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LIPIDS AND CARDIOVASCULAR DISEASE

Nonfasting versus fasting lipid profile for cardiovascular risk prediction

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Summary

Before 2009 essentially all societies, guidelines, and statements required fasting before measuring a lipid profile for cardiovascular risk prediction. This was mainly due to the increase seen in triglycerides during a fat tolerance test. However, individuals eat much less fat during a normal day and nonfasting triglycerides have been shown to be superior to fasting in predicting cardiovascular risk. Lipids and lipoproteins only change minimally in response to normal food intake: in four large prospective studies, maximal mean changes were +0.3 mmol/L (26 mg/dL) for triglycerides, −0.2 mmol/L (8 mg/dL) for total cholesterol, −0.2 mmol/L (8 mg/dL) for LDL cholesterol, and −0.1 mmol/L (4 mg/dL) for HDL cholesterol. Further, in 108,602 individuals from the Copenhagen General Population Study in random nonfasting samples, the highest versus the lowest quartile of triglycerides, total cholesterol, LDL cholesterol, remnant cholesterol, non-HDL cholesterol, lipoprotein(a), and apolipoprotein B were all associated with higher risk of both ischaemic heart disease and myocardial infarction. Finally, lipid-lowering trials using nonfasting blood samples for assessment of lipid levels found that reducing levels of nonfasting lipids reduced the risk of cardiovascular disease.

To date there is no sound scientific evidence as to why fasting should be superior to nonfasting when evaluating a lipid profile for cardiovascular risk prediction. Indeed, nonfasting samples rather than fasting samples have many obvious advantages. First, it would simplify blood sampling in the laboratory. Second, it would benefit the patient, avoiding the inconvenience of fasting and therefore needing to have blood drawn early in the day. Third, for individuals with diabetes, the risk of hypoglycaemia due to fasting would be minimised. Many countries are currently changing their guidelines towards a consensus on measuring a lipid profile for cardiovascular risk prediction in the nonfasting state, simplifying blood sampling for patients, laboratories, and clinicians worldwide.

Keywords: LDL cholesterol; postprandial; total cholesterol; triglycerides; risk scores.

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INTRODUCTION

For many years it has been the standard to measure a lipid profile in the fasting state for cardiovascular risk prediction. The reasons for this are, among others: (1) the theoretical dynamic changes that can occur in test results for some lipid components during a postprandial test; (2) in many laboratories low-density lipoprotein (LDL) cholesterol is estimated by the Friedewald equation [as either total cholesterol – high-density lipoprotein (HDL) cholesterol – triglycerides/2.2 in mmol/L; or total cholesterol – HDL cholesterol – triglycerides/5 in mg/dL], and since triglycerides are seen to vary according to fasting status during a fat-tolerance test, calculated LDL cholesterol is also affected; (3) because this is the way it has always been done; and (4) because fasting has been the standard, there is uncertainty about the cut-offs of nonfasting lipid measurements (Fig. 1).

Cholesterol circulates in the blood as five major components: LDL cholesterol, intermediate-density lipoproteins (IDL) cholesterol, HDL cholesterol, very low-density lipoproteins (VLDL) cholesterol, and chylomicrons and their remnants. Chylomicrons are the main component found after intestinal cells absorb triglyceride-containing food, and VLDL comprise most of the plasma triglycerides, therefore triglycerides theoretically change in response to fasting versus nonfasting status. During a regular day most people eat regularly, including three main meals and snacks in between, and therefore are in the fasting state only for a few hours in the morning before breakfast (Fig. 1). A lipid profile measured in the fasting state, therefore, will not reflect the true lipid and lipoprotein composition and concentration in plasma present throughout the day.

