & Deeb, 2001; Johansen & Hegele, 2011). These genetic forms of the chylomicronemia syndrome are rare; however, the more commonly observed forms are caused by the interplay of genetic factors with external factors (Yuan et al., 2007; Gotoda et al., 2012), which are typically poorly controlled diabetes mellitus, excessive alcohol intake, and high estrogen levels in some women, as mentioned above.

7. Lowering of remnant cholesterol levels using current approaches

In a recent consensus document (Chapman et al., 2011), therapeutic targeting of triglycerides ≥1.7 mmol/L (≥150 mg/dL) was recommended in patients at high risk of cardiovascular disease after lowering of LDL cholesterol below recommended levels, which includes lowering of remnant cholesterol. Elevated levels of remnant cholesterol can be lowered by lifestyle changes and by pharmacological therapy. Lifestyle changes that effectively lower remnant cholesterol levels include weight reduction (the most important), reduced alcohol intake, reduced dietary intake of saturated fat, smoking cessation, and increased physical activity (Chapman et al., 2011). The mechanisms by which lifestyle changes reduce remnant cholesterol levels are by decreasing hepatic secretion and by increasing hepatic clearance of VLDL particles.

Current pharmacological therapy that lowers remnant cholesterol levels includes statins, niacin, and fibrates. Randomized clinical intervention trials aimed at lowering LDL cholesterol levels with statins have shown that triglycerides and non-HDL cholesterol, the latter being LDL cholesterol plus remnant cholesterol, were also lowered during treatment (Grundy et al., 2004; Gotto & Moon, 2012); however, these findings were in post-hoc analyses, and most statin trials excluded participants with triglycerides >4 mmol/L (>350 mg/dL), making it impossible to know the potential benefit of statin treatment in those with elevated remnant cholesterol levels and triglycerides >4 mmol/L (>350 mg/dL). There have so far not been conducted randomized clinical intervention trials with statins among patients with hypertriglyceridemia with the primary target of lowering triglyceride and/or remnant cholesterol levels.

Randomized trials with niacin in addition to statin or fibrate therapy have lead to conflicting results regarding reduction in risk of cardiovascular disease (Carlson & Rosenhamer, 1988; Lavigne & Karas, 2013). The Coronary Drug Project showed that immediate release niacin 3 g/day was associated with a 26% reduction in non-fatal myocardial infarction after 7 years of follow-up and a 11% reduced all-cause mortality after 15 years of follow-up (Canner et al., 1986; Clofibrate and niacin in coronary heart disease, 1975); however, the AIM-HIGH study found no additional effect of adding extended-release niacin in addition to statin therapy in secondary prevention of cardiovascular disease (Boden et al., 2011). Importantly, the AIM-HIGH study requested additional LDL cholesterol lowering if the LDL cholesterol target was not met, even in the niacin placebo arm, making it very difficult to show an effect of niacin added to statin treatment. Results from the HPS2-THRIVE study, examining the effect of adding niacin to statin therapy in a wider range of patients are awaited, but preliminary results show lack of effect together with substantial side effects in the niacin group (Haynes et al., 2013; HPS2-THRIVE press release on preliminary results, 2013). Concerning remnant cholesterol it should be emphasized though, that both the AIM-HIGH and the HPS2-THRIVE studies recruited very few participants with particularly elevated remnant cholesterol levels, meaning that current evidence cannot educate us about the effect of niacin when given to high risk individuals with elevated remnant cholesterol levels. The only appropriate trial for this question was the trial by Carlson & Rosenhamer in, 1988 of combined niacin and fibrate treatment given to myocardial infarction survivors (Carlson & Rosenhamer, 1988). Although this trial showed a clear 26% reduction in total mortality and a 36% reduction in IHD mortality in the treatment group, these results should be interpreted cautiously as the trial was not blinded.

Randomized clinical intervention trials examining fibrates in prevention of cardiovascular disease have also produced conflicting results, primarily showing effect on reduction of risk of non-fatal myocardial infarction, but with no effect on risk of ischemic stroke or cardiovascular mortality (Jun et al., 2010). However, these trials mainly focused on elevated LDL cholesterol levels or reduced HDL cholesterol levels, and a meta-analysis of post-hoc analyses from several fibrate trials of subgroups of patients with elevated triglyceride levels, showed a large benefit of fibrates on risk of cardiovascular disease in these patients (Sacks et al., 2010; Chapman et al., 2011).

