HAL
open science

Remnant lipoproteins: are they equal to or more atherogenic than LDL?

Carlos a Aguilar Salinas, M. John Chapman

To cite this version:

Carlos a Aguilar Salinas, M. John Chapman. Remnant lipoproteins: are they equal to or more atherogenic than LDL?. Current Opinion in Lipidology, 2020, 31 (3), pp.132-139. 10.1097/MOL.0000000000000682 . hal-03014044

HAL Id: hal-03014044

https://hal.sorbonne-universite.fr/hal-03014044

Submitted on 19 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Title : Remnant lipoproteins: Are they equal to or more atherogenic than LDL ?

Authors : Carlos A. Aguilar Salinas[1,2,3] and M. John Chapman[4,5,6,7]

Authors' affiliations:

[1]Unidad de Investigación en Enfermedades Metabólicas. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Mexico City.
[2]Departamento de Endocrinología y Metabolismo. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Mexico City.
[3]Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Ave. Morones Prieto 3000, Monterrey, N.L., México, 64710.
[4] Endocrinology-Metabolism Division, Pitie-Salpetriere University Hospital, Paris 75651, France.
[5] Faculty of Medicine, Sorbonne University, Paris 75651, France.
[6] National Institute for Health and Medical Research (INSERM), Paris 75013, France
[7] Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia

Corresponding Author :

M John Chapman BSc (Hons), PhD, DSc, EFESC

Endocrinology-Metabolism Division, Pitie-Salpetriere University Hospital, Paris 75651, France.

Tel: 331.4875.6328

Email address: john.chapman@upmc.fr

ABSTRACT :

Purpose of review

To critically appraise new insights into the biology of remnant lipoproteins and their putative role in the pathophysiology of atherosclerotic cardiovascular disease, and to compare the atherogenicity of remnant particles with that of LDL.

Recent findings

New in vivo stable isotope tracer studies of the kinetics of apoB48- and apoB100-containing lipoproteins in postprandial conditions have revealed that apoB48-containing VLDL accumulated markedly in hypertriglyceridemic subjects. These intestinally-derived particles were cleared slowly, and represented up to 25% of circulating VLDL; as part of the remnant particle population, they may increase cardiovascular risk. Importantly, the PCSK9 inhibitor, evolocumab, was shown to reduce remnant levels (-29%) during the postprandial period in diabetic subjects on statin therapy, an effect which may be additive to that of LDL cholesterol reduction in conferring cardiovascular benefit. In recent Mendelian randomization studies, the effect of lowering triglyceride-rich lipoproteins or LDL cholesterol translated to similar clinical benefit per unit of apoB. Finally, in randomized trials involving statin-treated patients with ASCVD, remnant cholesterol levels were associated with coronary atheroma progression independently of LDL cholesterol.

Summary

Overall, data from observational studies in large cohorts, Mendelian randomization studies, meta-regression analyses and post-hoc analyses of randomized trials are consistent with the contention that remnants are highly atherogenic particles and contribute to the atherosclerotic burden in an equivalent manner to that of LDL.

Abbreviations :

Apo, apolipoprotein

ASCVD, atherosclerotic cardiovascular disease

VLDL, very low-density lipoproteins

IDL, intermediate-density lipoproteins

HDL, high-density lipoproteins

LDL, low-density lipoproteins

CE, cholesteryl ester

CETP, cholesteryl ester transfer protein

TGRL, triglyceride-rich lipoproteins

Sf, Svedberg flotation rate

Keywords:

Remnant cholesterol, LDL cholesterol, dysbetalipoproteinemia, apolipoprotein B, apolipoprotein E, apolipoprotein CIII ; matrix proteoglycans

Introduction :

Recent findings have furthered our understanding of the complex biology of circulating triglyceride-rich lipoproteins (TGRL) and their remnants, and of their relevance to the pathophysiology of atherosclerotic cardiovascular disease (ASCVD). Indeed, we now recognize that in both the fasting and post-prandial periods, remnant particles are produced by intravascular remodelling of both intestinally-derived chylomicrons and very low-density lipoproteins (VLDL) of hepatic origin. In plasma, such particles are distributed widely across the density intervals of both VLDL (d<1.006 g/mL; including chylomicrons defined as Sf >400) and intermediate density lipoproteins (IDL; d<1.006-1.019 g/mL)[1,2••]. Equally present as components of these highly heterogeneous, density-defined, lipoprotein fractions are newly-secreted chylomicrons and VLDL. Importantly, new evidence in human subjects shows that such VLDL may be of both intestinal as well as hepatic origin, the former containing apoB48 rather than apoB100. As a consequence, both intestinal chylomicrons and their remnants are identified by their content of a single copy of apolipoprotein (apo) B48, while particles isolated in the d<1.006 g/mL density fraction may contain either apoB48 or apoB100 according to their tissue origin [1,2••].

