Pathology (February 2019) **51(2)**, pp. 148–154

LIPIDS AND CARDIOVASCULAR DISEASE

Non-HDL-cholesterol and apolipoprotein B compared with LDL-cholesterol in atherosclerotic cardiovascular disease risk assessment

Stuart S. Carr[1], Amanda J. Hooper[1,2], David R. Sullivan[3], John R. Burnett[1,2]

[1]*School of Medicine, Faculty of Health and Medical Sciences, University of Western Australia, Perth, WA, Australia;* [2]*Department of Clinical Biochemistry, Royal Perth Hospital and Fiona Stanley Hospital Network, PathWest Laboratory Medicine, Perth, WA, Australia;* [3]*Department of Chemical Pathology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia*

Summary

Low density lipoprotein (LDL) is the predominant atherogenic lipoprotein particle in the circulation. Conventionally, a fasting lipid profile has been used for atherosclerotic cardiovascular disease (ASCVD) risk assessment. A non-fasting sample is now regarded as a suitable alternative to a fasting sample. In routine clinical practice, the Friedewald equation is used to estimate LDL-cholesterol, but it has limitations. Commercially available direct measures of LDL-cholesterol are not standardised. LDL-cholesterol is a well-established risk factor for ASCVD, being the primary therapeutic target in both primary and secondary prevention. Non-high-density lipoprotein (HDL)-cholesterol is a measure of the cholesterol content in the atherogenic lipoproteins, but it does not reflect the particle number. Non-HDL-cholesterol has the advantage over LDL-cholesterol of including remnant cholesterol and being independent of triglyceride variability, but it is compromised by the non-specificity bias of direct HDL-cholesterol methods used in the calculation. Apolipoprotein (apo) B, the major structural protein in very low-density lipoprotein, intermediate density lipoprotein, LDL and lipoprotein (a), is a measure of the number of atherogenic lipoproteins. ApoB methods are standardised, but the assay comes at an additional, albeit relatively low cost. Non-HDL-cholesterol and apoB are more accurate measures than LDL-cholesterol in hypertriglyceridaemic individuals, non-fasting samples, and in those with very-low LDL-cholesterol concentrations. Accumulating evidence suggests that non-HDL-cholesterol and apoB are superior to LDL-cholesterol in predicting ASCVD risk, and both have been designated as secondary targets in some treatment guidelines. We review the measurement, potential role, utility and current status of non-HDL-cholesterol and apoB when compared with LDL-cholesterol in ASCVD risk assessment.

Key words: Lipids; LDL-cholesterol; apolipoprotein B; non-HDL-cholesterol; atherosclerosis; cardiovascular disease; risk assessment.

Received 13 November, revised 25 November, accepted 25 November 2018
Available online 27 December 2018

INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of morbidity and mortality worldwide. Lipoproteins, particularly low density lipoprotein (LDL) and other apolipoprotein (apo) B-containing lipoproteins including very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and lipoprotein (a) [Lp(a)] play a fundamental role in the initiation and evolution of atherosclerosis. During atherogenesis, the cholesterol-rich, apoB-containing lipoproteins are retained and accumulate within vascular intima of the arterial wall,[1,2] and together with reactive immune and inflammatory mechanisms result in atherosclerotic plaque formation and progression.[3]

Genetic, epidemiological and clinical studies have clearly shown that plasma concentrations of LDL-cholesterol are directly related to the incidence of coronary events and cardiovascular deaths.[4] Elevated concentrations of apoB-containing lipoproteins, particularly LDL, are associated with an increased risk of developing ASCVD. Clinical trials using lipid-lowering drugs have unequivocally shown that lowering LDL-cholesterol results in significant reductions in both morbidity and mortality in patients with or without established coronary heart disease. Recently, studies using aggressive plasma LDL-cholesterol reduction as secondary prevention have demonstrated increased survival rates.[5] However, despite reductions in LDL-cholesterol with maximally tolerated statins and newer lipid-lowering agents, many people still experience cardiovascular events and/or ASCVD progression,[6] which may, in part, relate to triglyceride or the cholesterol content within triglyceride-rich lipoproteins.[7,8] Statin-treated patients with elevated triglyceride levels have been shown to be at increased ASCVD risk.[9] Moreover, the recent findings from the REDUCE-IT trial have shown that icosapent ethyl, a highly purified eicosapentaenoic acid ethyl ester, significantly reduces the risk of major ischaemic events, including cardiovascular death, in patients with elevated triglyceride levels despite statin therapy.[10]

LDL-cholesterol is a well-established risk factor for ASCVD, being the primary therapeutic target in both primary and secondary prevention according to global dyslipidaemia guidelines.[11–14] In some guidelines, non-high density lipoprotein (HDL)-cholesterol, a measure of cholesterol content of a wider

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: https://doi.org/10.1016/j.pathol.2018.11.006

range of atherogenic apoB-containing lipoproteins, and apoB, a measure of the number of atherogenic lipoprotein particles, have been designated as secondary treatment targets, which if increased, might lead to intensification of lipid-lowering therapy.

Under most conditions, LDL-cholesterol, non-HDL-cholesterol and apoB are highly correlated. However, in individuals with mild-moderate hypertriglyceridaemia and associated conditions, such as diabetes, obesity and the metabolic syndrome, discordance between these measures may occur.[15] These observations, together with the recent introduction of Australian guidelines for harmonised lipid reporting,[16] which recommended reporting of non-HDL-cholesterol in the standard lipid profile, highlight the need for clinicians to understand the status of these markers in ASCVD risk assessment.