Statins are the first choice of therapy in patients with hyperlipidemia; however, addition of fibrates or possibly niacin should be considered in patients who have hypertriglyceridemia, and thus elevated remnant cholesterol levels, despite optimal statin treatment and lifestyle interventions (Chapman et al., 2011). Although the addition of niacin to statin therapy can be questioned after the negative results of the AIM-HIGH (Boden et al., 2011) and HPS2-THRIVE (Haynes et al., 2013; HPS2-THRIVE press release on preliminary results, 2013) studies, as mentioned above, these trials were not designed to look at effects of niacin addition to statin in those with elevated levels of remnant cholesterol.

Taken together, there is a need for large randomized clinical intervention trials investigating if reducing remnant cholesterol levels (and triglyceride levels) in patients with elevated levels will reduce the risk of cardiovascular disease.

8. Future pharmacological approaches to lowering remnant cholesterol levels

Given the documented causal association between elevated levels of remnant cholesterol and increased risk of IHD (Jorgensen et al., 2013; Varbo et al., 2013a; Varbo et al., 2013b), and the lack of well documented effect in reducing IHD risk in those with elevated remnant cholesterol levels with statins, fibrates, and/or niacin, there is a clear need for new therapies to reduce remnant cholesterol as add on therapy to statins or to be used in statin intolerant patients.

One possibility is to use omega-3 fatty acids, or fish oil, to lower remnant cholesterol levels, although the use of such drugs have so far focused on lowering triglycerides to reduce risk of pancreatitis in those with triglycerides >5 mmol/L (440 mg/dL). However, recent trials have suggested that some omega-3 fatty acids may also reduce remnant cholesterol levels (Bays et al., 2012), but large randomized trials to detect reduced IHD risk after such treatment is awaited. So far randomized clinical intervention trials with fish oil in secondary prevention of

cardiovascular disease have had conflicting results (Casula et al., 2013; Kwak et al., 2012; Rizos et al., 2012).

Other possibilities include a large number of novel drugs in the process of being developed and tested for clinical use (Gotto & Moon, 2013). These include drugs targeting the production of apolipoprotein B with the *APOB* antisense oligonuclotide mipomersen (Raal et al., 2010), recently approved for use in patients with homozygous familial hypercholesterolemia in the US, and the MTP inhibitor lomitapide (Cuchel et al., 2007; Cuchel et al., 2013), also recently approved for use in patients with homozygous familial hypercholesterolemia, in the US and in Europe. Both mipomersen and lomitapide unfortunately have adverse effects including increasing hepatic lipid content, which so far limits the use to add on therapy to other lipid-lowering therapies in patients with homozygous familial hypercholesterolemia.

Another new line of drugs being developed are antibodies against plasma proprotein convertase subtilisin/kexin type 9 (PCSK9)(Lee & Hegele, 2013). PCSK9 binds to the LDL receptor and promotes its degradation, and inhibition of PCSK9 increases the number of LDL receptors on the liver, promoting LDL uptake from plasma, and possibly also uptake of remnants. Also, antisense inhibition of *APOC3*, encoding apolipoprotein C3, have in preclinical models and in a phase I clinical trial showed promising results regarding reduction of triglyceride levels (Graham et al., 2013). There has also been developed gene therapy for lipoprotein lipase deficiency, which has been shown to lower triglycerides in patients with severe hypertriglyceridemia (Gaudet et al., 2012), and are now approved for clinical use in Europe; lipoprotein lipase activators are likewise being developed (Eriksson et al., 2013). Finally, several drugs that inhibit CETP have been developed; however, results so far have either showed increased risk of cardiovascular disease of CETP inhibition on top of statins (Barter et al., 2007), or no effect on risk of cardiovascular disease (Schwartz et al., 2012), compared to statin therapy alone. Results from two additional randomized clinical intervention trials are awaited (Cannon et al., 2010; Nicholls et al., 2011).

Importantly, before any of these novel therapies, potentially to be used to lower remnant cholesterol/triglyceride levels, can reach clinical use, they need to be documented to be efficacious in reducing remnant cholesterol levels, to be largely free of side effects, and eventually to be shown to reduce risk of cardiovascular disease, either as an add on to statin therapy or by themselves in statin intolerant patients.

9. Conclusions and perspectives

In conclusion, evidence from observational studies, in-vitro and animal studies, and from genetic studies all support a causal association between elevated levels of remnant cholesterol and increased risk of IHD; however, even though post-hoc subgroup analyses of randomized trials using fibrates showed a benefit of lowering triglycerides or remnant cholesterol levels, large randomized clinical intervention trials with the primary target of lowering remnant cholesterol levels are still missing. Such positive trials may open the possibility for further reducing risk of IHD in many millions of high risk individuals worldwide.