To date there is no sound scientific evidence as to why fasting should be superior to nonfasting when evaluating a lipid profile for cardiovascular risk prediction. Indeed, nonfasting samples rather than fasting samples have many obvious advantages. First, it would simplify blood sampling in the laboratory. Second, it would benefit the patient, avoiding the inconvenience of fasting and therefore needing to have blood drawn early in the day. Third, for individuals with diabetes the risk of hypoglycaemia due to fasting would be minimised. Many countries are currently changing their guidelines, moving towards a consensus on measuring a lipid profile for cardiovascular risk prediction in the nonfasting state.[1–10]

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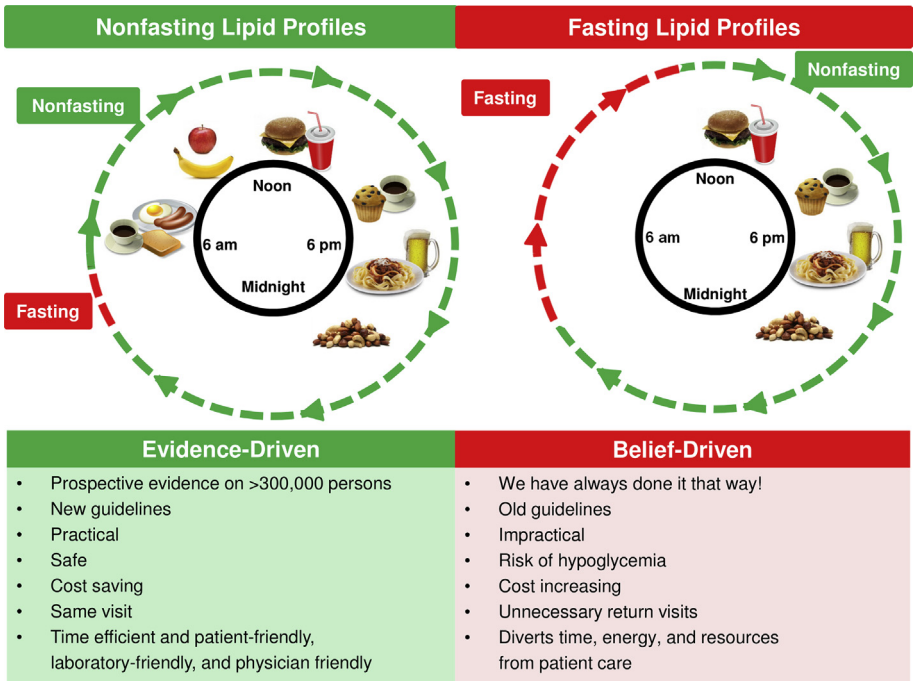

Fig. 1 Comparison of fasting and nonfasting lipid profiles. Direct comparison of arguments for and against use of random, nonfasting, and fasting blood sampling. Nonfasting blood sampling can occur anytime during the 24 hour cycle, irrespective of what and when the individual ate before blood sampling. By contrast, a fasting blood sample can only be drawn after a period without food intake for 8 or more hours, which often means that a natural small fast of a few hours in the early morning will be extended, possibly until noon, before the blood is drawn. Reprinted from *J Am Coll Cardiol* (Nordestgaard)[48] with permission from Elsevier.

THE LIPID PROFILE FOR CARDIOVASCULAR RISK PREDICTION

In a clinical setting, a standard lipid profile used for cardiovascular risk prediction includes total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides (Table 1). Worldwide, total cholesterol and HDL cholesterol are used in most risk estimator systems while LDL cholesterol is used in most randomised controlled trials with lipid-lowering therapy. Major evidence supports the role of total cholesterol and LDL cholesterol in atherosclerotic cardiovascular disease, and lowering of the two has been proven in abundance to lower the risk of cardiovascular disease and mortality.[6,11–13] In epidemiological studies, low levels of HDL cholesterol has been shown to be a strong independent risk factor for cardiovascular disease;[14,15] however, these associations have been proven not to be causal.[16–18] The role of HDL cholesterol in atherosclerotic progression might be more complex and several other roles have been suggested.[19–23]

Other lipids can be added to the standard lipid profile such as remnant cholesterol and non-HDL cholesterol, which both can be calculated without extra direct measurements or costs. Remnant cholesterol which basically includes triglyceride-rich lipoprotein cholesterol is calculated as: total cholesterol – LDL cholesterol – HDL cholesterol. Remnant cholesterol has been shown to be observationally and causally (from human genetics) associated with increased risk of cardiovascular disease and all-cause mortality.[24–28] Non-HDL cholesterol is calculated as total cholesterol – HDL cholesterol, and is equivalent to LDL cholesterol, remnant cholesterol, and cholesterol in lipoprotein(a) combined, and thereby represents the cholesterol content of all atherogenic particles. There is a clear association between non-HDL cholesterol levels and risk of cardiovascular disease, and a reduction in non-HDL cholesterol has been shown to lead to a reduction in cardiovascular disease events.[2,29–31]