We review the measurement, potential role, utility and current status of non-HDL-cholesterol and apoB when compared with LDL-cholesterol in ASCVD risk assessment.

LDL-CHOLESTEROL

In most fasting individuals, LDL particles comprise ~90% of the apoB-containing lipoproteins in the circulation. However, in routine clinical practice, rather than LDL, it is the cholesterol component of LDL, namely LDL-cholesterol, which is measured. Because of this, LDL-cholesterol has been the standard lipid measure used for ASCVD risk assessment. Furthermore, the effects of dietary patterns and macronutrients, particularly saturated and trans-unsaturated fats, are predominantly judged in terms of their effect on LDL-cholesterol.[17] LDL-cholesterol can be measured directly, but is typically calculated. Conventional randomised controlled trials, prospective epidemiological cohort studies, and more recently Mendelian randomisation studies have all shown a direct relationship between LDL-cholesterol and the incidence of coronary heart disease.[4,18] Although lowering of LDL-cholesterol to a target of <1.8 mmol/L decreases ASCVD risk, many people still experience cardiovascular events and/or atherosclerosis disease progression[6] and this 'residual risk', which is not identified by LDL-cholesterol, contributes to significant cardiovascular morbidity and mortality.[6,19,20]

LDL size and composition

When triglyceride levels are elevated, the presence of cholesterol ester transfer protein allows lipids such as cholesterol to be redistributed amongst lipoproteins according to concentration gradients. Cholesterol-rich lipoproteins such as LDL and HDL receive triglyceride in exchange for their cholesterol content. The triglyceride is catabolised, resulting in smaller, denser cholesterol-depleted lipoprotein particles. In the case of LDL, the cholesterol content of these small dense LDL no longer reflects the number of LDL particles.[21] Measurement of the diameter of small dense LDL may reflect the extent of this process, but it does not reflect the number of LDL particles and, therefore, it is not a reflection of ASCVD risk *per se*.

Direct LDL-cholesterol

Poor agreement between homogeneous (direct) LDL-cholesterol methods relates to the analytical issues involved with the selective measurement of cholesterol in LDL particles that can vary in size, density and composition.[22] All LDL particles are atherogenic, regardless of their size, and their cholesterol content can differ between individuals.[23] Small, dense LDL particles, which can be found in individuals with mild-moderate hypertriglyceridaemia and associated metabolic disorders, such as diabetes, obesity and the metabolic syndrome, contain less cholesterol than larger LDL particles. Indeed, it has been postulated that 'residual risk' might, in part, be explained by the 'atherogenic dyslipidaemia' consisting of elevated small dense LDL particles, hypertriglyceridaemia, reduced HDL-cholesterol, increased remnant lipoproteins, and postprandial hyperlipidaemia.[24]

Calculated LDL-cholesterol

In most clinical diagnostic laboratories, LDL-cholesterol is calculated by the Friedewald equation: LDL-cholesterol (in mmol/L) = Total cholesterol – HDL-cholesterol – VLDL-cholesterol, where VLDL-cholesterol is estimated as triglyceride/2.2 assuming a constant triglyceride:cholesterol ratio in VLDL particles.[25] The Friedewald equation has several caveats: it includes cholesterol present in IDL and Lp(a) and assumes that there are no chylomicron particles present. At triglyceride concentrations >4.5 mmol/L it underestimates LDL-cholesterol and is regarded as invalid. At low LDL-cholesterol concentrations (<1.8 mmol/L), especially when accompanied by triglyceride elevation, the Friedewald equation underestimates LDL-cholesterol when compared with a direct LDL-cholesterol measurement.[26,27] This observation has important clinical relevance owing to the introduction of the PCSK9 inhibitors, which when used in combination with statins, can result in extremely low LDL-cholesterol concentrations,[28] and alternative formulas to estimate LDL-cholesterol have been proposed.[29,30]

NON-HDL-CHOLESTEROL

Non-HDL-cholesterol is a measure of cholesterol content of the atherogenic apoB-containing lipoproteins: VLDL, IDL, LDL, chylomicron remnants and Lp(a) (Fig. 1).[31] Unlike LDL-cholesterol, it includes the atherogenic risk component of remnant lipoproteins, namely IDL and chylomicron

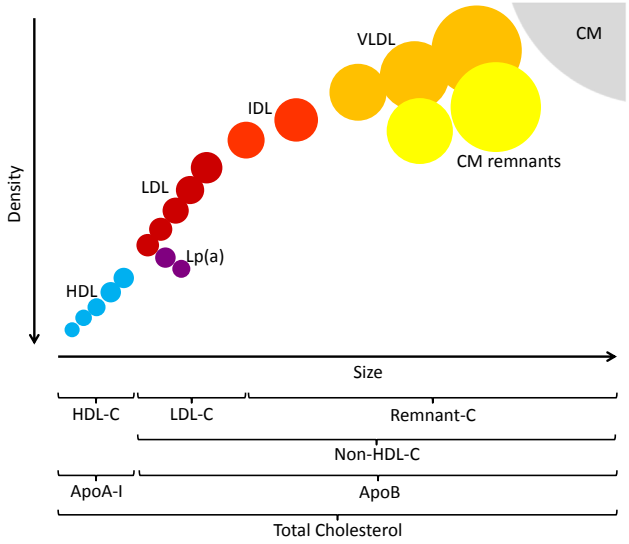

Fig. 1 Plasma lipoproteins separated according to density and size and their representative cholesterol and apolipoprotein markers.

