Review

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Apolipoproteins as markers and managers of coronary risk

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Summary

Coronary artery disease (CAD) is a major cause of morbidity and mortality in Western communities. Reliable indices of coronary risk assessment and targets for drug treatment are important to the management of patients. Although plasma LDL cholesterol is well established as a predictor of CAD, it may not be the best circulatory marker. Results from recent epidemiological studies and statin trials suggest that apolipoprotein B-100 (apoB), with or without apoA-I, is superior to LDL cholesterol in predicting coronary events. Measurements of apolipoproteins are internationally standardized, automated, cost-effective and more convenient and precise than those for LDL cholesterol. ApoB may also be preferable to the measurement of non-HDL cholesterol. Measurement of apolipoproteins (apoB and possibly apoA-I) should be routinely added to the routine lipid profile (cholesterol, triglycerides and high-density lipoprotein cholesterol) to assess the atherogenic potential of lipid disorders. This is particularly relevant to dyslipidaemias characterized by an elevation in plasma triglycerides. Apolipoproteins, especially apoB, could also replace the standard 'lipid profile' as a target for therapy in at-risk patients.

Introduction

The development of atherosclerosis involves the interaction of multiple metabolic and cellular processes.[1] Central to this are disorders of lipoprotein metabolism.[2] Epidemiological and clinical studies have consistently demonstrated that elevated concentration of low-density lipoprotein (LDL) cholesterol in plasma is associated with increased risk of cardiovascular disease (CVD).[3–5] The Adult Treatment Panel III (ATP) report of the National Cholesterol Education Program accordingly identifies elevated LDL cholesterol as the primary target of lipid-lowering therapy for reducing CVD risk.[6] Although the contribution of LDL cholesterol to the development of atherosclerosis is well accepted, its unique superiority over other circulating predictors of CVD is unclear. Several recent observations have resurrected an older notion that apolipoprotein (apo) B and apoA-I may in fact be more powerful lipid-related predictors of risk for CVD.[7–9] We review this evidence, and address the importance of apoB and apoA-I for clinical practice.

Apolipoproteins: role and significance in lipoprotein metabolism

To explain why apolipoproteins may be risk predictors requires a brief revision of lipoprotein metabolism.[10] Apolipoprotein B-100 (apoB) is the chief protein component constituent of the

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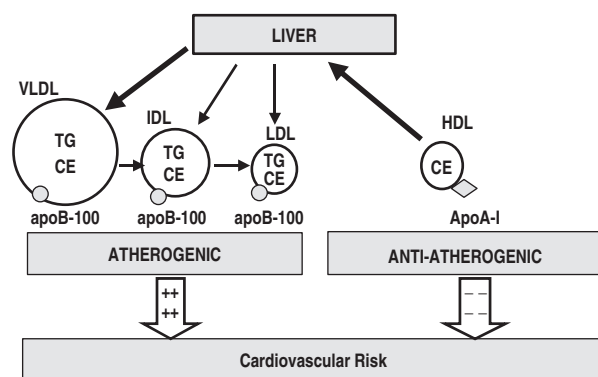*email: gfwatts@cyllene.uwa.edu.au*

Figure 1. Schematic summary of the endogenous pathways of the atherogenic and anti-atherogenic lipoproteins. Apo, apolipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; CE, cholesteryl esters; ++, increased risk; −−, decreased risk.

atherogenic very-low-density lipoprotein (VLDL), of intermediate-density lipoprotein (IDL) and of LDL particles, each particle including one apoB molecule. Hence, plasma apoB levels reflect the total numbers of atherogenic particles. In humans, VLDL particles carry endogenously synthesized triglyceride from the liver into plasma, where they undergo lipolysis to IDL by the action of lipoprotein lipase. IDL is lipolysed by hepatic lipase, converting to LDL, or taken up by the liver via the LDL receptor. ApoB is also essential for the binding of LDL particles to the LDL receptor for cellular uptake and degradation of LDL particles. ApoA-I is the major apolipoprotein constituent of the anti-atherogenic high-density lipoproteins (HDL). Levels of apoA-I are strongly associated with those of HDL cholesterol. ApoA-I is critically involved in removing excess cholesterol from tissues and incorporating it into HDL for reverse transport, either directly or indirectly via LDL to the liver. HDL also contains apoA-II, but its function and role in atherogenesis is unclear.

Figure 1 summarizes the main pathways of lipoprotein metabolism and the apolipoproteins involved. It is noteworthy that apoB, apoA-I and the apoB/apoA-I ratio reflect the status of the major atherogenic and anti-atherogenic pathways of lipoprotein metabolism. Accordingly, high apoB, high apoB/apoA-I ratio and low apoA-I levels in plasma indicate a high risk for CVD and vice versa. As with lipoprotein metabolism in general, the levels of apoB and apoA-I are controlled by genetic, environmental and hormonal factors.[10] Conditions that selectively elevate LDL cholesterol (e.g. familial hypercholesterolaemia) will increase apoB concentrations, and conditions which lower HDL cholesterol (e.g. type 2 diabetes) will lower apoA-I. However, this is not always the rule. An important exception is hypertriglyceridaemia in the insulin-resistant syndrome of central obesity, where LDL cholesterol levels are 'normal' yet apoB concentrations are elevated.[10,11] In this situation, the liver oversecretes VLDL-apoB particles loaded with triglycerides, which are then rapidly delipidated by hepatic lipase and converted to cholesterol-poor LDL, via a mechanism mediated by cholesteryl transfer protein, so that the plasma is rich in LDL-apoB particles depleted of cholesterol (Figure 2).