As a function of triglyceride pool size, and of the metabolic, genetic and nutritional background in a given individual, part of the hepatic apoB100- containing remnant pool is converted to cholesterol-rich LDL by further remodeling by lipases and cholesteryl ester transfer protein (CETP); the remainder is taken up by hepatocytes via multiple mechanisms [1-4]. The relative contents of apoE and apoCIII in TGRL particles are critical not only in the formation of VLDL remnants, but also in their rates of hepatic clearance relative to their propensity to form LDL [3•,5,6•].

A substantial body of evidence now attests to the indisputable causal role of LDL in the pathophysiology of ASCVD, and is based upon the fulfillment of eight key criteria of causality (Table 1)[7]. A critical question now arises: are remnants of triglyceride-rich lipoproteins, the

direct precursors of LDL, of similar, lesser or greater atherogenic potential than LDL particles themselves ? In this review, we present a comprehensive evaluation of recent evidence from pathophysiological, epidemiologic, genetic, Mendelian randomisation, and intervention studies that links remnant particles with atherosclerosis and cardiovascular events. Such evidence has prompted the proposal that TGRL and their remnants represent targets for the therapeutic lowering of circulating concentrations of these particles, with subsequent reduction in cardiovascular risk [3•,8,9].

Atherobiology of remnants :

The physicochemical characteristics of remnant particles are intimately linked to their atherogenic potential. Firstly, the particle size of small chylomicron and VLDL remnants (≈250-700 Å) is consistent with their entry by transcytosis into the arterial intima at sites of predilection of atherosclerotic lesion formation, where they may be retained by electrostatic interaction between their principal apolipoproteins (apoB and apoE) and matrix proteoglycans [1,3,4,8,9,10]. Importantly, and unlike LDL, remnants efflux slowly in relation to their rates of entry [11]. Furthermore, the enrichment of remnants in apoCIII amplifies their retention to matrix proteoglycan biglycan by an unidentified mechanism [12]. Significantly, the intravascular cholesteryl ester transfer protein (CETP)-mediated enrichment of remnants in cholesteryl ester (CE) confers them with elevated cholesterol content, thereby favouring arterial cholesterol accumulation. Indeed, it is estimated that a typical remnant in the IDL range of size and molecular weight containing some 30% by weight of cholesterol (after correction for the CE fatty acid) may contain up to a maximum of a fourfold greater absolute number of cholesterol molecules (up to ≈ 8600 per particle) as compared to an LDL particle (2000-2700)[3••,13]. Secondly, in addition to containing a single copy of either apoB48 or B100, the proteome of remnant particles is typically enriched in apoE relative to other TGRL, but equally features apoCIII, apoCII, apoCI, apoAV and angiopoietin-like protein (ANGPTL) -3, together with traces of apoAI and apoAII [1,4,8,9,14]. ApoE is a major determinant not only of the binding and uptake of remnants by both receptor-dependent and

receptor-independent mechanisms in hepatocytes, but importantly, the receptor-mediated endocytosis of native remnants by surface receptors (LDL receptor, LDL receptor-related protein 1) of subendothelial monocyte-derived macrophages [1,4,9,14]. Remnant lipoprotein uptake by intimal macrophages is thus a key driver of macrophage foam cell formation, the hallmark cell of the atherosclerotic plaque. Such remnant-mediated cholesterol accumulation favours macrophage M1 polarisation in the intimal microenvironment, with an enhanced inflammatory response [15]. Moreover, proinflammatory macrophages secrete a spectrum of factors which promote lesion development and ultimately destabilisation; these include matrix-degrading proteases, prooxidant enzymes, lipases, growth factors, proinflammatory cytokines, eicosanoids, proteoglycans and procoagulant factors [16]. Triglyceride-rich lipoproteins and their remnants may equally enhance local vascular inflammation in the subendothelial space by virtue of the release of free fatty acids and other hydrolytic lipid derivatives, such as proinflammatory lysolipids, upon lipolysis induced by lipoprotein lipase (LPL), phospholipases and sphingomyelinases [11].