remnants, and can be simply calculated in the fasting or non-fasting state at no additional cost: Non-HDL-cholesterol = Total cholesterol – HDL-cholesterol. Remnant cholesterol = Total cholesterol – HDL-cholesterol – LDL cholesterol and has been shown to be a causal factor for ischaemic heart disease.[32] Meta-analysis has shown non-HDL-cholesterol to be as good as LDL-cholesterol in ASCVD risk assessment and superior to LDL-cholesterol in individuals with mild-moderate hypertriglyceridaemia.[33]

Despite these advantages, non-HDL-cholesterol is not as widely used as LDL-cholesterol, which reflects the traditional reliance on LDL-cholesterol as the primary measure of ASCVD risk attributable to lipoproteins in epidemiological studies.[34] Consequently, LDL-cholesterol has also served as the main indicator of the efficacy of lipid-modifying therapy in randomised controlled trials.[35] This has been translated into LDL-cholesterol levels for target attainment during risk factor management.[36]

Non-HDL-cholesterol is simpler, more convenient and more predictive than LDL-cholesterol, but this is less widely recognised and hence less frequently used (Table 1). It is particularly useful in the setting of mild-moderate hypertriglyceridaemia, which is common (~14% of Australians aged 18 or over have a triglyceride $\geq$2.0 mmol/L)[37] and often accompanies associated cardiometabolic disorders such as diabetes, obesity and the metabolic syndrome.[7] As it is not inferior to LDL-cholesterol in other clinical settings, it should be employed more liberally. The targets defined for LDL-cholesterol can be converted to equivalent targets for non-HDL-cholesterol by increasing the values by 0.8 mmol/L.

Non-HDL-cholesterol is a more reliable measurement than LDL-cholesterol because the latter requires triglyceride for calculation by the Friedewald equation. Triglyceride measurement, even in the fasting state, is fraught with a degree of intra-individual variability, in part due to background diet.[38] On the other hand, direct measurement of both LDL-cholesterol and HDL-cholesterol could be criticised for their dependence on assay design features that selectively limit the access of reagents to cholesterol on different lipoproteins.[39] Nevertheless, non-HDL-cholesterol has an analytical advantage in this respect because the vulnerable direct measurement is a much smaller component of the overall result compared to direct LDL-cholesterol. Consequently, non-HDL-cholesterol results are more robust than LDL-cholesterol.

Care should be exercised in the presence of severe hypertriglyceridaemia, which corresponds to triglyceride levels exceeding 10 mmol/L.[40] In this situation, the direct measurement of LDL-cholesterol or HDL-cholesterol may be confounded by cholesterol in other fractions, particularly chylomicrons and their remnants.[41,42] In reality, the contribution of chylomicron cholesterol may lead to a situation in which non-HDL-cholesterol over-emphasises ASCVD risk when the main concern is the danger of developing acute pancreatitis.[43] Although treatment for severe hypertriglyceridaemia is often successful, the changes in HDL and LDL composition can resolve slowly and it may take several months for non-HDL-cholesterol to become a reliable indicator of ASCVD risk.

APOLIPOPROTEIN B

ApoB plays a central role in human lipoprotein metabolism. Full length apoB-100 is a large, amphipathic protein comprising 4536 amino acids, which is produced by the liver.[44] In contrast, the intestine, via a unique mRNA editing process, synthesises only the first 2152 amino acids (48%) of the protein, to produce apoB-48.[45] It is the editing process that determines the apoB isoform to be synthesised, which in turn dictates the type of lipoprotein, either chylomicron or VLDL particle, to be assembled. ApoB-48 is required for chylomicron production and apoB-100 is an essential structural component of VLDL and its metabolic products, IDL and LDL, as well as Lp(a). ApoB, unlike other apolipoproteins, is not readily exchangeable between lipoproteins.

Due to its large size, propensity to form insoluble aggregates in aqueous solutions, and its sensitivity to proteolytic degradation, the primary structure of apoB was not elucidated until 1986.[44] ApoB-100 (550 kDa) contains five super-domains, namely NH3-βα1-β1-α2-β2-α3-COOH, with the βα1 domain corresponding to the first ~20% of the protein, critical for binding with the molecular chaperone MTP and for the assembly of triglyceride-rich lipoproteins.[46] Amphipathic α-helices and β-sheets as well as short hydrophobic cluster domains are responsible for lipid binding and are thought to largely determine LDL particle diameter.[47] ApoB-100 contains an LDL-receptor binding domain that is involved in the uptake of LDL as well as VLDL and IDL from the circulation.