Epidemiology: apolipoproteins as risk predictors

Table 1 summarizes the epidemiological studies that have shown that apolipoproteins are better predictors of cardiovascular risk than conventionally measured lipids, specifically LDL cholesterol and HDL cholesterol.[12–21]

Quebec Cardiovascular Study. This was the first prospective study to demonstrate strongly that apoB was superior to cholesterol indices in predicting CHD risk.[12] In a sample of 2155 Canadian men followed for a period of 5 years for clinical signs of ischaemic heart disease (IHD), plasma apoB concentrations showed a strong association with onset of IHD (risk ratio 1.4, 95%CI 1.2–1.7) independent of triglycerides, HDL cholesterol, and total/HDL cholesterol ratio. Stepwise logistic regression analysis also revealed that apoB was a stronger correlate of IHD than the total/HDL cholesterol ratio. In the same study over 13 years of follow up, St-Pierre *et al.* recently reported that elevated plasma apoB levels remained an independent risk factor for IHD.[13] This study also found that the association of high levels of apoB and an increased risk of IHD was more obvious in men with relatively low level of LDL cholesterol.

Apolipoprotein-related Mortality Risk Study (AMORIS). A total of 175 553 individuals from Sweden were recruited and followed for an average of 5.5 years.[14] The relationships between fatal myocardial infarction (MI) and apolipoproteins and other lipid measures were examined. In multivariate analyses (after adjusting for age, total cholesterol and triglycerides), apoB, apoA-I and apoB/apoA-I ratio were all highly significant predictors of MI in both sexes. ApoB was also more significant than LDL cholesterol in prediction of risk of MI in both men and women. Receiver operating characteristics (ROC) analysis also showed that apoB had higher sensitivity and specificity than LDL cholesterol as a predictor variable in both sexes, especially in those
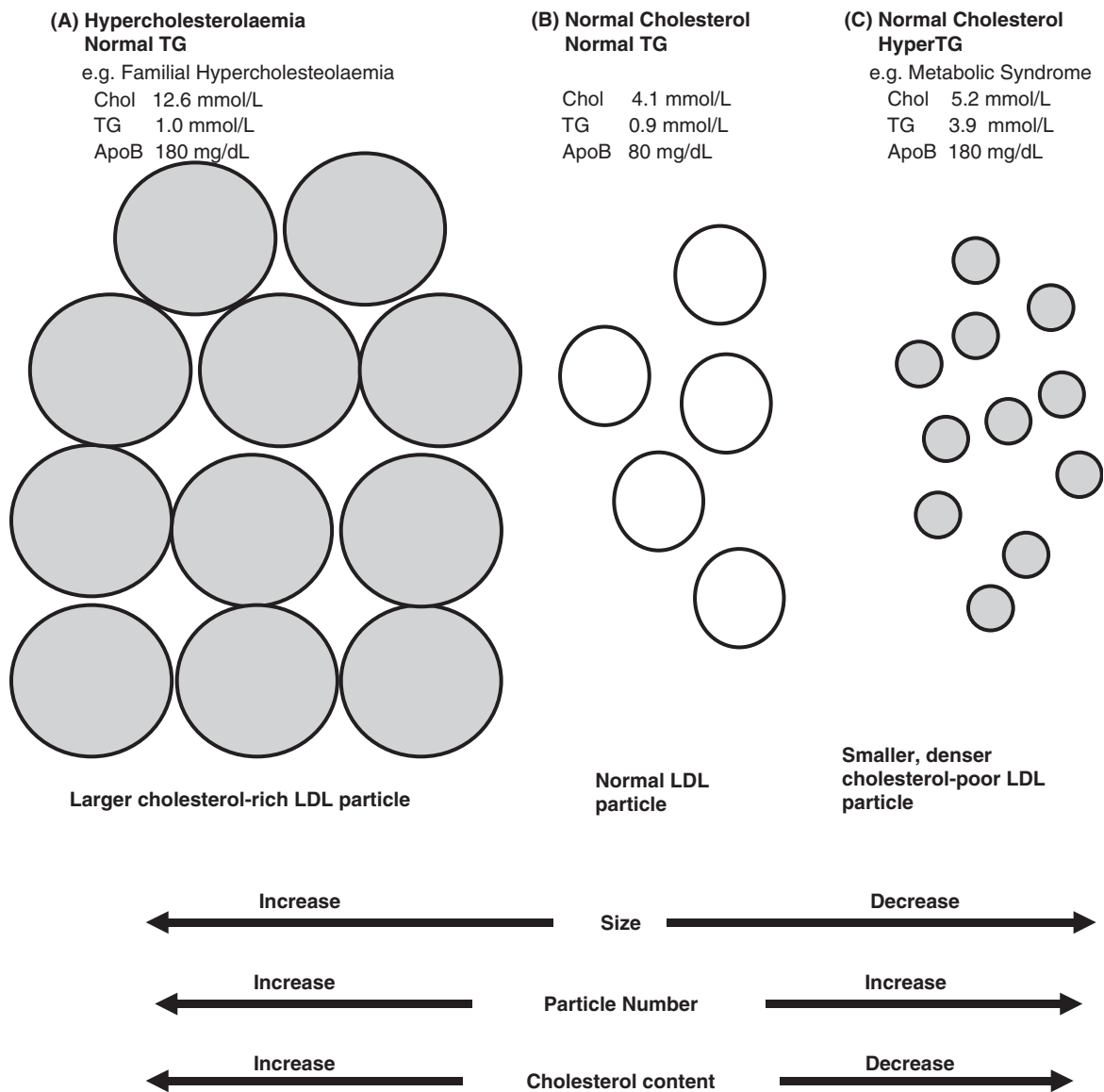
(A) Hypercholesterolaemia
    Normal TG
    e.g. Familial Hypercholesteolaemia
    Chol   12.6 mmol/L
    TG   1.0 mmol/L
    ApoB   180 mg/dL