LDL cholesterol can be either directly measured using various assays or calculated by the Friedewald equation as either: total cholesterol – HDL cholesterol – triglycerides/2.2 in mmol/L; or total cholesterol – HDL cholesterol – triglycerides/5 in mg/dL. This is usually only used for triglycerides <4.5 mmol/L or <400 mg/dL[32] and otherwise LDL cholesterol is directly measured. The Friedewald equation was developed on fasting material, but nonfasting and fasting samples correlate similarly when comparing calculated LDL cholesterol and measured LDL cholesterol (Fig. 2), also reported elsewhere.[33,34]

A minimal lipid profile including only triglycerides and total cholesterol can be used in countries where costs are a major issue such as developing countries.

An expanded lipid profile including lipoprotein(a) should be used in individuals at intermediate or high risk of cardiovascular disease, with premature cardiovascular disease, with familial hypercholesterolaemia, with recurrent cardiovascular disease despite statin treatment, and/or with consistent high LDL cholesterol despite high intensity lipid-lowering therapy.[35] Lipoprotein(a) is an independent causal risk factor for cardiovascular disease and is highly genetically determined.[36–39] Levels vary only modestly over time and therefore lipoprotein(a) measurements should not be repeated regularly.

Further, apolipoprotein B (apoB) can be included in an expanded lipid profile (Table 1). It is the structural protein of all non-HDL lipoproteins, all of which contain one single apoB molecule; therefore, apoB concentration is considered a measurement of all atherogenic particles in plasma.[40] The value of apoB over LDL cholesterol as a marker of

cardiovascular disease risk is controversial.[41–45] It involves an extra cost and further standardisation is needed to implement apoB in the standard lipid profile.[46]

Non-HDL cholesterol and remnant cholesterol can be included in the lipid profile as they come at no additional cost. Both are good predictors of cardiovascular disease; however, discordance can occur between apoB and non-HDL cholesterol in patients with dyslipidaemia.[47]

Additional measurements such as lipoprotein subfractions, other apolipoproteins, and metabolomic phenotyping have been introduced in some laboratories, but their added value in a clinical setting is still questionable.[48]

PATHOPHYSIOLOGY OF LIPIDS AND LIPOPROTEINS

Lipids and lipoproteins are atherogenic, but on the other hand also have vital functions in the human body. The primary role of triglycerides is to provide energy for muscles and other organs and excess energy is stored as triglycerides in adipose cells. Triglycerides are also used as insulation of the skin and as protection around organs.

Bile acids are produced from cholesterol in the liver, they allow lipids and water to mix in the intestines, and are essential in the breakdown and absorption of food including lipids and the fat-soluble vitamins A, D, E, and K from the intestines to the blood. The essential lipids, linolenic acid and linoleic acid, are used in the production of cell membranes, while oestrogen, testosterone, progesterone and the active form of vitamin D are all formed from cholesterol. About 25 percent of the cholesterol in the body originates from the diet while 75 percent is formed in the liver and other cells. Lipids are transported in the bloodstream in lipoprotein particles which consist of an outer layer containing phospholipids, free cholesterol, and proteins covering the core consisting of triglycerides and cholesterol ester.[49] Lipids from the diet are taken up by the intestine and secreted to the bloodstream as chylomicrons (Fig. 3). In the bloodstream chylomicrons will encounter capillary endothelial cells and the enzyme lipoprotein lipase will metabolise triglycerides in the chylomicrons. The chylomicron remnants are then subject to endocytosis by the liver and here metabolism of the chylomicron remnant lipoproteins will occur. Lipoprotein synthesis in the liver begins with production and release of VLDLs and again lipoprotein lipase will hydrolyse triglycerides leading to the remaining lipoprotein, IDL, then converted through the action of the triglyceride-degrading enzyme hepatic lipase into LDL. LDL particles are then taken up in the liver and other tissues via the LDL receptor.