Overall, the contribution of lipoproteins to the atherogenic burden depends on several factors, including plasma abundance and residence time, as well as endothelial entry rates via transcytosis, the degree of retention in arterial tissue and the propensity to elicit both foam cell formation and a maladaptive response. The remarkably greater abundance and long half-life of LDL particles (≈250 mg/dL; up to 3 days) relative to that of remnants argue strongly for LDL particles as the main target for prevention of ASCVD. However, recent estimates suggest that up to 30% of the cholesterol load in apoB-containing lipoproteins is transported in remnant particles under nonfasting conditions [17]. In this context, in vivo kinetic studies of remnant particle metabolism, performed under metabolic conditions during which the arterial wall is most exposed to these particles, ie. the post-prandial period, are of immediate relevance. Indeed, TGRL and remnant levels are typically present at elevated levels over periods as long as 8 hours or more depending on the lipolytic capacity of the individual. Thus, using a new stable isotope-based tracer approach and an integrated non-steady state multicompartmental model, Björnson and colleagues showed that intestinally-derived, apoB48-

containing chylomicrons and VLDL remain in plasma for a markedly longer period of time than previously observed (several hours in the VLDL range instead of a few minutes)[2••]. Such particles may be remodelled to remnants, particularly in hypertriglyceridemic subjects. Consequently, apoB48-containing lipoproteins potentially contribute significantly to the remnant-related atherogenic burden. Considered together, the above pathophysiological and metabolic evidence highlights the multiple, interactive and mutually complementary mechanisms which underlie the atherogenicity of both apoB48- and apoB100-containing remnants.

Epidemiologic studies of remnant cholesterol:

Extensive observational evidence has linked plasma accumulation of both LDL and remnants with the incidence of cardiovascular events [18-22]. The association between LDL cholesterol levels and the incidence of cardiovascular events has been the subject of multiple studies with many thousands of participants [7]. Comparable evidence for remnants is strong but less substantial. The Copenhagen General Population Study and the Copenhagen City Heart Study, in which nearly 90,000 subjects have participated, provides seminal information however [14,19-22]. Confirmatory reports have been published in Chinese populations [23] and in selected groups as exemplified by type 2 diabetes [24]. Nonetheless, the strength of the evidence is limited by methodological issues, as the concentration of remnant particles was estimated indirectly using levels of either plasma triglycerides or calculated remnant cholesterol (as total cholesterol - LDL-cholesterol - HDL-cholesterol). Plasma triglyceride is not an ideal marker, as it may be determined in part by the presence of other particles that are not strongly associated with atherosclerosis (ie. chylomicrons and large VLDLs); a similar comment applies to determination of remnant cholesterol levels when measured in a corresponding manner (14,19,20,21,22-24). In addition, the contribution of remnants could be under-represented in many studies, as determinations have frequently been performed under fasting conditions. Despite such potential limitations, those subjects in the highest versus the lowest percentile of non-fasting remnant cholesterol concentration in the prospective Copenhagen cohorts displayed a 2.4 fold increased

risk for ischemic heart disease [19]. When an equivalent comparison was made for LDL-cholesterol, the corresponding hazard ratio for risk was 2.3. The hazard ratio for myocardial infarction attained a maximum of 3.4 for remnant cholesterol and 4.7 for LDL-cholesterol. Remarkably, remnant cholesterol concentration was superior in predicting all-cause mortality relative to LDL cholesterol [19].

Evidence from Mendelian randomization studies:

Sets of genetic variants that are implicated in the determination of LDL cholesterol levels are strongly associated with cardiovascular events, thereby constituting robust evidence that these particles exert a causal role in atherosclerosis [7]. In contrast, the case for remnants is more complex as the genetic variants that influence their circulating concentrations exert pleiotropic effects, having additional targets and impact on lipid and lipoprotein metabolism [14,22,25]. However, a recent analysis based on the UK biobank (n=654,783 participants) compared the association between coronary heart disease (n=91,129 cases) and sets of genetic variants that themselves are associated with lower levels of either LDL (via the LDL receptor) or triglycerides (by modulating lipoprotein lipase (LPL) activity)[26••]. As LPL action represents a critical step in the progressive transformation of chylomicrons or VLDL to remnants, this approach has the potential to compare the contribution of LDL relative to that of remnants with respect to cardiovascular risk. Thus, variants associated with lower triglyceride levels should equally have an effect on apoB concentrations in order to be associated with CHD, even if this effect is associated with a small increment in LDL cholesterol concentrations. Remarkably, both LPL and LDLR scores were associated with similar reduction in risk of CHD per 10mg/dL lower level of apoB [26••]. This finding indicates that the effect of lowering TGRL on the one hand, and LDL cholesterol on the other, translates into clinical benefit which is proportional to the absolute reduction in their content of apoB. Such evidence again suggests that a particle per particle equivalence may exist when comparing the atherogenic potential of TGRL and remnant particles with that of LDL.