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Conflict of interest disclosures

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References

Alipour, A., Van Oostrom, A. J., Izraeljan, A., Verseyden, C., Collins, J. M., Frayn, K. N., et al. (2008). Leukocyte activation by triglyceride-rich lipoproteins. *Arterioscler Thromb Vasc Biol 28*, 792–797.

Austin, M.A. (1991). Plasma triglyceride and coronary heart disease. *Arterioscler Thromb 11*, 2–14.

Bansal, S., Buring, J. E., Rifai, N., Mora, S., Sacks, F. M., & Ridker, P.M. (2007). Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA 298*, 309–316.

Barter, P. J., Caulfield, M., Eriksson, M., Grundy, S. M., Kastelein, J. J., Komajda, M., et al. (2007). Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med 357*, 2109–2122.

Bays, H. E., Braeckman, R. A., Ballantyne, C. M., Kastelein, J. J., Otvos, J.D., Stirtan, W. G., et al. (2012). Icosapent ethyl, a pure EPA omega-3 fatty acid: Effects on lipoprotein particle concentration and size in patients with very high triglyceride levels (the MARINE study). *J Clin Lipidol 6*, 565–572.

Benlian, P., De Gennes, J. L., Foubert, L., Zhang, H., Gagne, S. E., & Hayden, M. (1996). Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. *N Engl J Med 335*, 848–854.

Boden, W. E., Probstfield, J. L., Anderson, T., Chaitman, B. R., Desvignes-Nickens, P., Koprowicz, K., et al. (2011). Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med 365*, 2255–2267.

Brunzell, J.D., & Deeb, S. S. (2001). Familial lipoprotein lipase deficiency, Apo C-II deficiency, and hepatic lipase deficiency. In C. R. Scriver, A. L. Beaudet, W. S. Sly, & D. Valle (Eds.), *The metabolic and molecular bases of inherited diseases* (pp. 2789–2816) (8th ed.). Mc-Graw-Hill, Medical Publishing Division.

Canner, P. L., Berge, K. G., Wenger, N. K., Stamler, J., Friedman, L., Prineas, R. J., et al. (1986). Fifteen year mortality in Coronary Drug Project patients: Long-term benefit with niacin. *J Am Coll Cardiol 8*, 1245–1255.

Cannon, C. P., Shah, S., Dansky, H. M., Davidson, M., Brinton, E. A., Gotto, A.M., et al. (2010). Safety of anacetrapib in patients with or at high risk for coronary heart disease. *N Engl J Med 363*, 2406–2415.

Carlson, L. A., & Rosenhamer, G. (1988). Reduction of mortality in the Stockholm Ischaemic Heart Disease Secondary Prevention Study by combined treatment with clofibrate and nicotinic acid. *Acta Med Scand 223*, 405–418.

Casula, M., Soranna, D., Catapano, A. L., & Corrao, G. (2013). Long-term effect of high dose omega-3 fatty acid supplementation for secondary prevention of cardiovascular outcomes: A meta-analysis of randomized, double blind, placebo controlled trials. *Atheroscler Suppl 14*, 243–251.

Chapman, M. J., Ginsberg, H. N., Amarenco, P., Andreotti, F., Boren, J., Catapano, A. L., et al. (2011). Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: Evidence and guidance for management. *Eur Heart J 32*, 1345–1361.

Clofibrate, niacin in coronary heart disease (1975). *JAMA 231*, 360–381.

Cuchel, M., Bloedon, L. T., Szapary, P. O., Kolansky, D.M., Wolfe, M. L., Sarkis, A., et al. (2007). Inhibition of microsomal triglyceride transfer protein in familial hypercholesterolemia. *N Engl J Med 356*, 148–156.

Cuchel, M., Meagher, E. A., du Toit, T. H., Blom, D. J., Marais, A.D., Hegele, R. A., et al. (2013). Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: A single-arm, open-label, phase 3 study. *Lancet 381*, 40–46.

Davies, M. J. (1996). Stability and instability: Two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. *Circulation 94*, 2013–2020.

Devaraj, S., Vega, G., Lange, R., Grundy, S. M., & Jialal, I. (1998). Remnant-like particle cholesterol levels in patients with dysbetalipoproteinemia or coronary artery disease. *Am J Med 104*, 445–450.

Di Angelantonio, E., Sarwar, N., Perry, P., Kaptoge, S., Ray, K. K., Thompson, A., et al. (2009). Major lipids, apolipoproteins, and risk of vascular disease. *JAMA 302*, 1993–2000.