(B) Normal Cholesterol
    Normal TG

    Chol   4.1 mmol/L
    TG   0.9 mmol/L
    ApoB   80 mg/dL

(C) Normal Cholesterol
    HyperTG
    e.g. Metabolic Syndrome
    Chol   5.2 mmol/L
    TG   3.9 mmol/L
    ApoB   180 mg/dL

Larger cholesterol-rich LDL particle

Normal LDL particle

Smaller, denser cholesterol-poor LDL particle

Increase	Size	Decrease
Increase	Particle Number	Increase
Increase	Cholesterol content	Decrease

Figure 2. Low-density lipoprotein particle number, size and composition in different metabolic states: (**A**) familial hypercholesterolaemia; (**B**) normolipidaemia; and (**C**) metabolic syndrome. Each LDL particle includes one apoB molecule. Chol, cholesterol; TG, triglycerides.

with normal/low LDL cholesterol level. Similar findings were also observed in individuals aged 70 years or older.

Second Northwick Park Heart Study (NPHSII). Here 2508 healthy middle-aged UK men were recruited to examine the relative values of apoB and other lipid variables in predicting CHD risk over 6 years of follow-up.[15] In univariate analyses, the risk ratios for LDL cholesterol, apoB, apoA-I and apoB/apoA-I ratio were 2.67, 2.90, 0.52 and 3.58, respectively. Clearly, the apoB/apoA-I ratio conferred the highest relative risk of CHD.

MONICA/KORA Augsburg Cohort Study. After recruitment, 1414 men and 1436 women aged 35–64 years without a prior coronary event

were followed-up for 13 years.[16] ApoB and apoB/apoA-I ratio had predictive power similar to that of total cholesterol/HDL cholesterol in both sexes, after adjustment for age, smoking, alcohol, BMI, diabetes, and hypertension.

Health Professionals Follow-up Study. This prospective study compared the predictive value of apoB with that of LDL cholesterol in 746 diabetic men followed-up for 6 years.[17] In both univariate and multivariate hazard models, the risk ratio for apoB was higher than those for LDL cholesterol. The area under the ROC curve for the CVD-risk prediction model with apoB was larger than that for LDL cholesterol, indicating a better risk prediction for apoB than for LDL cholesterol. The predictive

Table 1 Comparison of LDL cholesterol and apolipoproteins as coronary (or cardiovascular) risk predictors in observational and statin trials

Study	n	LDL cholesterol	ApoB	ApoA-I	ApoB/apoA-I ratio	Predictor scale
Observational studies						
Quebec Study[12]	2155 (men)	NA	1.4 (1.2–1.7)	0.85 (0.7–1.0)	NA	Change of 1 SD
AMORIS[14]	98 722 (men)	1.14 (1.01–1.28)	1.33 (1.17–1.51)	0.67 (0.62–0.71)	1.23 (1.18–.27)	Change of 1 SD
	76 831 (women)	0.85 (0.69–1.05)	1.53 (1.25–1.88)	0.74 (0.67–0.81)	1.38 (1.25–1.52)	
NPHSII[15]	2505 (men)	2.67 (1.62–4.41)	2.90 (1.82–4.64)	0.52 (0.33–0.84)	3.58 (2.08–6.19)	Top vs. lowest quartile
HPS[17]	746 (diabetic men)	1.74 (0.99–3.06)	2.31 (1.23–4.35)	NA	NA	Top vs. lowest quartile
WHS[18]	15 632 (women)	1.62 (1.17–2.25)	2.50 (1.68–3.72)	0.57 (0.42–0.77)	3.01 (2.01–4.50)	Top vs. lowest quintile
NHS[19]	32 826 (women)	1.4 (1.2–1.6)	1.8 (1.5–2.2)	NA	NA	Increase of 1 SD
AFCAPS/TexCAPS[22]	3304 (men and women)	1.44 (NA)*	1.39 (NA)*	NA	NA	Top vs. lowest tertiles
LIPID (placebo)[23]	4502 (men and women)	1.28 (1.0–1.44)	2.07 (1.32–3.22)	0.41 (0.24–0.69)	NA	Change of 1 unit
Statin trials						
AFCAPS/TexCAPS[22]	3301 (men and women)	1.26 (NA)*	1.66 (NA)*	NA	NA	Top vs. lowest tertiles
LIPID[23]	4386 (men and women)	1.08 (0.94–1.23)	1.49 (1.02–2.17)	0.61 (0.39–0.93)	NA	Change of 1 unit
Leiden Heart Study[25]	848 (men and women)	1.16 (0.80–1.67)	3.21 (1.10–9.35)	0.20 (0.08–0.49)	NA	High vs. low group

Data are risk ratios (95%CI). *Data from Sniderman *et al.* (reference 8).

power of non-HDL cholesterol was as strong as that of apoB in these hazard models. However, the ratio of total to HDL cholesterol was the best predictor of CVD in this cohort of diabetic men.