Atherosclerosis development starts by the endothelium of the artery being injured or diseased together with entrance of lipoproteins into the intima. Then monocytes are activated and transformed into foam cells in the arterial intima. Foam cells collect cholesterol and triglycerides from the bloodstream.[50–54] LDL cholesterol and remnant cholesterol can penetrate the arterial wall, but not diffuse further and therefore are trapped in the arterial media. LDL particles cannot be

Table 1 Minimal, standard, and expanded lipid profiles, nonfasting or fasting

Measurement	Measurements in plasma or serum as part of lipid profiles			Minimal lipid profile	Standard lipid profile	Expanded lipid profile	Single measurement (not advised)	Additional measurements
	Lipid	Lipoprotein	Apolipoprotein					
Advantage Disadvantage				Inexpensive No lipoprotein measurements	Low cost None	Relatively low cost None	None Overlooked elevated triglycerides and remnant cholesterol	None Expensive, largely unnecessary measurements
Triglycerides	√			√	√	√		
Total cholesterol	√			√	√	√	√	
LDL cholesterol[a]		√			√	√	√	
HDL cholesterol		√			√	√		
Remnant cholesterol[b]		√			√	√		
Non-HDL cholesterol[c]		√			√	√		
Lipoprotein(a)		√				√		
Apolipoprotein B			√			(√)		√
Apolipoprotein A1			√					√
Lipoprotein subfractions		√						√
Other apolipoproteins			√					√
Metabolomic phenotyping	√	√	√					√

[a] LDL cholesterol can either be measured directly or calculated by the Friedewald equation if triglycerides are <400 mg/dL (4.5 mmol/L): total cholesterol − HDL cholesterol − triglycerides/2.2 (all in mmol/L; or triglycerides/5 with values in mg/dL), with direct measurement of LDL cholesterol at triglyceride concentrations ≥400 mg/dl (4.5 mmol/L).

[b] Remnant cholesterol (= triglyceride-rich lipoprotein cholesterol) is calculated as total cholesterol − LDL cholesterol − HDL cholesterol, using random, nonfasting or fasting lipid profiles; if LDL cholesterol is also calculated, then remnant cholesterol is equivalent to triglycerides/2.2 in mmol/L and to triglycerides/5 in mg/dL.

[c] Non-HDL cholesterol is calculated as total cholesterol − HDL cholesterol and is equivalent to LDL and remnant cholesterol combined. Reprinted from *J Am Coll Cardiol* (Nordestgaard)[48] with permission from Elsevier.