Eriksson, M., Larsson, M., Caraballo, R., Enquist, P. A., Elofsson, M., & Olivecrona, G. (2013). Novel small molecule lipoprotein lipase activator LP071 improves plasma lipid parameters in vivo. *Paper presented at the 81st Annual Congress of the European Atherosclerosis Society, Lyon 2–5 June 2013*.

Falk, E., Shah, P. K., & Fuster, V. (1995). Coronary plaque disruption. *Circulation 92*, 657–671.

Freiberg, J. J., Tybjaerg-Hansen, A., Jensen, J. S., & Nordestgaard, B. G. (2008). Nonfasting triglycerides and risk of ischemic stroke in the general population. *JAMA 300*, 2142–2152.

Frikke-Schmidt, R., Nordestgaard, B. G., Agerholm-Larsen, B., Schnohr, P., & Tybjærg-Hansen, A. (2000). Context-dependent and invariant associations between lipids, lipoproteins, and apolipoproteins and apolipoprotein E genotype. *J Lipid Res 41*, 1812–1822.

Fukushima, H., Kugiyama, K., Sugiyama, S., Honda, O., Koide, S., Nakamura, S., et al. (2001). Comparison of remnant-like lipoprotein particles in postmenopausal women with and without coronary artery disease and in men with coronary artery disease. *Am J Cardiol 88*, 1370–1373.

Fukushima, H., Sugiyama, S., Honda, O., Koide, S., Nakamura, S., Sakamoto, T., et al. (2004). Prognostic value of remnant-like lipoprotein particle levels in patients with coronary artery disease and type II diabetes mellitus. *J Am Coll Cardiol 43*, 2219–2224.

Gaudet, D., Methot, J., & Kastelein, J. (2012). Gene therapy for lipoprotein lipase deficiency. *Curr Opin Lipidol 23*, 310–320.

Giannattasio, C., Zoppo, A., Gentile, G., Failla, M., Capra, A., Maggi, F. M., et al. (2005). Acute effect of high-fat meal on endothelial function in moderately dyslipidemic subjects. *Arterioscler Thromb Vasc Biol 25*, 406–410.

Gofman, J. W., Hanig, M., Jones, J. B., Lauffer, M.A., Lawry, E. Y., Lewis, L. A., et al. (1956). Evaluation of serum lipoprotein and cholesterol measurements as predictors of clinical complications of atherosclerosis; report of a cooperative study of lipoproteins and atherosclerosis. *Circulation 14*, 691–742.

Gotoda, T., Shirai, K., Ohta, T., Kobayashi, J., Yokoyama, S., Oikawa, S., et al. (2012). Diagnosis and management of type I and type V hyperlipoproteinemia. *J Atheroscler Thromb 19*, 1–12.

Gotto, A.M., Jr., & Moon, J. E. (2012). Recent clinical studies of the effects of lipid-modifying therapies. *Am J Cardiol 110*, 15A–26A.

Gotto, A.M., Jr., & Moon, J. E. (2013). Pharmacotherapies for lipid modification: Beyond the statins. *Nat Rev Cardiol 10*, 560–570.

Graham, M. J., Lee, R. G., Bell, T. A., III, Fu, W., Mullick, A. E., Alexander, V. J., et al. (2013). Antisense oligonucleotide inhibition of apolipoprotein C-III reduces plasma triglycerides in rodents, nonhuman primates, and humans. *Circ Res 112*, 1479–1490.

Grant, P. J. (2007). Diabetes mellitus as a prothrombotic condition. *J Intern Med 262*, 157–172.

Grundy, S. M., Cleeman, J. I., Merz, C. N., Brewer, H. B., Jr., Clark, L. T., Hunninghake, D. B., et al. (2004). Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation 110*, 227–239.

Hanada, H., Mugii, S., Okubo, M., Maeda, I., Kuwayama, K., Hidaka, Y., et al. (2012). Establishment of chemiluminescence enzyme immunoassay for apolipoprotein B-48 and its clinical applications for evaluation of impaired chylomicron remnant metabolism. *Clin Chim Acta 413*, 160–165.

Havel, R. J. (2000). Remnant lipoproteins as therapeutic targets. *Curr Opin Lipidol 11*, 615–620.

Havel, R. J. (2010). Triglyceride-rich lipoproteins and plasma lipid transport. *Arterioscler Thromb Vasc Biol 30*, 9–19.