Evidence from genetic disorders of lipid metabolism:

Monogenic conditions, as exemplified by elevated circulating concentrations of LDL in familial hypercholesterolemia (FH), and of lipoprotein remnant particles in dysbetalipoproteinemia, are natural experiments in which the respective atherogenic roles of these lipoproteins can be prospectively assessed. These disorders are characterised by selective clearance defects giving rise to LDL or remnant accumulation, the latter primarily in the IDL density interval [27,28]. In FH, the genetic defect is located mainly in the LDL receptor, and to a lesser degree in the apoB and PCSK9 genes [27]. By contrast, the most common cause of dysbetalipoproteinemia is homozygosity for the receptor binding-defective form of apoE (apo *E2/E2* genotype), which is the main ligand for the family of LDL receptors that remove remnants from plasma [28]. ApoE2/E2 status requires the coexistence of a secondary cause of hyperlipidemia for phenotypic expression with severe accumulation of remnants. Although both conditions are associated with a markedly increased risk for premature ASCVD, there are major differences in the clinical expression between these two entities. The age of onset of hyperlipidemia is greater in dysbetalipoproteinemia; as a consequence, dysbetalipoproteinemia cases may present normal lipid concentrations for many years. In contrast, hypercholesterolemia is present at birth in FH. In addition, the clinical phenotypes tend to be distinct; thus, palmar crease xanthomata are seen in dysbetalipoproteinemia, while this cutaneous manifestation is rare in FH. Both disorders feature premature coronary atherosclerosis; peripheral artery disease is as common as coronary atherosclerosis however in dysbetalipoproteinemia [29]. These observations suggest that the impact of the plasma accumulation of LDL particles on the arterial wall is not the same as that for remnants, and may differ according to sites in the arterial tree. Regrettably, the lack of registries of patients with dysbetalipoproteinemia precludes comparison of the magnitude of cardiovascular risk with that in FH. Finally, in some cases of FH, concurrent accumulation of both LDL and remnants occurs, with more severe atherosclerotic lesions resulting [30]. Furthermore, remnant cholesterol concentration was an

independent predictor of the presence of cardiovascular disease in FH patients (n=282) in this study [30].

Evidence from randomized controlled studies:

LDL-centered therapies, notably statins, ezetimibe and PCK9 antibodies, have consistently proven to be effective in reducing the incidence of cardiovascular events; some 95% of the beneficial effects of such therapies can be accounted for by the magnitude of the absolute LDL cholesterol reduction [1]. Interestingly however, Taskinen and colleagues have recently provided evidence that the PCSK9 inhibitor, evolocumab, significantly reduces remnant cholesterol levels (-29%) during the postprandial response in diabetic subjects on background statin therapy, a finding consistent with the higher absolute cardiovascular risk reduction observed in patients with diabetes (2.7%) versus patients without diabetes (1.6%) in the Fourier trial [31•, 32].

Ideally, clinical intervention trials in dyslipidemic patients with lipid lowering agents targeted specifically to remnant particles would permit a more direct comparison of their atherogenic potential relative to that of LDL. The design of such trials would prove singularly challenging however, particularly given the intricate metabolic relationships of remnants with their upstream TGRL precursors and downstream LDL products, and the need to neutralise the component of cardiovascular risk due to LDL and possibly Lp(a). Moreover, the link between lipolysis of TGRL and the role of the sequestration of surface lipolytic fragments to maintenance of the HDL pool should not be ignored. Nonetheless, remnant lipoproteins (measured as remnant-cholesterol) were clearly identified as a component of the residual risk in ASCVD patients with on-statin LDL cholesterol at the guideline-recommended goal of <70mg/dL, consistent with the proposal that therapeutic targeting of remnants is desirable [32].

Fibrates, niacin and omega-3 fatty acids are currently indicated for the treatment of hypertriglyceridemic states; statins, ezetimibe and PCSK9 inhibitors exert modest effects (typically <15%) which depend largely on triglyceride levels at baseline [6•,14,33]. However, fibrates, niacin and

omega-3 fatty acid preparations influence concentrations of all major lipoprotein classes by direct or indirect mechanisms, have minor effects on LDL cholesterol, and therefore cannot be considered simply as remnant-lowering agents. In particular, the effect of fibrates and niacin in the prevention of cardiovascular events has been inconsistent, particularly when evaluated in combination with statin therapy [6•,14,33]. These drugs lower apoB concentrations to a minor degree (≤20%)[16,14,33]; consideration of issues including study design, the enrolment of patients who were moderately hypertriglyceridemic at most, together with methodological issues, may explain the negative or neutral results of prevention trials based on fibrate and niacin therapies [14,33].