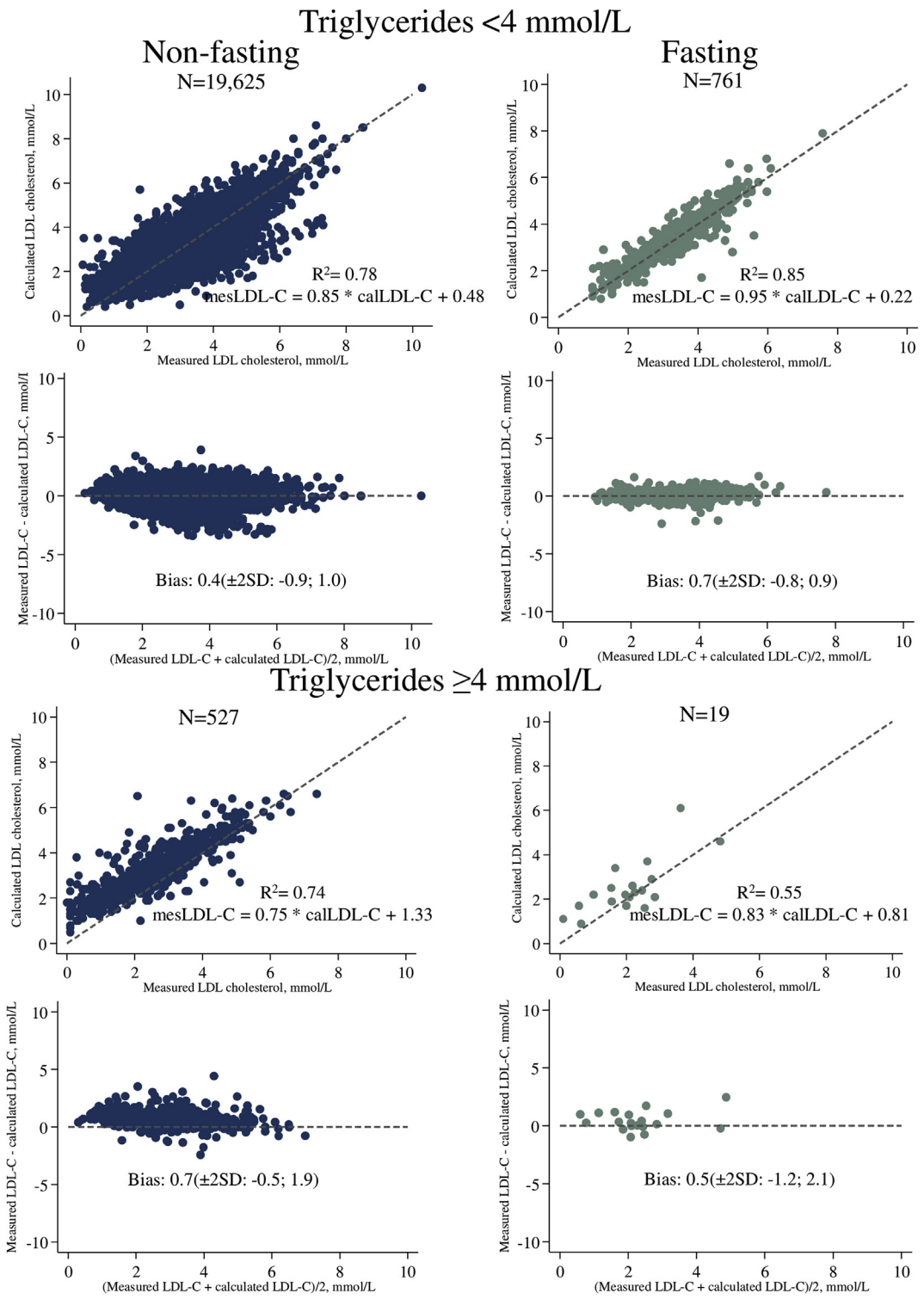

Fig. 2 Comparison of calculated low-density lipoprotein cholesterol using the Friedewald equation with low-density lipoprotein cholesterol measured directly using random nonfasting and fasting lipid profiles. Mes, measured; Cal, calculated using the Friedewald equation (low-density lipoprotein cholesterol = total cholesterol – high-density lipoprotein cholesterol – triglycerides/2.2; all values in mmol/L; if values are in mg/dL then use triglycerides/5). Based on unpublished data from 5906 individuals participating in the Copenhagen City Heart Study 2001–2003 examination and 15,026 individuals from the Copenhagen General Population Study 2014–2016 examination. Adapted with permission from *Eur Heart J* (Nordestgaard *et al.*).[8]

taken up before they are modified, whereas remnant particles can be engulfed by macrophages without modification.[55,56]

Large VLDL particles and chylomicron particles cannot cross the arterial wall because of their larger size (Fig. 4).[57,58] Like HDL, LDL, IDL, chylomicron remnants and small VLDL, lipoprotein(a) can transfer into the intima; however, it has not been shown that lipoprotein(a) is a key factor in atherosclerotic growth and the exact pathophysiology of lipoprotein(a) is not fully understood.[39] When LDL cholesterol and remnant cholesterol is elevated in plasma over many

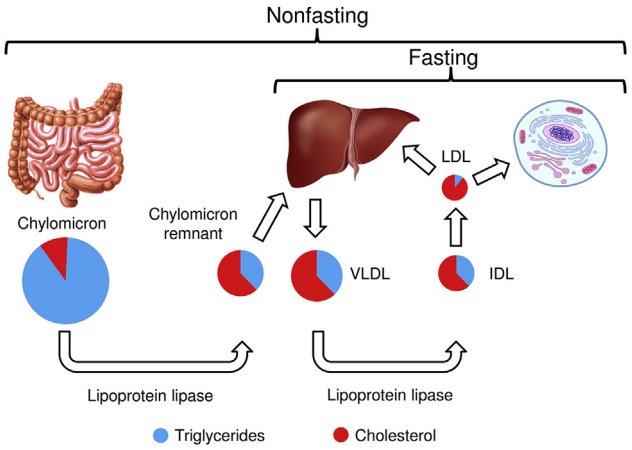

Fig. 3 Atherogenic lipoproteins present in the blood during periods of fasting and nonfasting. During fasting, only liver-derived lipoproteins are present in plasma, whereas in the nonfasting state, intestinal-derived lipoproteins are likewise found in plasma. IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein. Reprinted from *J Am Coll Cardiol* (Nordestgaard)[48] with permission from Elsevier.