Havel, R. J., & Kane, J. P. (2001). Introduction: Structure and metabolism of plasma lipoproteins. In C. R. Scriver, A. L. Beaudet, W. S. Sly, & D. Valle (Eds.), *The metabolic and molecular bases of inherited diseases* (pp. 2705–2716) (8th ed.). Mc-Graw-Hill, Medical Publishing Division.

Haynes, R., Jiang, L., Hopewell, J. C., Li, J., Chen, F., Parish, S., et al. (2013). HPS2-THRIVE randomized placebo-controlled trial in 25 673 high-risk patients of ER niacin/laropiprant: Trial design, pre-specified muscle and liver outcomes, and reasons for stopping study treatment. *Eur Heart J 34*, 1279–1291.

Hegele, R. A., Ban, M. R., Hsueh, N., Kennedy, B.A., Cao, H., Zou, G. Y., et al. (2009). A polygenic basis for four classical Fredrickson hyperlipoproteinemia phenotypes that are characterized by hypertriglyceridemia. *Hum Mol Genet 18*, 4189–4194.

Higashi, K., Ito, T., Nakajima, K., Yonemura, A., Nakamura, H., & Ohsuzu, F. (2001). Remnant-like particles cholesterol is higher in diabetic patients with coronary artery disease. *Metabolism 50*, 1462–1465.

Hiki, M., Shimada, K., Ohmura, H., Kiyanagi, T., Kume, A., Sumiyoshi, K., et al. (2009). Serum levels of remnant lipoprotein cholesterol and oxidized low-density lipoprotein in patients with coronary artery disease. *J Cardiol 53*, 108–116.

Hokanson, J. E., & Austin, M.A. (1996). Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: A meta-analysis of population-based prospective studies. *J Cardiovasc Risk 3*, 213–219.

Hopkins, P. N., Wu, L. L., Hunt, S.C., & Brinton, E. A. (2005). Plasma triglycerides and type III hyperlipidemia are independently associated with premature familial coronary artery disease. *J Am Coll Cardiol 45*, 1003–1012.

HPS2-THRIVE press release on preliminary results (2013). http://www.thrivestudy.org/press_release.htm

Imke, C., Rodriguez, B.L., Grove, J. S., McNamara, J. R., Waslien, C., Katz, A.R., et al. (2005). Are remnant-like particles independent predictors of coronary heart disease incidence? The Honolulu Heart study. *Arterioscler Thromb Vasc Biol 25*, 1718–1722.

Inoue, T., Uchida, T., Kamishirado, H., Takayanagi, K., Hayashi, T., Morooka, S., et al. (2004). Remnant-like lipoprotein particles as risk factors for coronary artery disease in elderly patients. *Horm Metab Res 36*, 298–302.

Johansen, C. T., & Hegele, R. A. (2011). Genetic bases of hypertriglyceridemic phenotypes. *Curr Opin Lipidol 22*, 247–253.

Johansen, C. T., Kathiresan, S., & Hegele, R. A. (2011). Genetic determinants of plasma triglycerides. *J Lipid Res 52*, 189–206.

Johansen, C. T., Wang, J., Lanktree, M. B., McIntyre, A.D., Ban, M. R., Martins, R. A., et al. (2011). An increased burden of common and rare lipid-associated risk alleles contributes to the phenotypic spectrum of hypertriglyceridemia. *Arterioscler Thromb Vasc Biol 31*, 1916–1926.

Jorgensen, A.B., Frikke-Schmidt, R., West, A. S., Grande, P., Nordestgaard, B. G., & Tybjaerg-Hansen, A. (2013). Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. *Eur Heart J 34*, 1826–1833.

Jun, M., Foote, C., Lv, J., Neal, B., Patel, A., Nicholls, S. J., et al. (2010). Effects of fibrates on cardiovascular outcomes: A systematic review and meta-analysis. *Lancet 375*, 1875–1884.

Karpe, F., Boquist, S., Tang, R., Bond, G. M., De, F. U., & Hamsten, A. (2001). Remnant lipoproteins are related to intima-media thickness of the carotid artery independently of LDL cholesterol and plasma triglycerides. *J Lipid Res 42*, 17–21.

Kathiresan, S., Melander, O., Guiducci, C., Surti, A., Burtt, N.P., Rieder, M. J., et al. (2008). Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. *Nat Genet 40*, 189–197.

Kiss-Toth, E., Bagstaff, S. M., Sung, H. Y., Jozsa, V., Dempsey, C., Caunt, J. C., et al. (2004). Human tribbles, a protein family controlling mitogen-activated protein kinase cascades. *J Biol Chem 279*, 42703–42708.