Genome-wide and other association studies have identified polymorphisms in *APOB* that are associated with LDL-

Table 1 Advantages, disadvantages and targets for LDL-cholesterol, non-HDL-cholesterol and apoB

Analyte	Advantages	Disadvantages	Targets
LDL-cholesterol	• Widely available • Strong evidence base • Well defined risk cutpoints/targets • 1° target in guidelines	• Friedewald equation is not valid if triglyceride >4.5 mmol/L • Affected by increased Lp(a) • Poor agreement between direct methods	<3.0 mmol/L (low risk) <2.5 mmol/L (high risk) <1.8 mmol/L (very high risk)
Non-HDL-cholesterol	• No fasting requirement • Does not require triglyceride to be $\leq$4.5 mmol/L • Includes remnant cholesterol • No additional cost	• Not well understood • Arbitrary risk cut points/targets • 2° target in guidelines	<3.8 mmol/L (moderate risk) <3.3 mmol/L (high risk) <2.6 mmol/L (very high risk)
ApoB	• No fasting requirement • Standardised method • Useful in individuals with 'atherogenic dyslipidaemia' and those with very low LDL-cholesterol concentrations	• Not widely available • Not well understood • Arbitrary risk cutpoints/targets • 2° target in guidelines • Additional cost	<1.0 g/L (high risk) <0.8 g/L (very high risk)

cholesterol concentrations.[48] Rare mutations, mostly causing truncated apoB species, reduce apoB production and/or increase its clearance, resulting in familial hypobetalipoproteinaemia, a disorder characterised by low LDL-cholesterol concentrations and protection from ASCVD.[49,50] In contrast, apoB missense mutations in the LDL-receptor binding region, such as p.Arg3527Gln, result in impaired clearance of LDL particles and cause familial hypercholesterolaemia.[51]

ApoB measurement

ApoB can be measured in routine clinical laboratories using commercially available immunonephelometric or immunoturbidimetric methods on a variety of automated instrument platforms, all of which use polyclonal antibodies specific to apoB. The majority of those immunoassays measure total apoB (apoB-100 and apoB-48). Even in the postprandial state, apoB-48-containing chylomicron particles in healthy individuals are very low (usually <1%).[52] Efforts have been made to improve method-method variability by harmonisation and the analytical performance of these methods, in terms of accuracy and precision, has been shown to be superior to that of LDL-cholesterol.[53]

Although a fasting serum sample is recommended, non-fasting samples are acceptable. Similar to other proteins measured using light scattering techniques, apoB is affected by turbidity, which can be found in severe hypertriglyceridaemia. In healthy subjects the biological within-individual variation for apoB ranges between 3 and 12%[54] and during pregnancy apoB increases by 56% in the third trimester.[55] Reference intervals for apoB have been shown to be age, sex, ethnicity and population dependent.[56–58] Similar to LDL-cholesterol, clinical decision cut points for apoB of 1.00 g/L and 1.20 g/L have been proposed which correspond to the 50th and 75th percentile, respectively,[52] with values greater than the former regarded as moderate and the latter a high ASCVD risk.

ApoB standardisation

The standardisation of apoB methods has been challenging, due to both the lack of a primary reference method and suitable primary standard; noting that apoB cannot be solubilised in purified form owing to self-association and irreversible matrix aggregation. However, a collaborative initiative during the late 1980s and early-mid 1990s between the International Federation for Clinical Chemistry (IFCC) and the various diagnostic companies, achieved international standardisation and harmonisation of apoB methods with the development and adoption of WHO/IFCC SP3-07 (more recently SP3-08), a secondary matrix-matched, serum-based reference material, with an assigned value traceable to an ultracentrifuged LDL (d 1.030–1.050 kg/L) pool, for assay calibration.[53,59–61] This secondary reference material was to be an interim solution until an accurate value-assigned primary reference material and primary reference method were developed. More recently, liquid chromatography tandem mass spectrometry, owing to its unique capability of providing results with SI traceability, has been proposed as a candidate reference procedure to standardise apoB measurement.[62]

LDL SUBCLASSES AND PARTICLE NUMBER

Nuclear magnetic resonance (NMR) spectroscopy can provide a measure of the LDL particle number and size along with information on other lipoprotein subclasses.[23] This subfractionation technique has been shown to be at least equivalent to non-HDL-cholesterol and apoB in ASCVD risk assessment.[63] The method depends on the deconvolution of complex NMR spectra according to correlation with traditional ultracentrifuge-based methods. Furthermore, high-field NMR spectrometers require very low temperatures and are therefore reliant on liquid nitrogen and other major infrastructure. Consequently, the method is not widely available, is expensive, and is not standardised.

CARDIOVASCULAR RISK ASSESSMENT

Population-based studies have identified differences in ASCVD risk prediction between LDL-cholesterol, non-HDL-cholesterol and apoB. Both non-HDL-cholesterol and apoB have been shown to be superior to LDL-cholesterol as markers of ASCVD risk.[64] A meta-analysis from 12 independent epidemiological studies including 233,455 participants and 22,950 events reported that the relative risk ratios of fatal or non-fatal ischaemic cardiovascular events were lowest for LDL-cholesterol (RR 1.25), intermediate for non-HDL-cholesterol (RR 1.34) and highest for apoB (RR 1.43).[33] Over 10 years, a high-risk treatment strategy in US adults using non-HDL-cholesterol would potentially prevent 300,000 more clinical events than an LDL-cholesterol strategy, and an apoB strategy 500,000 more than a non-HDL-cholesterol strategy. However, not all studies have supported the superiority of apoB over non-HDL-cholesterol and LDL-cholesterol.[65,66] A meta-analysis of seven randomised controlled trials showed that the clinical benefit of statin therapy relates more to changes in apoB than to changes in LDL-cholesterol or non-HDL-cholesterol.[67]

ASCVD risk assessment is central to clinical management because it quantifies the absolute risk of a cardiovascular event in the ensuing time period.[36] It avoids the inadequacy of previous strategies based on relative risk, which tended to deal with cardiovascular risk factors, including lipids, in isolation. Assessment of absolute risk allows simultaneous consideration of all major risk factors including smoking, hypertension and diabetes. Indeed, one of the strengths is that it captures the tendency of risk factors to cluster in individuals who are obese or insulin resistant. Non-modifiable risk factors are amongst the strongest influences on future ASCVD risk, but absolute risk calculators are limited in their ability to encompass family history. They are effective in making adjustment for gender, but the effect of age tends to be dominant. As a result, there is a danger that absolute ASCVD risk calculators will favour the aggressive treatment of the elderly and a reluctance to treat younger high-risk patients. Comparison of the application of individual risk calculators within diverse populations has revealed substantial differences that imply a need for local within-population calibration.