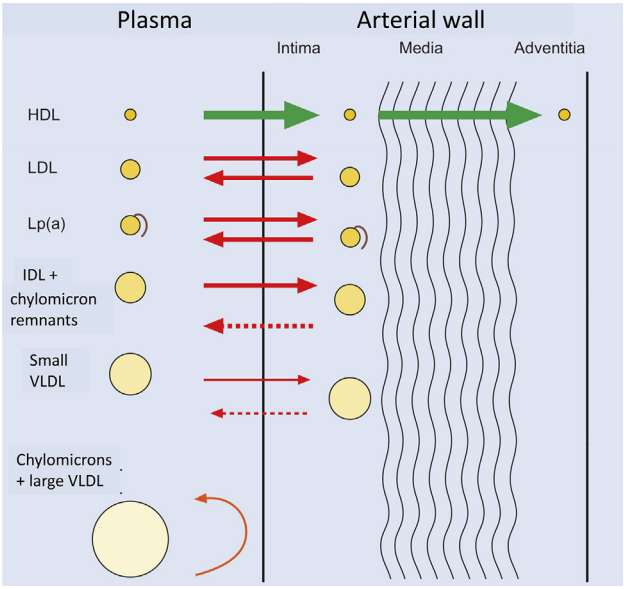

Fig. 4 Transfer of lipoproteins between plasma and the arterial intima. This figure depicts the relative speed by which different lipoproteins enter and leave the arterial intima, and thereby which lipoproteins get trapped preferentially in the intima. First, the larger the lipoprotein diameter, the fewer that enter the intima, where chylomicrons and large very low-density lipoprotein (VLDL) are simply too large to enter. Second, although high-density lipoprotein (HDL) is small enough to penetrate the media and leave via the adventitia, other lipoproteins are so large that they can only leave the intima via the lumen of the artery. Because back transport is against a blood pressure gradient, the largest lipoproteins, such as intermediate-density lipoprotein (IDL), chylomicron remnants, and small VLDL, get trapped preferentially in the intima. Lp(a), lipoprotein(a). Reprinted from *J Am Coll Cardiol* (Nordestgaard)[48] with permission from Elsevier.

years, atherosclerotic progression will occur. As lipids are trapped in foam cells and atherosclerotic plaques grow, they thicken the artery wall and bulge into the bloodstream. These plaques narrow the artery reducing blood flow. Myocardial infarction may occur if a plaque is ruptured and a blood clot will block the artery leading to reduced oxygen delivery to the affected area.

In individuals with isolated hypercholesterolaemia, when plasma cholesterol is elevated and plasma triglycerides are <2 mmol/L (<176 mg/dL), the main atherogenic cholesterol in plasma is in LDL (Fig. 5). In combined hyperlipidaemia with elevated plasma cholesterol and plasma triglycerides between 2 and 10 mmol/L (176 and 880 mg/dL), the atherogenic cholesterol consists of that in LDL, VLDL, IDL, and chylomicron remnants. Finally, in severe hypertriglyceridaemia when both plasma cholesterol and triglycerides are highly elevated [triglycerides >10 mmol/L (>880 mg/dL)], the atherogenic lipoproteins are as before LDL, VLDL, IDL, and chylomicron remnants. At very high triglyceride levels the risk of pancreatitis is very high,[59] but the atherogenic risk is somewhat lower than for combined hyperlipidaemia, simply because a large fraction of cholesterol is carried in chylomicrons and large VLDLs (shown in gold on right of Fig. 5) that are not able to penetrate into the arterial intima (Fig. 4).