Kohler, H. P., & Grant, P. J. (2000). Plasminogen-activator inhibitor type 1 and coronary artery disease. *N Engl J Med 342*, 1792–1801.

Kolovou, G. D., Mikhailidis, D. P., Kovar, J., Lairon, D., Nordestgaard, B. G., Ooi, T. C., et al. (2011). Assessment and clinical relevance of non-fasting and postprandial triglycerides: An expert panel statement. *Curr Vasc Pharmacol 9*, 258–270.

Kronenberg, F., & Utermann, G. (2013). Lipoprotein(a): Resurrected by genetics. *J Intern Med 273*, 6–30.

Kugiyama, K., Doi, H., Takazoe, K., Kawano, H., Soejima, H., Mizuno, Y., et al. (1999). Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. *Circulation 99*, 2858–2860.

Kwak, S. M., Myung, S. K., Lee, Y. J., & Seo, H. G. (2012). Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular disease: A meta-analysis of randomized, double-blind, placebo-controlled trials. *Arch Intern Med 172*, 686–694.

Lamon-Fava, S., Herrington, D.M., Reboussin, D.M., Sherman, M., Horvath, K. V., Cupples, L. A., et al. (2008). Plasma levels of HDL subpopulations and remnant lipoproteins predict the extent of angiographically-defined coronary artery disease in postmenopausal women. *Arterioscler Thromb Vasc Biol 28*, 575–579.

Langsted, A., Freiberg, J. J., Tybjaerg-Hansen, A., Schnohr, P., Jensen, G. B., & Nordestgaard, B. G. (2011). Nonfasting cholesterol and triglycerides and association with risk of myocardial infarction and total mortality: The Copenhagen City Heart Study with 31 years of follow-up. *J Intern Med 270*, 65–75.

Lavigne, P.M., & Karas, R. H. (2013). The current state of niacin in cardiovascular disease prevention: A systematic review and meta-regression. *J Am Coll Cardiol 61*, 440–446.

Lee, P., & Hegele, R. A. (2013). Current Phase II proprotein convertase subtilisin/kexin 9 inhibitor therapies for dyslipidemia. *Expert Opin Investig Drugs 22*, 1411–1423.

MacArthur, J. P., Bishop, J. R., Stanford, K. I., Wang, L., Bensadoun, A., Witztum, J. L., et al. (2007). Liver heparan sulfate proteoglycans mediate clearance of triglyceride-rich lipoproteins independently of LDL receptor family members. *J Clin Invest 117*, 153–164.

Masuoka, H., Ishikura, K., Kamei, S., Obe, T., Seko, T., Okuda, K., et al. (1998). Predictive value of remnant-like particles cholesterol/high-density lipoprotein cholesterol ratio as a new indicator of coronary artery disease. *Am Heart J 136*, 226–230.

Masuoka, H., Kamei, S., Ozaki, M., Kawasaki, A., Shintani, U., Ito, M., et al. (2000). Predictive value of remnant-like particle cholesterol as an indicator of coronary artery stenosis in patients with normal serum triglyceride levels. *Intern Med 39*, 540–546.

Masuoka, H., Kamei, S., Wagayama, H., Ozaki, M., Kawasaki, A., Tanaka, T., et al. (2000). Association of remnant-like particle cholesterol with coronary artery disease in patients with normal total cholesterol levels. *Am Heart J 139*, 305–310.

McNamara, J. R., Shah, P. K., Nakajima, K., Cupples, L. A., Wilson, P. W., Ordovas, J. M., et al. (2001). Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women: Results from the Framingham Heart Study. *Atherosclerosis 154*, 229–236.

Miwa, K., Makita, T., Ishii, K., Okuda, N., & Taniguchi, A. (2004). High remnant lipoprotein levels in patients with variant angina. *Clin Cardiol 27*, 338–342.

Mora, S., Rifai, N., Buring, J. E., & Ridker, P.M. (2008). Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation 118*, 993–1001.

Moyer, M. P., Tracy, R. P., Tracy, P. B., van't Veer, C., Sparks, C. E., & Mann, K. G. (1998). Plasma lipoproteins support prothrombinase and other procoagulant enzymatic complexes. *Arterioscler Thromb Vasc Biol 18*, 458–465.

Nakajima, K., Nakano, T., & Tanaka, A. (2006). The oxidative modification hypothesis of atherosclerosis: The comparison of atherogenic effects on oxidized LDL and remnant lipoproteins in plasma. *Clin Chim Acta 367*, 36–47.