The original epidemiological data on which absolute risk calculators are based included information that has subsequently been omitted. Established ASCVD is sufficient to warrant risk factor intervention in all cases, so this aspect is not considered. Likewise, left ventricular hypertrophy on electrocardiogram is not routinely considered. This may explain why some biomarkers, such as cardiac troponin or B-

type natriuretic peptide, which can powerfully enhance cardiovascular risk assessment, have not found a place in routine practice.[68] Indeed, ASCVD risk assessment has leap-frogged these opportunities by applying non-invasive imaging techniques that detect the presence of sub-clinical cardiovascular disease. In the future, these techniques may erode the role of non-HDL-cholesterol and apoB in ASCVD risk assessment, but these measurements may become even more prominent in the intervention strategies to prevent the progression of ASCVD.

DISCORDANCE

LDL-cholesterol, non-HDL-cholesterol and apoB are all markers of ASCVD risk. Under most conditions, the three markers are highly correlated; however, the cholesterol mass of the apoB-containing particles is variable. The technique of discordance analysis has been used to compare these related and metabolically linked variables to identify which is the most accurate marker of ASCVD risk.[69]

In individuals with mild-moderate hypertriglyceridaemia and associated cardiometabolic disorders such as diabetes, obesity and the metabolic syndrome, discordance can occur as a result of the predominance of small, dense, cholesterol-poor LDL, and therefore LDL-cholesterol may not accurately reflect LDL particle concentration or its effect on ASCVD risk.[15,70] The discordance causes LDL-cholesterol to underestimate the true ASCVD risk, whereas under these conditions, apoB more accurately reflects the causal effect of LDL on ASCVD risk. A number of discordance studies including the Health Professionals Follow-up Study, Woman's Health Study, INTERHEART study and the Framingham Heart Study all support the concept that ASCVD risk is more closely related to the concentration of atherogenic lipoprotein particles than to the mass of cholesterol carried by them.[71–75]

Differences in LDL particle size, density and composition that underlie discordance have led to public and professional confusion.[69] The qualitative changes in LDL particle size, as reflected by diameter and 'type', are only atherogenic if accompanied by an increase in particle number, as demonstrated in the Quebec Heart Study.[76] Conversely, elevated LDL-cholesterol due to an increased number of qualitatively normal LDL still represents an increase in the number of atherogenic particles. Therefore, normal LDL particle size, density and diameter must not dissuade clinicians from treating elevated LDL-cholesterol levels because they are present in increased numbers. This would also be reflected by the accompanying non-HDL-cholesterol, and apoB concentrations and the LDL particle number.

LIPID GUIDELINES

It is important to maintain and enhance the opportunity to provide guideline input concerning screening, diagnostic and laboratory monitoring aspects of ASCVD risk and other major public health issues. This will assist the judicious use of testing and the cost-effective selection of high-risk individuals for pharmacological and non-pharmacological intervention. A comparative analysis of lipid treatment guidelines has been the topic of a recent review.[77]

A non-fasting sample is now regarded as a suitable alternative to a fasting sample,[78,79] with this recommendation endorsed by European, Canadian and US societies and incorporated into guidelines.[11–13] These same guidelines recommend LDL-cholesterol as the primary target (<2.5 and <1.8 mmol/L, respectively, for patients at high-risk and very high-risk). Some guidelines also recommend non-HDL-cholesterol (<3.3 and <2.6 mmol/L) and apoB (<1.0 and <0.8 g/L) as secondary (or alternative) targets.[11,13] Interestingly, using a novel more accurate estimation of LDL-cholesterol than that of the Friedewald equation, it was shown that secondary non-HDL-cholesterol targets only altered management in a small proportion of individuals and that apoB targets were only modestly useful after LDL-cholesterol targets were achieved.[80]

Ideally, guidelines should also include consideration of the current and future role of emerging biomarkers, which in the case of ASCVD risk assessment includes lipoproteins and other candidates.[81] Guidelines aspire to be a comprehensive evidence-based consideration of all aspects of disease management, and as such, should include discussion of tests designed to monitor response and guarantee the safety of interventions.

HARMONISED LIPID REPORTING

Quality healthcare includes mechanisms to minimise the deviation of tests, procedures and management strategies from best practice. Consensus guidelines allow populations to be managed according to the relevant evidence base. This leads to the establishment of agreed medical decision points, thresholds and targets that facilitate consistent decision-making. Lipid metabolism is an area in which efforts have been successful in harmonising the information and advice provided in connection with results such as LDL-cholesterol, non-HDL-cholesterol and apoB. The recent introduction of Australian guidelines for harmonised lipid reporting,[16] which recommended the reporting of non-HDL-cholesterol as part of the standard lipid profile, highlight the need for clinicians to understand the status of this and other biomarkers in ASCVD risk assessment. Once established, such a framework should facilitate the introduction and dissemination of relevant scientific advances.