A recent meta-regression analysis of trial level data has addressed the generic question as to whether reduction in triglyceride levels translates into cardiovascular benefit across three lipid-lowering therapeutic classes, and included fibrates, niacin and omega-3 fatty acids; data from an earlier meta-regression of statin trials was equally integrated into the analysis [34]. Although triglyceride lowering translated overall to a lower risk of vascular events, even after adjustment for changes in LDL cholesterol, these findings should be regarded with caution, as driven by the positive results obtained with high dose eicosapentanoic acid (EPA)(icosapent ethyl; 4g/day) in high risk patients on background statin therapy in the REDUCE-IT trial [35••]. Indeed, although the cardiovascular benefit (a 25% reduction in the primary composite cardiovascular endpoint in the intervention group relative to placebo) in the REDUCE-IT trial occurred across a wide range of baseline triglyceride levels, the mechanism(s) which underlies such benefit remains speculative. Nonetheless, we cannot exclude the possibility that hypertriglyceridemic participants with either ASCVD or diabetes and other risk factors in REDUCE-IT, and who would be anticipated to exhibit an amplified postprandial response with elevated remnant levels, may have gained major benefit from attenuation of remnant atherogenicity consistent with a plasma triglyceride reduction on treatment of 20% versus placebo.

The relationship of remnant cholesterol levels to the progression of atherosclerotic disease is of special interest. In this context, Elshazly and colleagues estimated the contribution of remnant cholesterol to changes in % coronary atheroma volume (determined by intravascular ultrasound) and

2-year major cardiovascular events in 10 clinical trials (n=5754) involving both statin and non-statin therapies [36•]. Remnant cholesterol was determined as non-HDL cholesterol – LDL cholesterol. In multivariable analyses, on-treatment remnant cholesterol was significantly correlated with change in plaque volume, even after adjusting for LDL cholesterol and other risk factors. A positive correlation was equally found between the highest on-treatment remnant cholesterol quartile and the incidence of cardiovascular events as compared to the lowest. Overall, the correlations were stronger for on-treatment remnant cholesterol than for on-treatment LDL cholesterol. Thus, these reports support the contention that remnant cholesterol constitutes a complimentary target to LDL cholesterol for the prevention of cardiovascular events.

Conclusions

Table 1 summarizes and compares the evidence for key criteria supporting a causal role for remnants on the one hand, and LDL on the other, in the pathophysiology of ASCVD. Indeed, both LDL and remnants fulfill eight criteria consistent with the assumption of causality (Table 1). However, the relative contribution of LDL relative to that of remnants to the atherogenic process may differ substantially as a function of the nutritional, metabolic, genetic and pharmacological profile in any given individual [37]. Nonetheless, we submit that particle for particle, the weight of the evidence to date is consistent with a markedly similar atherogenic potential for both an LDL particle and a cholesterol-, apoE-rich remnant. Remnant particles may therefore be considered as a target for therapy, and especially in very high and high risk statin-treated patients with established ASCVD in which guideline recommended LDL cholesterol goals have been achieved, but in whom residual atherosclerotic cardiovascular disease risk subsists [8,9,38•].

It is in this light that the ongoing development of innovative agents which will have potentially major impact on remnant levels is especially encouraging; notable among them are pemafibrate, a new generation fibrate currently under evaluation in a cardiovascular outcome trial (PROMINENT) in dyslipidemic diabetic subjects, as well as agents targeted to upregulate TGRL lipolysis- and potentially hepatic remnant clearance - by virtue of the inhibition of apoCIII or ANGPTL3 [40,41].

Finally, a review of the multiple facets of the atherogenicity of remnant particles leads inevitably to the identification of some of the key, unsolved questions of both clinical and scientific relevance, which are summarised in Table 2. Such questions can be anticipated to stimulate further research – and ultimately to advance our understanding of the complex biology of remnant particles.

KEY POINTS:

1) In vivo turnover data in non-fasting hypertriglyceridemic subjects indicate that the intestine produces apoB48-containing VLDL in addition to chylomicrons, and that such VLDL give rise to remnants with prolonged plasma residence.
2) The evidence base for both LDL and remnant particles fulfils eight key criteria of causality for ASCVD.
3) On a particle for particle basis, the atherogenic potential of a cholesterol-rich remnant is similar to that of an LDL particle.
4) Remnant cholesterol constitutes a complimentary target to that of LDL cholesterol in the prevention of ASCVD.
5) Recent findings (epidemiological, meta-analyses and post hoc analyses of randomized trials) confirm the atherogenic risk associated with plasma remnant accumulation.

Acknowledgements

None

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest for the preparation of this review.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as • of special interest, •• of outstanding interest.