Nakajima, K., Nakano, T., Tokita, Y., Nagamine, T., Inazu, A., Kobayashi, J., et al. (2011). Postprandial lipoprotein metabolism: VLDL vs chylomicrons. *Clin Chim Acta 412*, 1306–1318.

Nakajima, K., Saito, T., Tamura, A., Suzuki, M., Nakano, T., Adachi, M., et al. (1993). Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. *Clin Chim Acta 223*, 53–71.

Nakamura, T., Takano, H., Umetani, K., Kawabata, K., Obata, J. E., Kitta, Y., et al. (2005). Remnant lipoproteinemia is a risk factor for endothelial vasomotor dysfunction and coronary artery disease in metabolic syndrome. *Atherosclerosis 181*, 321–327.

Nicholls, S. J., Brewer, H. B., Kastelein, J. J., Krueger, K. A., Wang, M.D., Shao, M., et al. (2011). Effects of the CETP inhibitor evacetrapib administered as monotherapy or in combination with statins on HDL and LDL cholesterol: A randomized controlled trial. *JAMA 306*, 2099–2109.

Nordestgaard, B. G., Benn, M., Schnohr, P., & Tybjaerg-Hansen, A. (2007). Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA 298*, 299–308.

Nordestgaard, B. G., Chapman, M. J., Ray, K., Boren, J., Andreotti, F., Watts, G. F., et al. (2010). Lipoprotein(a) as a cardiovascular risk factor: Current status. *Eur Heart J 31*, 2844–2853.

Nordestgaard, B. G., & Freiberg, J. J. (2011). Clinical relevance of non-fasting and postprandial hypertriglyceridemia and remnant cholesterol. *Curr Vasc Pharmacol 9*, 281–286.

Nordestgaard, B. G., Langsted, A., & Freiberg, J. J. (2009). Nonfasting hyperlipidemia and cardiovascular disease. *Curr Drug Targets 10*, 328–335.

Nordestgaard, B. G., Stender, S., & Kjeldsen, K. (1988). Reduced atherogenesis in cholesterol-fed diabetic rabbits. Giant lipoproteins do not enter the arterial wall. *Arteriosclerosis 8*, 421–428.

Nordestgaard, B. G., Tybjaerg-Hansen, A., & Lewis, B. (1992). Influx in vivo of low density, intermediate density, and very low density lipoproteins into aortic intimas of genetically hyperlipidemic rabbits. Roles of plasma concentrations, extent of aortic lesion, and lipoprotein particle size as determinants. *Arterioscler Thromb 12*, 6–18.

Nordestgaard, B. G., Wootton, R., & Lewis, B. (1995). Selective retention of VLDL, IDL, and LDL in the arterial intima of genetically hyperlipidemic rabbits in vivo. Molecular size as a determinant of fractional loss from the intima-inner media. *Arterioscler Thromb Vasc Biol 15*, 534–542.