CONCLUSION

Accumulating evidence suggests that non-HDL-cholesterol and apoB are superior to LDL-cholesterol in predicting ASCVD risk, and both have been designated as secondary targets in some treatment guidelines. At this juncture, it remains to be shown whether their use will improve cardiovascular outcomes in routine clinical care.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Address for correspondence: Dr John R. Burnett, Department of Clinical Biochemistry, PathWest Laboratory Medicine, Royal Perth Hospital, GPO Box X2213, Perth, WA 6847, Australia. E-mail: john.burnett@health.wa.gov.au

References

1. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell* 2015; 161: 161–72.
2. Skalen K, Gustafsson M, Rydberg EK, *et al*. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. *Nature* 2002; 417: 750–4.

3. Libby P, Loscalzo J, Ridker PM, *et al*. Inflammation, immunity, and infection in atherothrombosis: JACC review topic of the week. *J Am Coll Cardiol* 2018; 72: 2071–81.
4. 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; 38: 2459–72.
5. Schwartz GG, Steg PG, Szarek M, *et al*. Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med* 2018; 379: 209710–7.
6. Sampson UK, Fazio S, Linton MF. Residual cardiovascular risk despite optimal LDL cholesterol reduction with statins: the evidence, etiology, and therapeutic challenges. *Curr Atheroscler Rep* 2012; 14: 1–10.
7. Sandesara PB, Virani SS, Fazio S, *et al*. The forgotten lipids: triglycerides, remnant cholesterol, and atherosclerotic cardiovascular disease risk. *Endocr Rev* 2018 Oct 11: (Epub ahead of print).
8. Nordestgaard BG. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology, genetics, and biology. *Circ Res* 2016; 118: 547–63.
9. Nichols GA, Philip S, Reynolds K, *et al*. Increased cardiovascular risk in hypertriglyceridemic patients with statin-controlled LDL cholesterol. *J Clin Endocrinol Metabol* 2018; 103: 3019–27.
10. Bhatt DL, Steg PG, Miller M, *et al*. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N Engl J Med* 2018 Nov 10: (Epub ahead of print).
11. Anderson TJ, Gregoire J, Pearson GJ, *et al*. 2016 Canadian Cardiovascular Society Guidelines for the management of dyslipidemia for the prevention of cardiovascular disease in the adult. *Can J Cardiol* 2016; 32: 1263–82.
12. Catapano AL, Graham I, De Backer G, *et al*. 2016 ESC/EAS guidelines for the management of dyslipidaemias: the task force for the management of dyslipidaemias of the European society of cardiology (ESC) and European atherosclerosis society (EAS) developed with the special contribution of the European Assocciation for cardiovascular prevention & rehabilitation (EACPR). *Atherosclerosis* 2016; 253: 281–344.
13. Stone NJ, Robinson JG, Lichtenstein AH, *et al*. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American college of cardiology/American heart association task force on practice guidelines. *J Am Coll Cardiol* 2014; 63: 2889–934.
14. Grundy SM, Stone NJ, Bailey AL, *et al*. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Circulation* 2018 Nov 8: (Epub ahead of print).
15. Cantey EP, Wilkins JT. Discordance between lipoprotein particle number and cholesterol content: an update. *Curr Opin Endocrinol Diabetes Obes* 2018; 25: 130–6.
16. Australasian Association of Clinical Biochemists Harmonisation of Lipids Expert Group. Harmonised lipid reporting – recommendations from the harmonisation workshop 2018. DGD01. Aug 2018; cited Nov 2018. https://www.aacb.asn.au/documents/item/5185
17. Siri-Tarino PW, Krauss RM. Diet, lipids, and cardiovascular disease. *Curr Opin Lipidol* 2016; 27: 323–8.
18. Cholesterol Treatment Trialists Collaboration, Baigent C, Blackwell L, *et al*. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010; 376: 1670–81.
19. Reith C, Armitage J. Management of residual risk after statin therapy. *Atherosclerosis* 2016; 245: 161–70.
20. Shapiro MD, Fazio S. From lipids to inflammation: new approaches to reducing atherosclerotic risk. *Circ Res* 2016; 118: 732–49.
21. Diffenderfer MR, Schaefer EJ. The composition and metabolism of large and small LDL. *Curr Opin Lipidol* 2014; 25: 221–6.
22. Ramasamy I. Update on the laboratory investigation of dyslipidemias. *Clin Chim Acta* 2018; 479: 103–25.
23. Mora S, Otvos JD, Rifai N, *et al*. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation* 2009; 119: 931–9.
24. Stahel P, Xiao C, Hegele RA, *et al*. The atherogenic dyslipidemia complex and novel approaches to cardiovascular disease prevention in diabetes. *Can J Cardiol* 2018; 34: 595–604.