1. Havel RJ. Triglyceride-rich lipoprotein remnants, in: N. Rifai, G. Warnick, M. Dominiczak (Eds.), Handbook of Lipoprotein Testing, 25 AACC Press, Washington D. C., 1997, pp. 451–464.
2. ••Björnson E, Packard CJ, Adiels M, *et al*. Apolipoprotein B48 metabolism in chylomicrons and very low-density lipoproteins and its role in triglyceride transport in normo- and hypertriglyceridemic human subjects. J Intern Med. 2019; Dec 17. doi: 10.1111/joim.13017.

   Important and novel in vivo kinetic studies of chylomicron and VLDL metabolism in the postprandial period highlighting the formation of intestinally-derived, apoB48-containing remnants.
3. •Boren J, Chapman MJ, Krauss RM, *et al*. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2020. doi:10.1093/eurheartj/ehz962.

   A comprehensive, integrated review of mechanisms underlying the atherogenicity of LDL particles.
4. Mahley RW, Huang Y. Atherogenic remnant lipoproteins: role for proteoglycans in trapping, transferring, and internalizing. J. Clin. Invest. 2007; 117: 94–98.
5. Zheng C, Khoo C, Ikewaki K, Sacks FM. Rapid turnover of apoCIII-containing triglyceride-rich lipoproteins contributing to the formation of LDL subfractions. J Lipid Res. 2007; 48:1190-1203.
6. •Packard CJ. Triglyceride lowering 2.0: Back to the Future. Eur Heart J. 2020;41;95-98.

   Metabolic insights into remnant formation as a function of triglyceride level.
7. Ference BA, Ginsberg HN, Graham I, *et al*. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017 Aug 21;38(32):2459-2472.

8. Havel RJ. Remnant lipoproteins as therapeutic targets. Curr Opin Lipidol. 2000;11:615-620.
9. Dallinga-Thie GM, Kroon J, Boren J, Chapman MJ. Triglyceride-rich lipoproteins and Remnants: Targets for Therapy? Curr Cardiol Rep.2016; 18:67.
10. Boren J, Williams KJ. The central role of arterial retention of cholesterol-rich apolipoprotein B-containing lipoproteins in the pathogenesis of atherosclerosis : a triumph of simplicity. Curr Opin Lipidol. 2016;27:473-483.
11. Schwartz EA, Reaven PD. Lipolysis of triglyceride-rich lipoproteins, vascular inflammation and atherosclerosis. Biochim Biophys Acta. 2012; 1821:858-866.
12. Olin-Lewis K, Krauss RM, La Belle M, et al. ApoC-III content of apoB-containing lipoproteins is associated with binding to the vascular proteoglycan biglycan. J Lipid Res. 2002; 43:1969–1977.
13. Chapman MJ, Laplaud PM, Luc G et al. Further resolution of the low density lipoprotein spectrum in normal human plasma: physicochemical characteristics of discrete subspecies separated by density gradient ultracentrifugation. J Lipid Res. 1988;29:442-458.
14. Nordestgaard BG. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology, genetics, and biology. Circ Res 2016; 118:547–563
15. Pourcet B, Staels B. Alternative macrophages in atherosclerosis: not always protective! J Clin Invest. 2018;128:910-912.
16. Lusis AL. Atherosclerosis. Nature. 2000; 407:233-241.
17. Balling M, Langsted A, Afzal S, et al. A third of nonfasting plasma cholesterol is in remnant lipoproteins: Lipoprotein subclass profiling in 9293 individuals. Atherosclerosis 2019;286:97-104.
18. Prospective Studies Collaboration, Lewington S, Whitlock G, Clarke R, *et al*. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet. 2007;370:1829–1839.

19. Varbo A, Freiberg JJ, Nordestgaard BG. Extreme nonfasting remnant cholesterol vs extreme LDL cholesterol as contributors to cardiovascular disease and all-cause mortality in 90000 individuals from the general population. Clin Chem. 2015;61:533–543.

20. Varbo A, Benn M, Nordestgaard BG. Remnant cholesterol as a cause of ischemic heart disease: evidence, definition, measurement, atherogenicity, high risk patients, and present and future treatment. Pharmacol Ther. 2014;141:358-67.

21. Varbo A, Nordestgaard BG. Remnant lipoproteins. Curr Opin Lipidol. 2017 ; 28 :300-307.

22. Varbo A, Benn M, Tybjaerg-Hansen A, Nordestgaard BG. Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. Circulation. 2013;128:1298–1309.

23. Xiang QY, Tian F, Lin QZ, *et al*. Comparison of remnant cholesterol levels estimated by calculated and measured LDL-C levels in Chinese patients with coronary heart disease. Clin Chim Acta. 2020;500:75-80.