Women's Health Study. This studied 15 632 initially healthy US women aged 45 years or older over a 10-year period.[18] Under multivariate analysis, the risk ratios for LDL cholesterol (direct assay), non-HDL cholesterol, apoB and apoA-I were 1.62, 2.51, 2.50 and 0.57, respectively. Despite the authors concluding that non-HDL cholesterol and the ratio of total cholesterol to HDL cholesterol were as good as or better than apolipoprotein fractions in the prediction of future CVD events, apoB was in fact the single most significant lipid-related predictor of the occurrence of CVD events in the study. hs-CRP was also a strong predictor of CVD, with risk ratio of 2.98 (95%CI 1.90–4.67).

Nurses Health Study. This prospective study estimated the relative risk for lipids and apolipoproteins as predictors of CHD in 32 826 US women over 8 years of follow-up.[19] The risk ratios for apoB, triglyceride, LDL cholesterol, total cholesterol and HDL cholesterol were 1.8, 1.5, 1.4, 1.3 and 0.6, respectively, indicating that apoB levels were more strongly associated with increased CHD incidence than was LDL cholesterol. Under multivariate analysis, HDL cholesterol and its related ratios were the strongest contributors to predicting CHD. However, the study did not measure apoA-I, and could not therefore assess the predictive value of apoB/apoA-I.

Thrombo Study. This investigated the predictive role of haemostatic and lipid variables on risk of recurrent coronary events in 1045 MI patients with an average follow-up of 26 months.[20] High apoB and low apoA-I levels were significant predictors of recurrent coronary events, independent of other lipid variables such as total cholesterol, LDL cholesterol and triglycerides. The risk ratios for apoB and apoA-I were 1.82 and 0.54, respectively.

InterHeart Study. This was a case-control observation study that assessed the relative importance of risk factors for CHD in 15 152 cases and 14 820 controls recruited from 52 countries worldwide.[21] The ratio of apoB to apoA-I (OR 3.25, 95%CI 2.82–3.76) was the strongest risk factor in predicting MI, followed by current smoking (2.87, 95%CI 2.58–3.19), psychosocial factors (2.67, 95%CI 2.21–3.22), diabetes (2.37, 95%CI 2.07–2.71), hypertension (1.91, 95%CI 1.74–2.10) and abdominal obesity (1.62, 95%CI 1.45–1.80). Table 2 presents odd ratios for risk of MI in men and women. Similar odds ratios were seen in women and men for the association of MI with raised apoB/apoA-I ratio, smoking, and abdominal obesity.

Table 2 Odds ratios (OR) for risk factors associated with myocardial infarction in the INTERHEART study, after adjustment for age and geographical region

Risk factor	Sex	OR	99%CI
ApoB/apoA-I ratio	F	4.42	3.43–5.70
	M	3.76	3.23–4.38
Diabetes	F	4.26	3.51–5.18
	M	2.67	2.36–3.02
Psychosocial index	F	3.49	2.41–5.04
	M	2.58	2.11–3.14
Smoking	F	2.86	2.36–3.48
	M	3.05	2.78–3.33
Hypertension	F	2.95	2.57–3.39
	M	2.32	2.12–2.53
Abdominal obesity	F	2.26	1.90–2.68
	M	2.24	2.03–2.47
Fruits/vegetables	F	0.58	0.48–0.71
	M	0.74	0.66–0.83
Exercise	F	0.48	0.39–0.59
	M	0.77	0.69–0.85
Alcohol	F	0.41	0.32–0.53
	M	0.88	0.81–0.96

For further details, see Yusuf *et al.* (reference 21).

However, the increased risk associated with hypertension and diabetes seemed to be greater in women than in men.

In summary, the above data provide compelling evidence that apolipoproteins are the best lipid-related predictors to CVD in a wide range of subpopulations, including men, women, type 2 diabetics and different ethnic groups.

Clinical trials: apolipoproteins as predictors of treatment benefits

Results from several major statin trials have also demonstrated the superiority of apoB and apoA-I over cholesterol indices as predictors of treatment benefits on cardiovascular outcomes (Table 1).[22–25]

Air Force/Texas Coronary Atherosclerosis Prevention Study(AFCAPS/TexCAPS). This was a primary prevention study of the effect of lovastatin in reducing the occurrence of coronary events in 6605 asymptomatic individuals with average LDL cholesterol and below-average HDL cholesterol.[22] Although levels of LDL cholesterol, HDL cholesterol and apoB at entry were significant predictors of a first acute major coronary event, only on-treatment apoB and apoB/apoA-I ratio were predictive of subsequent risk.