25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499–502.
26. Martin SS, Blaha MJ, Elshazly MB, *et al*. Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. *J Am Coll Cardiol* 2013; 62: 732–9.
27. Meeusen JW, Snozek CL, Baumann NA, *et al*. Reliability of calculated low-density lipoprotein cholesterol. *Am J Cardiol* 2015; 116: 538–40.
28. Shapiro MD, Tavori H, Fazio S. PCSK9: from basic science discoveries to clinical trials. *Circ Res* 2018; 122: 1420–38.
29. Whelton SP, Meeusen JW, Donato LJ, *et al*. Evaluating the atherogenic burden of individuals with a Friedewald-estimated low-density lipoprotein cholesterol <70 mg/dL compared with a novel low-density lipoprotein estimation method. *J Clin Lipidol* 2017; 11: 1065–72.
30. Sathiyakumar V, Park J, Golozar A, *et al*. Fasting versus nonfasting and low-density lipoprotein cholesterol accuracy. *Circulation* 2018; 137: 10–9.
31. Verbeek R, Hovingh GK, Boekholdt SM. Non-high-density lipoprotein cholesterol: current status as cardiovascular marker. *Curr Opin Lipidol* 2015; 26: 502–10.
32. Varbo A, Benn M, Tybjaerg-Hansen A, *et al*. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol* 2013; 61: 427–36.
33. Sniderman AD, Williams K, Contois JH, *et al*. A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. *Circ Cardiovasc Qual Outcome* 2011; 4: 337–45.
34. Zhang X, Liu J, Wang M, *et al*. Twenty-year epidemiologic study on LDL-C levels in relation to the risks of atherosclerotic event, hemorrhagic stroke, and cancer death among young and middle-aged population in China. *J Clin Lipidol* 2018; 12. 1179–89e4.
35. Mihaylova B, Emberson J, Blackwell L, *et al*. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet* 2012; 380: 581–90.
36. National Vascular Disease Prevention Alliance. *Guidelines for the Management of Absolute Cardiovascular Disease Risk*. Melbourne: National Stroke Foundation, 2012.
37. Australian Bureau of Statistics. *Australian Health Survey: Biomedical Results for Chronic Diseases, 2011-12*. Canberra: Australian Bureau of Statistics, 2013.
38. Smith SJ, Cooper GR, Myers GL, *et al*. Biological variability in concentrations of serum lipids: sources of variation among results from published studies and composite predicted values. *Clin Chem* 1993; 39: 1012–22.
39. Perera NJ, Burns JC, Perera RS, *et al*. Adjustment of direct high-density lipoprotein cholesterol measurements according to intercurrent triglyceride corrects for interference by triglyceride-rich lipoproteins. *J Clin Lipidol* 2010; 4: 305–9.
40. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. *Lancet* 2014; 384: 626–35.
41. Langlois MR, Descamps OS, van der Laarse A, *et al*. Clinical impact of direct HDLc and LDLc method bias in hypertriglyceridemia. A simulation study of the EAS-EFLM Collaborative Project Group. *Atherosclerosis* 2014; 233: 83–90.
42. Nauck M, Warnick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. *Clin Chem* 2002; 48: 236–54.
43. Ewald N, Hardt PD, Kloer HU. Severe hypertriglyceridemia and pancreatitis: presentation and management. *Curr Opin Lipidol* 2009; 20: 497–504.
44. Chen SH, Yang CY, Chen PF, *et al*. The complete cDNA and amino acid sequence of human apolipoprotein B-100. *J Biol Chem* 1986; 261: 12918–21.
45. Powell LM, Wallis SC, Pease RJ, *et al*. A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. *Cell* 1987; 50: 831–40.
46. Segrest JP, Jones MK, De Loof H, *et al*. Structure of apolipoprotein B-100 in low density lipoproteins. *J Lipid Res* 2001; 42: 1346–67.
47. Gordon SM, Pourmousa M, Sampson M, *et al*. Identification of a novel lipid binding motif in apolipoprotein B by the analysis of hydrophobic cluster domains. *Biochim Biophys Acta Biomembr* 2017; 1859: 135–45.
48. Teslovich TM, Musunuru K, Smith AV, *et al*. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 2010; 466: 707–13.
49. Whitfield AJ, Barrett PHR, van Bockxmeer FM, *et al*. Lipid disorders and mutations in the APOB gene. *Clin Chem* 2004; 50: 1725–32.
50. Burnett JR, Bell DA, Hooper AJ, *et al*. Clinical utility gene card for: familial Hypobetalipoproteinaemia (APOB). *Eur J Hum Genet* 2015; 23: 889.
51. Boren J, Ekstrom U, Agren B, *et al*. The molecular mechanism for the genetic disorder familial defective apolipoprotein B100. *J Biol Chem* 2001; 276: 9214–8.
52. Contois JH, McConnell JP, Sethi AA, *et al*. Apolipoprotein B and cardiovascular disease risk: position statement from the AACC lipoproteins and vascular diseases division working group on best practices. *Clin Chem* 2009; 55: 407–19.