24. Nguyen SV, Nakamura T, Uematsu M, *et al.* Remnant lipoproteinemia predicts cardiovascular events in patients with type 2 diabetes and chronic kidney disease. J Cardiol. 2017;69(3):529-535.

25. Varbo A, Benn M, Tybjærg-Hansen A, *et al*. Remnant cholesterol as a causal risk factor for ischemic heart disease. J Am Coll Cardiol. 2013;61:427–436. doi: 10.1016/j. jacc.2012.08.1026.

26. ••Ference BA, Kastelein JJP, Ray KK, *et al*. Association of Triglyceride-Lowering LPL Variants and LDL-C-Lowering LDLR Variants With Risk of Coronary Heart Disease. JAMA. 2019;321:364-373.26.

Evidence from Mendelian randomization studies suggesting that the lowering of TGRL and LDL cholesterol levels may be proportional to the absolute reduction in apoB.

27. Santos RD, Gidding SS, Hegele RA, *et al*. International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. Defining severe familial hypercholesterolaemia and the

implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. Lancet Diabetes Endocrinol. 2016 Oct;4:850-61. doi: 10.1016/S2213-8587(16)30041-9.

28. Marais D. Dysbetalipoproteinemia: an extreme disorder of remnant metabolism. Curr Opin Lipidol. 2015;26:292-7
29. Koopal C, Retterstol K, Sjouke B, *et al*. Vascular risk factors, vascular disease, lipids and lipid targets in patients with familial dysbetalipoproteinemia:a European cross-sectional study. Atherosclerosis.2015;240:90–97.
30. Tada H, Kawashiri MA, Nohara A, *et al.* Remnant-like particles and coronary artery disease in familial hypercholesterolemia. Clin Chim Acta. 2018;482:120-123.
31. •Taskinen MR, Björnson E, Andersson L, *et al*. Impact of proprotein convertase subtilisin/kexin type 9 inhibition with evolocumab on the postprandial responses of triglyceride-rich lipoproteins in type II diabetic subjects. J Clin Lipidol. 2019. pii: S1933-2874(19)30363-0.
A PCSK9 inhibitor is demonstrated to significantly reduce TGRL levels in the postprandial period in type II diabetic subjects.
32. Fujihara Y, Nakamura T, Horikoshi T, *et al*. Remnant Lipoproteins Are Residual Risk Factor for Future Cardiovascular Events in Patients With Stable Coronary Artery Disease and On-Statin Low-Density Lipoprotein Cholesterol Levels <70 mg/dL. Circ J. 2019;83:1302-1308.
33. Laufs U, Parhofer KG, Ginsberg HN, Hegele RA. Clinical review on triglycerides. Eur Heart J. 2020;41:99-109c.
34. Marston NA, Giugliano RP, Im K, *et al.* Association Between Triglyceride Lowering and Reduction of Cardiovascular Risk Across Multiple Lipid-Lowering Therapeutic Classes: A Systematic Review and Meta-Regression Analysis of Randomized Controlled Trials. Circulation. 2019;140:1308-1317.
35. ••Bhatt DL, Steg PG, Miller M, *et al*. REDUCE-IT Investigators. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. N Engl J Med 2019;380:11–22.

Intervention with high dose EPA (4g/day) in statin-treated, high risk patients in the REDUCE-IT trial translated into marked cardiovascular benefit over 4.9 years of follow-up relative to placebo.

36. •Elshazly MB, Mani P, Nissen S, *et al.* Remnant cholesterol, coronary atheroma progression and clinical events in statin-treated patients with coronary artery disease. Eur J Prev Cardiol. 2019:2047487319887578.

    The earliest imaging approach to analysis of the relation of remnant cholesterol to coronary plaque progression.

37. Aguilar-Salinas CA, Tusie-Luna T, Pajukanta P. Genetic and environmental determinants of the susceptibility of Amerindian derived populations for having hypertriglyceridemia. Metabolism. 2014; 63:887-94.

38. •Mach F, Baigent C, Catapano AL, *et al*. ESC Scientific Document Group. 2019. ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41:111–188.

    New Guidelines from ESC/ESC for lipid management in dyslipidemic patients, with recommendations for lower LDL cholesterol goals in subjects at elevated risk, and for therapeutic approaches to risk reduction in hypertriglyceridemic states.

39. Pradhan AD, Paynter NP, Everett BM, *et al.* Rationale and design of the Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients with Diabetes (PROMINENT) study. Am Heart J. 2018;206:80–93.

40. Florentin M, Kostapanos MS, Anagnostis P, Liamis G. Recent developments in pharmacotherapy for hypertriglyceridemia: what's the current state of the art? Expert Opin Pharmacother. 2020;21:107-120.