Long-Term Intervention with Pravastatin in Ischemic Disease (LIPID) Trial. The relationships of

baseline and on-study lipids with subsequent CHD events were examined in 9014 CHD patients after treatment with pravastatin for 1 year.[23] Baseline apoB and apoA-I were stronger predictors of CHD events than LDL cholesterol and HDL cholesterol. The adjusted risk ratios for apoB, apoA-I, LDL cholesterol and HDL cholesterol were 2.07, 0.41, 1.28 and 0.52, respectively. The unadjusted on-treatment concentrations of apoB and apoA-I were predictive of a subsequent coronary event (RR 1.49, 95%CI 1.02–2.17 and 0.61; 95%CI 0.39–0.93, respectively), whereas on-treatment concentrations of LDL cholesterol or HDL cholesterol were not.

Scandinavian Simvastatin Survival Study (4S). Lipoprotein changes and reduction in the incidence of major CHD events were examined in 4444 CHD patients randomized to receive simvastatin or placebo over a median follow-up of 5 years.[24] Baseline apoB was significant predictor of CHD for patients in the placebo and treatment groups, but LDL cholesterol only predicted CHD risk for patients in the placebo group. The on-treatment concentrations of LDL cholesterol and apoB were both predictive of major coronary events after 1 year of treatment. The risk reductions (%) for each additional 1% lipid reduction for LDL cholesterol and apoB were 1.7% (95%CI 0.9–2.4) and 1.1% (95%CI 0.3–1.8), respectively.

Leiden Heart Study. This studied 848 patients (675 men, 173 women) with angiographically-proven CAD who received effective statin treatment.[25] In univariate analysis, the risk ratios for on-treatment LDL cholesterol, HDL cholesterol, apoB and apoA-I were 1.16 (95%CI 0.80–1.67), 0.37 (95%CI 0.17–0.80), 3.21 (95%CI 1.10–9.35) and 0.20 (95%CI 0.08–0.49), suggesting that apoB and apoA-I were superior to LDL cholesterol for predicting vascular events. Under multivariate analysis, on-treatment apoB and apoA-I were the only significant predictors for subsequent MI and all-cause mortality, after adjusting for total cholesterol, triglycerides, gender, diabetes, age and smoking. The risk ratios for apoB and apoA-I were 7.94 (95%CI 1.09–57.2) and 0.29 (95%CI 0.09–0.97), respectively.

These data show that changes in plasma apoB levels in response to treatment with a statin may be better predictors of clinical benefit than changes in LDL cholesterol.

Methodological aspects: advantages of measuring apolipoproteins

There are several methodological advantages that support the use of apolipoproteins over LDL cholesterol, particularly for apoB.

Routine LDL cholesterol is usually calculated from measurements of total cholesterol, triglycerides and HDL cholesterol in fasting blood sample using the Friedewald formula, which is valid only if fasting triglycerides are <4.5 mmol/l.[26] Calculated LDL cholesterol does not reflect the true value in patients with metabolic syndrome, diabetes mellitus, nephrotic syndrome or liver disease.[27] Moreover, the technical errors of calculating by the Friedewald equation are up to 20%, especially in subjects with LDL cholesterol levels <3.0 mmol/l.[28]

Measurement of HDL cholesterol requires an additional precipitation procedure to remove apoB-containing particles in plasma that is technically problematic. This introduces greater analytical error when estimating non-HDL or LDL cholesterol is used, rather than a simple, direct assay of apoB. The full lipid profile (cholesterol, triglycerides, HDL cholesterol and LDL cholesterol) requires the subject to fast for at least 12 h, whereas measurement of apoB does not required fasting. Although there are methods to measure LDL cholesterol and HDL cholesterol directly, these tests are not yet internationally standardized and are expensive. In contrast, the tests for measuring apoB and apoA-I are widely available, internationally standardized and automated.[29] The assay can be performed on frozen or non-fasting samples, with excellent precision of technical errors (usually <5%).

Table 3 summarizes the relative technical merits of the measurement of apoB, non-HDL cholesterol and LDL cholesterol in a routine laboratory. The estimated direct laboratory costs (Australian dollars: A$1 ≈ £0.43) for apoB, non-HDL cholesterol and calculated LDL cholesterol and direct LDL cholesterol are A$15, A$8, A$11 and A$7 per test, respectively. These costs may translate into consumer unit costs of A$65 for apoB, A$40 for non-HDL cholesterol and A$60 for standard lipid profile (total cholesterol, total triglyceride, HDL cholesterol and LDL cholesterol). The cost of replacing the standard lipid profile with apoB is therefore comparable. While measurement of apoB is at present more expensive than LDL cholesterol and non-HDL cholesterol, we estimate from data presented in Tables 1 and 4 that apoB gives an incremental risk prediction for CHD of 20% relative to LDL cholesterol and 10% relative to non-HDL cholesterol. This suggests an average additional medical cost of A$0.25–A$2.5 per 1% risk prediction compared with non-HDL cholesterol and LDL cholesterol, which we consider to be cost-efficient. The relative practical and economic merits of using these measures would, however, require more detailed evaluation in clinical practice. The economic case for adding the measurement of apoA-I to

Table 3 Comparison of practical aspects of the measurements of apoB, non-HDL cholesterol and LDL cholesterol