TIME SINCE LAST MEAL

Lipids and lipoproteins only change minimally in response to normal food intake as has been shown previously in both men, women, and children in large studies from several countries including both nonfasting and fasting samples.[60–64] Normal food intake was defined as whatever that individual ate on the particular day of blood sampling, no restrictions were made. This naturally differed between individuals and between countries. For triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol, the maximum change for 108,245 individuals from the Copenhagen General Population Study was +0.3, −0.2, −0.2, and −0.1 mmol/L (26, 8, 8, and 4 mg/dL) (Fig. 6). The corresponding numbers for 26,330 individuals from the Women's Health Study were +0.2, −0.1, −0.2, and 0.0 mmol/L (18, 4, 8, and 0 mg/dL), for 12,744 children from the National Health and Nutrition Examination Survey +0.1, −0.1, −0.1, and 0 mmol/L (9, 4, 4, and 0 mg/dL) (Fig. 7), and finally for 209,180 men and women from Calgary Laboratory Services +0.3, 0, −0.1, 0 mmol/L (26, 0, 4, and 0 mg/dL) (Fig. 8). Overall levels of total cholesterol and LDL cholesterol decrease slightly in response to normal food intake and in general this occurs from 1 to 4 hours following a meal. At the same interval, albumin has been shown to decrease, most likely in response to fluid intake during a meal, and most likely the decreases observed in cholesterol are also a response to fluid intake.[60] When fasting before blood sampling patients are allowed to drink water and therefore this would still influence the results. For triglycerides and remnant cholesterol there is a minor increase in plasma levels following normal food intake for 1–7 hours after a meal (Fig. 9); this occurs because of an increased level of chylomicrons chylomicron remnants originating from the intestine. Lipoprotein(a), apoB, and apolipoprotein A1 do not change in response to normal food intake (Fig. 6). In particular, it has been shown in 34,829 individuals from the Copenhagen General Population Study and the Copenhagen City Heart Study that lipoprotein(a) does not change in response to normal food intake.[65]

NONFASTING SAMPLES AND PREDICTIVE VALUE

Fasting requires not eating or drinking (except for water) for >8 hours; therefore, during a regular 24-hour cycle most individuals are mainly in a nonfasting state (Fig. 1, left panel). One argument for fasting before a lipid profile is because of the variations seen in triglycerides and LDL cholesterol due