41. Tsimikas S. RNA-targeted therapeutics for lipid disorders. Curr Opin Lipidol. 2018;29:459-466.

Legend to Figure 1:

A comparison of the key biological features of LDL as compared to remnant particles in relation to their atherogenicity. The figure highlights the key aspects of the evidence supporting the atherogenicity of both classes of particles, and emphasises the lack of experimental evidence (principally placebo-controlled, randomised intervention trials) for remnants.

Analogy

Plausibility

Temporality

Coherence

LDL

Experimental evidence

- Elevated particle numbers in plasma
- Up to 2700 cholesterol molecules / particle
- Half-life = up to 3 days
- Bind matrix proteoglycans via apoB100

Specificity

Dose response relationship

Strength of association

Consistency

Temporality

Plausibility

Analogy

Coherence

Remnants

Experimental evidence

- Lower particle numbers in plasma as compared to LDL
- Up to 8600 cholesterol molecules/IDL particle
- Half-life = < 24 hours
- Bind matrix proteoglycans and biglycan via apoE, apoCIII and apoB; low intimal efflux rates

Specificity

Consistency

Strength of association

Dose response relationship

Table 1. Criteria for causality: comparison of evidence supporting the relationship of low-density lipoprotein (LDL) and of remnants with the pathophysiology of premature atherosclerotic cardiovascular disease (ASCVD).

Criterion	**LDL**	**Remnants**
Plausibility	Strong experimental evidence [3,4,7,10,16]	No animal model is representative of human remnant metabolism [1,2]. Remnant components are found in human atherosclerotic plaques [4,10,12]
Strength	Monogenic and polygenic lifelong elevations lead to higher lifetime risk of ASCVD [3,27,]	Monogenic and polygenic lifelong elevations lead to higher lifetime risk of ASCVD [14,22,26,28]
Biological gradient	A dose-dependent association between the absolute magnitude of exposure to LDL and risk of ASCVD [3,7,10,27]	A dose-dependent association between the absolute magnitude of exposure to remnant cholesterol and risk of ASCVD [13,14,19,22,26,30,34,35]
Temporal sequence	Exposure to elevated LDL precedes the onset of ASCVD [3,7,27]	Exposure to elevated remnant cholesterol precedes the onset of ASCVD [14,20,22,26,28,37]
Specificity	LDL is associated with ASCVD independent of other risk factors [3,7]	Remnant cholesterol is associated with ASCVD independent of other risk factors [14,20,22,26,28]
Consistency	Multiple sources of evidence demonstrate a dose-dependent, log-linear association between the absolute magnitude of exposure to LDL and risk of ASCVD [3,7,27]	Observational and post-hoc analyses of intervention trials show a dose-dependent association between the absolute magnitude of exposure to remnants and risk of ASCVD [14,22,26,28]
Coherence	Multiple sources of evidence replicate the association LDL-ASCVD [3,7,10,16,27]	Several sources of evidence replicate the association of remnants with premature ASCVD [14,20,22,26,28]
Reduction in risk with intervention	Multiple randomized trials evaluating LDL lowering therapies demonstrate that reducing LDL cholesterol reduces the risk of ASCVD events [3,7,27]	Post-hoc analyses of randomized trials and meta-regression analyses suggest that lowering remnant cholesterol reduces the risk of ASCVD events; additional evidence is needed. [8,14,35]

Modified from reference [7].

Table 2. Unsolved questions and new avenues to further understanding of the role of remnant particles in the pathophysiology of ASCVD.

Unsolved questions	**Potential source of evidence**
Can a unifying definition for remnant particles be proposed?	Basic research focused on remnant biology in man
Does estimated remnant cholesterol provide complimentary information beyond that obtained from non-HDL cholesterol for assessment of atherogenic remnant burden?	Large outcome-based observational surveys or post hoc analyses of randomized controlled studies
Are direct homogeneous assays for measuring remnant cholesterol a useful tool in clinical practice?	User surveys, cost-effectiveness analysis
Can a treatment goal be proposed for remnant cholesterol in high-risk, dyslipidemic patients?	Post hoc analyses of randomized controlled studies
Does estimation of remnant cholesterol add clinically-relevant information to facilitate selection of add-on therapies in high risk, statin-treated dyslipidemic patients at LDL-cholesterol goal?	Post hoc analyses of randomized controlled studies
New avenues	
Precision medicine	Mendelian randomization studies, randomized controlled studies
ApoCIII-based therapies	Randomized controlled studies
New generations of fibrates	Randomized controlled studies
ANGPTL3-based drugs	Randomized controlled studies
Acyl-coA: diacylglycerol acyltransferase (DGAT)-1-based therapies	Randomized controlled studies