Nordestgaard, B. G., & Zilversmit, D. B. (1988). Large lipoproteins are excluded from the arterial wall in diabetic cholesterol-fed rabbits. *J Lipid Res 29*, 1491–1500.
Oi, K., Shimokawa, H., Hirakawa, Y., Tashiro, H., Nakaike, R., Kozai, T., et al. (2004). Postprandial increase in plasma concentrations of remnant-like particles: An independent risk factor for restenosis after percutaneous coronary intervention. *J Cardiovasc Pharmacol 44*, 66–73.
Ooi, T. C., & Nordestgaard, B. G. (2011). Methods to study postprandial lipemia. *Curr Vasc Pharmacol 9*, 302–308.
Raal, F. J., Santos, R. D., Blom, D. J., Marais, A.D., Charng, M. J., Cromwell, W. C., et al. (2010). Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: A randomised, double-blind, placebo-controlled trial. *Lancet 375*, 998–1006.
Ramasamy, I. (2013). Recent advances in physiological lipoprotein metabolism. *Clin Chem Lab Med 12*, 1–33.
Rapp, J. H., Lespine, A., Hamilton, R. L., Colyvas, N., Chaumeton, A. H., Tweedie-Hardman, J., et al. (1994). Triglyceride-rich lipoproteins isolated by selected-affinity anti-apolipoprotein B immunosorption from human atherosclerotic plaque. *Arterioscler Thromb 14*, 1767–1774.
Rizos, E. C., Ntzani, E. E., Bika, E., Kostapanos, M. S., & Elisaf, M. S. (2012). Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: A systematic review and meta-analysis. *JAMA 308*, 1024–1033.
Sacks, F. M., Carey, V. J., & Fruchart, J. C. (2010). Combination lipid therapy in type 2 diabetes. *N Engl J Med 363*, 692–694.
Sakata, K., Miho, N., Shirotani, M., Yoshida, H., Takada, Y., & Takada, A. (1998). Remnant-like particle cholesterol is a major risk factor for myocardial infarction in vasospastic angina with nearly normal coronary artery. *Atherosclerosis 136*, 225–231.
Sambola, A., Osende, J., Hathcock, J., Degen, M., Nemerson, Y., Fuster, V., et al. (2003). Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. *Circulation 107*, 973–977.
Schwartz, G. G., Olsson, A. G., Abt, M., Ballantyne, C. M., Barter, P. J., Brumm, J., et al. (2012). Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med 367*, 2089–2099.
Shaikh, M., Wootton, R., Nordestgaard, B. G., Baskerville, P., Lumley, J. S., La Ville, A. E., et al. (1991). Quantitative studies of transfer in vivo of low density, Sf 12–60, and Sf 60–400 lipoproteins between plasma and arterial intima in humans. *Arterioscler Thromb 11*, 569–577.
Song, J., Park, H., Hong, S. H., Lee, H. K., Park, Y. B., & Kim, J. Q. (2000). Remnant-like particle cholesterol levels in Korean patients with coronary artery disease and non-insulin dependent diabetes mellitus. *Clin Chem Lab Med 38*, 427–432.
Stalenhoef, A. F., & de Graaf, J. (2008). Association of fasting and nonfasting serum triglycerides with cardiovascular disease and the role of remnant-like lipoproteins and small dense LDL. *Curr Opin Lipidol 19*, 355–361.
Takeichi, S., Yukawa, N., Nakajima, Y., Osawa, M., Saito, T., Seto, Y., et al. (1999). Association of plasma triglyceride-rich lipoprotein remnants with coronary atherosclerosis in cases of sudden cardiac death. *Atherosclerosis 142*, 309–315.
Teslovich, T. M., Musunuru, K., Smith, A. V., Edmondson, A.C., Stylianou, I. M., Koseki, M., et al. (2010). Biological, clinical and population relevance of 95 loci for blood lipids. *Nature 466*, 707–713.
Ting, H. J., Stice, J. P., Schaff, U. Y., Hui, D. Y., Rutledge, J. C., Knowlton, A. A., et al. (2007). Triglyceride-rich lipoproteins prime aortic endothelium for an enhanced inflammatory response to tumor necrosis factor-alpha. *Circ Res 100*, 381–390.
Varbo, A., Benn, M., Tybjaerg-Hansen, A., Grande, P., & Nordestgaard, B. G. (2011). TRIB1 and GCKR polymorphisms, lipid levels, and risk of ischemic heart disease in the general population. *Arterioscler Thromb Vasc Biol 31*, 451–457.
Varbo, A., Benn, M., Tybjaerg-Hansen, A., Jorgensen, A.B., Frikke-Schmidt, R., & Nordestgaard, B. G. (2013). Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol 61*, 427–436.
Varbo, A., Benn, M., Tybjaerg-Hansen, A., & Nordestgaard, B. G. (2013). Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, while elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. *Circulation 128*, 1298–1309.
Varbo, A., Nordestgaard, B. G., Tybjaerg-Hansen, A., Schnohr, P., Jensen, G. B., & Benn, M. (2011). Nonfasting triglycerides, cholesterol, and ischemic stroke in the general population. *Ann Neurol 69*, 628–634.
Wang, L., Gill, R., Pedersen, T. L., Higgins, L. J., Newman, J. W., & Rutledge, J. C. (2009). Triglyceride-rich lipoprotein lipolysis releases neutral and oxidized FFAs that induce endothelial cell inflammation. *J Lipid Res 50*, 204–213.
Willer, C. J., Sanna, S., Jackson, A. U., Scuteri, A., Bonnycastle, L. L., Clarke, R., et al. (2008). Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet 40*, 161–169.
Yuan, G., Al-Shali, K. Z., & Hegele, R. A. (2007). Hypertriglyceridemia: Its etiology, effects and treatment. *CMAJ 176*, 1113–1120.
Zheng, X. Y., & Liu, L. (2007). Remnant-like lipoprotein particles impair endothelial function: Direct and indirect effects on nitric oxide synthase. *J Lipid Res 48*, 1673–1680.
Zilversmit, D. B. (1979). Atherogenesis: A postprandial phenomenon. *Circulation 60*, 473–485.