	ApoB	Non-HDL cholesterol	LDL cholesterol	
			Calculated	Direct
Fasting conditions	Not required	Not required	Required	Not required
Number of analytic steps	1	2	3	1
Use of recognized standard	Yes	No	No	No
Viability of frozen samples	Yes	No	No	Yes
Calculation required to estimate	No	TC–HDLC	TC–HDLC–TG/2.2	No
Risk of analytic imprecision	Lower	Higher	Higher	Lower
Potential for near-patient testing	Yes	Yes	Yes	Yes
Laboratory cost per test (A$)*	15	TC = 3, HDLC = 5	TC = 3, HDLC = 5, TG = 3	7

TC, total cholesterol; TG, total triglycerides; HDLC, HDL cholesterol; LDLC, LDL cholesterol. *Direct laboratory costs for testing in hospital laboratory (2005); for further discussion, see text. A$≈£0.43

Table 4 Logistic regression models (adjusted for age) examining the relative prediction of CHD risk and surrogate markers of CVD by apoB, non-HDL cholesterol and LDL cholesterol

Model	Predictor(s)	CHD risk equivalent	Extra-coronary plaques	High coronary calcium
1	ApoB	1.90 (1.53–2.37)	1.37 (1.16–1.61)	1.35 (1.09–1.68)
2	Non-HDL cholesterol	1.78 (1.43–2.21)	1.31 (1.11–1.56)	1.33 (1.07–1.64)
3	LDL cholesterol	1.47 (1.19–1.81)	1.19 (1.01–1.39)	1.26 (1.01–1.55)
4	ApoB	1.90 (1.17–3.05)	1.43 (0.98–2.07)	1.31 (0.80–2.15)
	Non-HDL cholesterol	1.00 (0.63–1.61)	0.96 (0.65–1.39)	1.04 (0.63–1.72)
5	ApoB	2.38 (1.68–3.35)	1.58 (1.21–1.07)	1.37 (0.96–1.94)
	LDL cholesterol	0.75 (0.53–1.05)	0.82 (0.65–1.56)	0.99 (0.70–1.40)
6	Non-HDL cholesterol	8.40 (4.19–16.8)	2.95 (1.73–5.02)	1.85 (0.91–3.73)
	LDL cholesterol	0.20 (0.10–0.39)	0.43 (0.25–0.73)	0.71 (0.35–1.44)

Data are odds ratios (95%CI). For further details see Simon *et al.* (reference 35)

the lipid profile may not be as well justified as that for apoB.

What about non-HDL cholesterol?

The superiority of apoB in predicting CHD risk compared with LDL cholesterol is in our view compelling. However, several epidemiological studies have suggested that non-HDL cholesterol is a better predictor of cardiovascular mortality than LDL cholesterol.[17,18,30–32] In the Nurses' Health Study, in 921 diabetic women, non-HDL cholesterol was a stronger predictor of CHD risk than apoB.[32] The multivariate-adjusted risk ratios for non-HDL cholesterol and apoB were 1.97 (95%CI 1.14–3.43) and 1.78 (95%CI1.02–3.11), respectively. Given that plasma non-HDL cholesterol concentration is simply derived from the standard lipid profile with no additional cost, one could argue that non-HDL cholesterol should be used as a cost-effective,

surrogate measure for apoB in clinical practice, consistent with the NCEP ATP III guidelines.[3,6] However, it remain uncertain whether non-HDL cholesterol or apoB is better at predicting risk. In some studies, apoB has been superior to non-HDL cholesterol in predicting vascular risk and benefit from statin therapy.[22,25]

Non-HDL cholesterol is not always strongly associated with apoB, especially in the presence of elevated triglyceride levels.[33] Unlike apoB, which reflects the total particle numbers of VLDL, IDL and LDL, non-HDL cholesterol provides an estimate of total cholesterol content of these lipoproteins in plasma derived as total cholesterol minus HDL cholesterol. As indicated before (Figure 2), in individuals with obesity and the metabolic syndrome, LDL cholesterol and/or non-HDL cholesterol concentrations may not be elevated even in the presence of high apoB levels. Sniderman *et al.* reported only moderate agreement between non-HDL cholesterol and apoB, with >30% of subjects

having discordant levels in a cohort of 2103 men without CAD at the onset of the Quebec Cardiovascular Study.[33] The authors concluded that non-HDL cholesterol did not appear to be an adequate surrogate for apoB. Kim *et al.* recently used similar statistical analyses to determine the degree of agreement between non-HDL chlesterol and apoB in 1181 individuals without CAD.[34] They reported that non-HDL cholesterol was only moderately concordant with apoB (kappa statistic 45%) across the quintile range for both analytes. A recent study by Simon *et al.* compared the validity of plasma apoB, non-HDL cholesterol and LDL cholesterol concentrations in predicting cardiovascular risk, defined by Framingham Risk Score, and subclinical atherosclerosis, defined as presence of extra-coronary plaques and high coronary calcium deposit.[35] The authors reported that apoB was consistently a stronger predictor of CHD risk (and peripheral and coronary atherosclerosis) than either non-HDL cholesterol or LDL cholesterol (Table 4). For example, the risk of having CHD risk was increased by 90% with apoB, while it was increased only by 78% with non-HDL cholesterol and 46% with LDL cholesterol. More importantly, the predictive power of non-HDL cholesterol was lost when apoB and non-HDL cholesterol were both introduced in the prediction model. (Table 4). This again suggests that apoB is a more powerful predictor of CVD risk than non-HDL cholesterol and LDL cholesterol. As we estimated earlier, measurement of apoB incurs an additional cost of A\$0.25–A\$2.5 per 1% risk prediction, but we consider this to be cost-efficient in clinical practice.