53. Marcovina SM, Albers JJ, Kennedy H, *et al*. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. IV. Comparability of apolipoprotein B values by use of International reference material. *Clin Chem* 1994; 40: 586–92.
54. Marcovina SM, Gaur VP, Albers JJ. Biological variability of cholesterol, triglyceride, low- and high-density lipoprotein cholesterol, lipoprotein(a), and apolipoproteins A-I and B. *Clin Chem* 1994; 40: 574–8.
55. Piechota W, Staszewski A. Reference ranges of lipids and apolipoproteins in pregnancy. *Eur J Obstet Gynecol Reprod Biol* 1992; 45: 27–35.
56. Contois JH, McNamara JR, Lammi-Keefe CJ, *et al*. Reference intervals for plasma apolipoprotein B determined with a standardized commercial immunoturbidimetric assay: results from the Framingham offspring study. *Clin Chem* 1996; 42: 515–23.
57. Leino A, Impivaara O, Kaitsaari M, *et al*. Serum concentrations of apolipoprotein A-I, apolipoprotein B, and lipoprotein(a) in a population sample. *Clin Chem* 1995; 41: 1633–6.
58. Jungner I, Marcovina SM, Walldius G, *et al*. Apolipoprotein B and A-I values in 147576 Swedish males and females, standardized according to the World Health Organization-International Federation of Clinical Chemistry first international reference materials. *Clin Chem* 1998; 44: 1641–9.
59. Albers JJ, Marcovina SM. Standardization of apolipoprotein B and A-I measurements. *Clin Chem* 1989; 35: 1357–61.
60. Marcovina SM, Albers JJ, Dati F, *et al*. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. *Clin Chem* 1991; 37: 1676–82.
61. Albers JJ, Marcovina SM, Kennedy H. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. II. Evaluation and selection of candidate reference materials. *Clin Chem* 1992; 38: 658–62.
62. Delatour V, Clouet-Foraison N, Gaie-Levrel F, *et al*. Comparability of lipoprotein particle number concentrations across ES-DMA, NMR, LC-MS/MS, immunonephelometry, and VAP: in search of a candidate reference measurement procedure for apoB and non-HDL-P standardization. *Clin Chem* 2018; 64: 1485–95.
63. Cole TG, Contois JH, Csako G, *et al*. Association of apolipoprotein B and nuclear magnetic resonance spectroscopy-derived LDL particle number with outcomes in 25 clinical studies: assessment by the AACC lipoprotein and vascular diseases division working group on best practices. *Clin Chem* 2013; 59: 752–70.
64. de Nijs T, Sniderman A, de Graaf J. ApoB versus non-HDL-cholesterol: diagnosis and cardiovascular risk management. *Crit Rev Clin Lab Sci* 2013; 50: 163–71.
65. Emerging Risk Factors C, Di Angelantonio E, Sarwar N, *et al*. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009; 302: 1993–2000.
66. Emerging Risk Factors C, Di Angelantonio E, Gao P, *et al*. Lipid-related markers and cardiovascular disease prediction. *JAMA* 2012; 307: 2499–506.
67. Thanassoulis G, Williams K, Ye K, *et al*. Relations of change in plasma levels of LDL-C, non-HDL-C and apoB with risk reduction from statin therapy: a meta-analysis of randomized trials. *J Am Heart Assoc* 2014; 3: e000759.
68. Tonkin AM, Blankenberg S, Kirby A, *et al*. Biomarkers in stable coronary heart disease, their modulation and cardiovascular risk: the LIPID biomarker study. *Int J Cardiol* 2015; 201: 499–507.
69. Sniderman AD, Lamarche B, Contois JH, *et al*. Discordance analysis and the Gordian Knot of LDL and non-HDL cholesterol versus apoB. *Curr Opin Lipidol* 2014; 25: 461–7.
70. Langlois MR, Chapman MJ, Cobbaert C, *et al*. Quantifying atherogenic lipoproteins: current and future challenges in the era of personalized medicine and very low concentrations of LDL cholesterol. A Consensus Statement from EAS and EFLM. *Clin Chem* 2018; 64: 1006–33.
71. Pischon T, Girman CJ, Sacks FM, *et al*. Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. *Circulation* 2005; 112: 3375–83.
72. Mora S, Buring JE, Ridker PM. Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events. *Circulation* 2014; 129: 553–61.
73. Lawler PR, Akinkuolie AO, Ridker PM, *et al*. Discordance between circulating atherogenic cholesterol mass and lipoprotein particle concentration in relation to future coronary events in women. *Clin Chem* 2017; 63: 870–9.
74. Sniderman AD, Islam S, Yusuf S, *et al*. Discordance analysis of apolipoprotein B and non-high density lipoprotein cholesterol as markers of cardiovascular risk in the INTERHEART study. *Atherosclerosis* 2012; 225: 444–9.
75. Pencina MJ, D'Agostino RB, Zdrojewski T, *et al*. Apolipoprotein B improves risk assessment of future coronary heart disease in the Framingham Heart Study beyond LDL-C and non-HDL-C. *Eur J Prev Cardiol* 2015; 22: 1321–7.
76. Lamarche B, Moorjani S, Lupien PJ, *et al*. Apolipoprotein A-I and B levels and the risk of ischemic heart disease during a five-year follow-up of men in the Quebec cardiovascular study. *Circulation* 1996; 94: 273–8.
77. Tibrewala A, Jivan A, Oetgen WJ, *et al*. A comparative analysis of current lipid treatment guidelines: nothing stands still. *J Am Coll Cardiol* 2018; 71: 794–9.
78. Nordestgaard BG, Langsted A, Mora S, *et al*. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cutpoints-a Joint Consensus Statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. *Clin Chem* 2016; 62: 930–46.
79. Nordestgaard BG. A test in context: lipid profile, fasting versus nonfasting. *J Am Coll Cardiol* 2017; 70: 1637–46.
80. Sathiyakumar V, Park J, Quispe R, *et al*. Impact of novel low-density lipoprotein-cholesterol assessment on the utility of secondary non-high-density lipoprotein-C and apolipoprotein B targets in selected worldwide dyslipidemia guidelines. *Circulation* 2018; 138: 244–54.
81. Mossakowska TJ, Saunders CL, Corbett J, *et al*. Current and future cardiovascular disease risk assessment in the European Union: an international comparative study. *Eur J Public Health* 2018; 28: 748–54.