Targets for treatment

Although clinical evidence clearly demonstrates that apoB and apoA-I are significantly associated with CAD risk, they have not been generally accepted as therapeutic targets by the various bodies providing lipid-regulating guidelines.[6,36,37] However, a target apoB level of <90 mg/dl for patients with CAD or at high risk of CAD has been suggested by the Canadian Cardiovascular Society.[38] Based on the known strong positive relationship between non-HDL cholesterol and apoB, a target levels for apoB has been proposed by Grundy as an updated revision of the NCEP ATPIII guidleines.[3] Regarding cut-off values for apoA-I, Walldius *et al.* defined cut-off apoA-I levels of 115 mg/dl for males, and 125 mg/dl for females,[9] corresponding to apoB/apoA-I ratios of 0.9 and 0.8, respectively. Table 5 summarizes therapeutic goals for LDL cholesterol, non-HDL cholesterol and apoB. The ATPIII does not specify a

Table 5 Comparison of therapeutic targets for LDL cholesterol, non-HDL cholesterol and ApoB when treating dyslipidaemia

Therapeutic target	mg/dl	mmol/l
LDL cholesterol		
CHD and/or CHD risk equivalents*	<100	<2.6
>2 risk factors	<130	<3.4
0–1 risk factor	<160	<4.1
Non-HDL cholesterol		
CHD and/or CHD risk equivalents*	<130	<3.4
>2 risk factors	<160	<4.1
0–1 risk factor	<190	<4.9
ApoB		
CHD and/or CHD risk equivalents*	<90	$<17 \times 10^{-4}$
>2 risk factors	<110	$<22 \times 10^{-4}$
0–1 risk factor	<130	$<25 \times 10^{-4}$

*For further details see Grundy *et al.* (reference 3).

goal for triglycerides and HDL cholesterol. Raising plasma HDL cholesterol to a level >1.2 mmol/l (>45 mg/dl) and lowering plasma triglycerides to <2.3 mmol/l (<200 mg/dl) are desirable in high-risk individuals, as recommended by the American Diabetes Association (ADA) guidelines for diabetic dyslipidaemia.[36] Recently reported Australian guidelines recommend lowering triglycerides to <1.5 mmol/l in high risk individuals (annual risk >3% for year), a level below which the plasma concentration of apoB should be <1.0 g/l in subject with the metabolic syndrome and type 2 diabetes.[39]

Achieving apolipoprotein targets

Although statins are effective in lowering LDL cholesterol, they do not always achieve a commensurate reduction in plasma apoB concentrations. This is well illustrated by data from the ACCESS study referring to a subgroup of 1889 patients (67% with CHD and 20% with ⩾2 risk factors).[40] In this study, atorvastatin lowered LDL cholesterol and apoB from 178 mg/dl to 102 mg/dl (–42%) and from 170 mg/dl to 114 mg/dl (–32%), respectively. As seen in Figure 3, while target plasma LDL cholesterol levels were on average achieved on treatment in subjects with CHD and/or CHD risk equivalents, target plasma apoB levels were not achieved with therapy (Table 3). Clearly, there is a potentially serious 'therapeutic gap' in apoB relative to LDL cholesterol in response to statin treatment in high-risk coronary patients. This discordance suggests
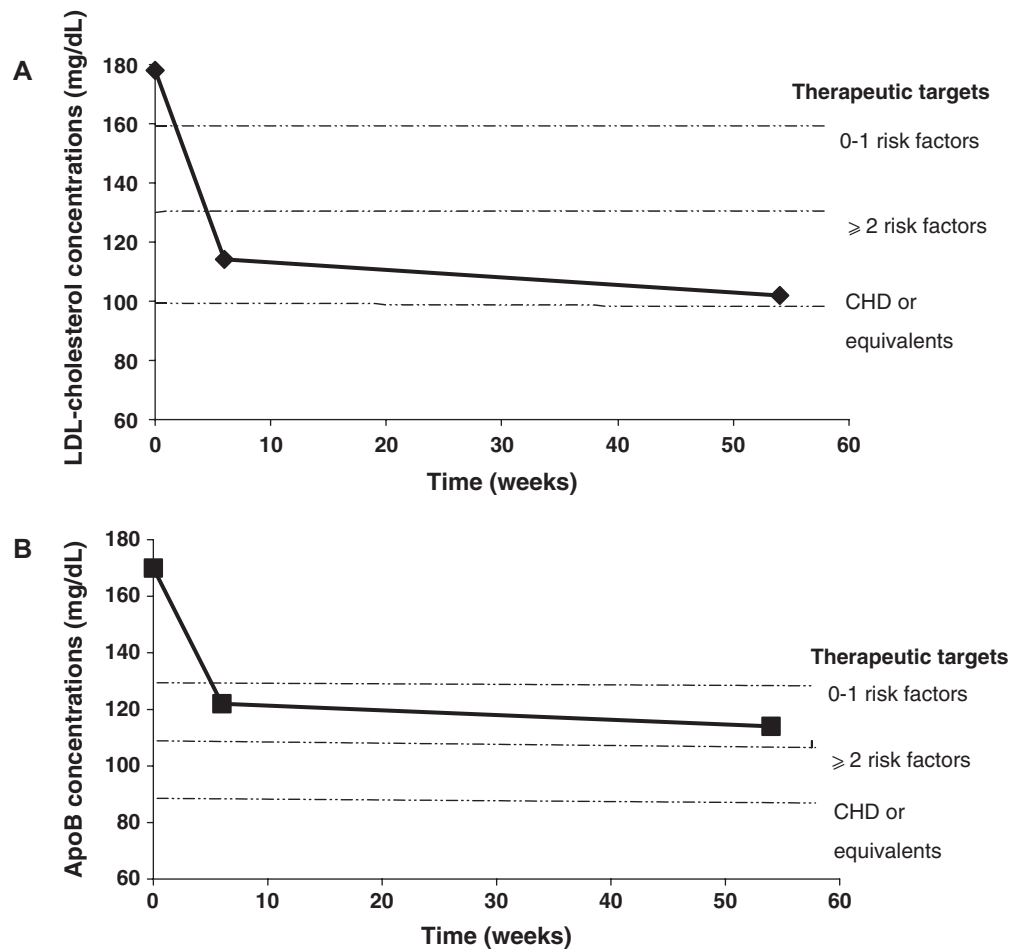
Figure 3. Effect of atorvastatin on LDL cholesterol (**A**) and apoB levels (**B**) in the ACCESS study (reference 40). Dotted lines indicate corresponding therapeutic targets at different risk levels. While plasma LDL cholesterol targets may be attained with statins, there is a significant 'treatment gap' in attaining apoB targets (reference 5).

that patients could be undertreated if LDL cholesterol is used as the sole target for statin therapy.

Recent findings from the Treat to New Targets Study (TNT),[41] the Heart Protection study (HPS)[42] and the Pravastatin or Atorvastatin Evaluation and Infection Trial (PROVE IT)[43] support a more aggressive LDL cholesterol goal of <1.8 mmol/l for patients at very high risk for CHD, compared to the previously recommended level of <2.6 mmol/l.[5] This might have been predicted by the 'gap' in the LDL cholesterol and apoB reductions shown in Figure 3. It follows that an optional LDL cholesterol lowering strategy to reduce CAD risk should include apoB targets as well. Alternatively, targeted pharmacotherapies could focus on apoB targets. In view of these observations, more powerful statins (such as rosuvastatin) or use of conventional statins in combination with fibrates, niacin or ezetimibe may be required to achieve apoB goals. Similar data on apoA-I goal relative to HDL cholesterol goals are not available, so no clear recommendation can be made

here. The efficacy of a therapeutic regimen based on apoB or apoB/apoA-I in decreasing CAD events requires to be formally investigated.

Which patients will benefit most from the use of apolipoproteins as risk predictors?

Clinically, one needs to identify which patients will benefit most from the use of apolipoproteins as risk predictors. In our view, apoB will be particularly useful in assessing patients who are hypertriglyceridaemic, with either mixed hyperlipidaemia or as an isolated abnormality. Thus, apoB should be measured in dyslipidaemias associated with metabolic syndrome, obesity, type 2 diabetes mellitus, chronic kidney disease and familial hyperbetalipoproteinaemia, since all such patients will have elevated plasma apoB. Such patients should receive targeted

treatment with lifestyle modification, statins and/or fibrates. Hypertriglyceridaemia (hyperTg) due to excess alcohol, high carbohydrate intake and abnormal lipoprotein lipase activity will not usually be associated with elevated apoB levels. These patients will only normally require nutritional and lifestyle changes. There are also patients with premature CHD, with normal cholesterol and marginal elevation of triglycerides (hyperTg hyperapoB), who are likely to have FCH, and who should routinely be assessed by measuring apoB, which should be the primary focus of drug treatment.[44] Despite there being an additional expense in measuring apoB (Table 3), we would argue that the benefits for risk prediction and management of dyslipidaemic patients outweigh the costs.

Conclusions

Results from both prospective epidemiological risk studies and lipid-lowering trials clearly demonstrate that the use of apolipoproteins is at least as good as, and often better than, LDL cholesterol, non-HDL cholesterol and various cholesterol ratios in estimating CAD risk. This may be particularly so in patients with features of the metabolic syndrome.

Pathophysiologically-based evidence and technical advantages of measuring apolipoproteins, especially apoB, also favour their clinical use in the routine assessment and treatment of dyslipidaemias. We recommend that measurement of apolipoproteins (apoB and possibly apoA-I) be routinely added to the routine lipid profile (cholesterol, triglycerides and high-density lipoprotein cholesterol) to assess the atherogeneity of a lipid disorder. This is particularly relevant to dyslipidaemias characterized by an elevation in plasma triglycerides. Apolipoproteins, especially apoB, could also replace the 'lipid profile' as a target for therapy in at risk patients.

Nevertheless, it is important to note that a single risk factor cannot estimate absolute risk of future cardiovascular events. Inclusion of a combination of demonstrable risk factors, including lipid and non-lipid variables (e.g. CRP, smoking or age) within predictive algorithms will provide a more accurate prediction of an individual's overall risk of CVD. Future studies should also examine the role of other lipoprotein, such as apoB-48, apoC-III and apoA-V, as predictors of CAD in different populations.

Acknowledgements

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