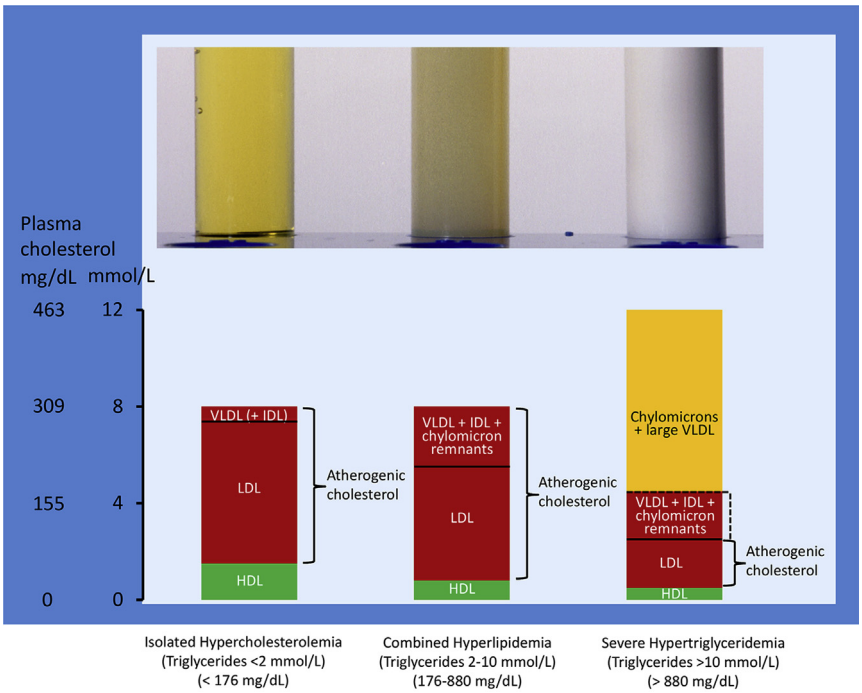

Fig. 5 Cholesterol in atherogenic lipoproteins in different types of hyperlipidaemia. (Top) The visual appearance of the three types of hyperlipidaemia: isolated hypercholesterolaemia; combined hyperlipidaemia; and severe hypertriglyceridaemia. (Bottom) Distribution of atherogenic cholesterol in different lipoproteins, all shown in red. For severe hypertriglyceridaemia, some cholesterol is found in chylomicrons and large very low-density lipoprotein (VLDL) that likely are not atherogenic (shown in gold), as these lipoproteins are too large to enter into the intima. Neutral cholesterol in high-density lipoprotein (HDL) is shown in green. IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein. Reprinted from *J Am Coll Cardiol* (Nordestgaard)[48] with permission from Elsevier.

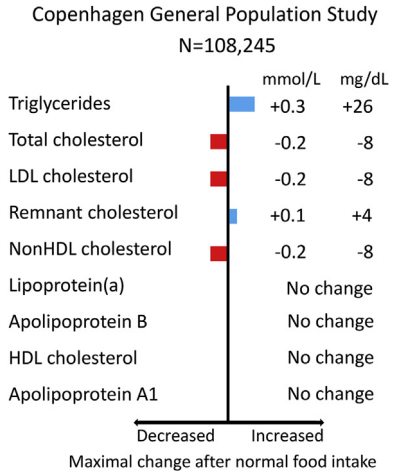

Fig. 6 Mean maximal change in lipids, lipoproteins, and apolipoproteins in random, nonfasting compared with fasting lipid profiles in 108,245 individuals from the Copenhagen General Population Study. HDL, high-density lipoprotein; LDL, low-density lipoprotein. Reprinted from *J Am Coll Cardiol* (Nordestgaard)[48] with permission from Elsevier.

to fat intake; however, in 5538 individuals from Herlev University Hospital, fasting and nonfasting levels were similar when measuring triglycerides (Fig. 10). All 5538 patients had both fasting and nonfasting triglyceride measurements; overall for individuals with nonfasting samples the median level was 1.41 (interquartile range 0.96–2.06) mmol/L [125 (85–183) mg/dL] and for fasting samples 1.37 (0.97–2.04) mmol/L [121 (86–181) mg/dL]. For triglyceride levels 0–4.0 mmol/L (0–354 mg/dL), fasting levels were 0.01–0.05 mmol/L (0.9 4 mg/dL) lower than nonfasting samples, and for triglyceride levels >4.0 mmol/L (>354 mg/dL), fasting levels were 0.27 mmol/L (24 mg/dL) higher than nonfasting levels. For individuals with and without diabetes mellitus, triglyceride levels were also similar for fasting and nonfasting samples. For 4141 individuals with both fasting and nonfasting measurements of LDL cholesterol from Herlev University Hospital, overall nonfasting levels were 2.6 (2.0–3.5) mmol/L [101 (77–135) mg/dL] and fasting levels were 2.5 (1.9–3.3) mmol/L [97 (73–128) mg/dL]. Also, as previously mentioned in several large-scale studies, the maximal increase of triglycerides was 0.3 mmol/L (27 mg/dL) at 3–4 hours after normal food intake (Fig. 6–9). If non-fasting plasma triglycerides are >5 mmol/L (440 mg/dL), a fasting blood sample could be considered; however, this is generally not necessary as most likely a single very high triglyceride measurement due to very high fat intake before blood sampling will be followed by another nonfasting measurement with lower triglyceride concentration.[8]

Flagging abnormal values in laboratories is usually done on the basis of age- and sex-specific reference intervals (the 2.5th to 97.5th percentiles). However, because of the unhealthy lifestyle in most Western populations, the upper reference limits for lipids and lipoproteins are very high; therefore, cutpoints rather than reference intervals are often used for flagging.[8]

The effect of food intake on plasma lipids and lipoproteins seems minimal; however, the question remains of whether nonfasting samples are equally solid as risk predictors for cardiovascular disease and mortality as nonfasting samples. In 16,161 individuals from the National Health and Nutrition Examination Survey III (NHANES-III), a national representative database of the US population with a follow-up time of 14 years, it was found that nonfasting LDL cholesterol levels had similar prognostic value compared with fasting LDL cholesterol levels for prediction of all-cause and

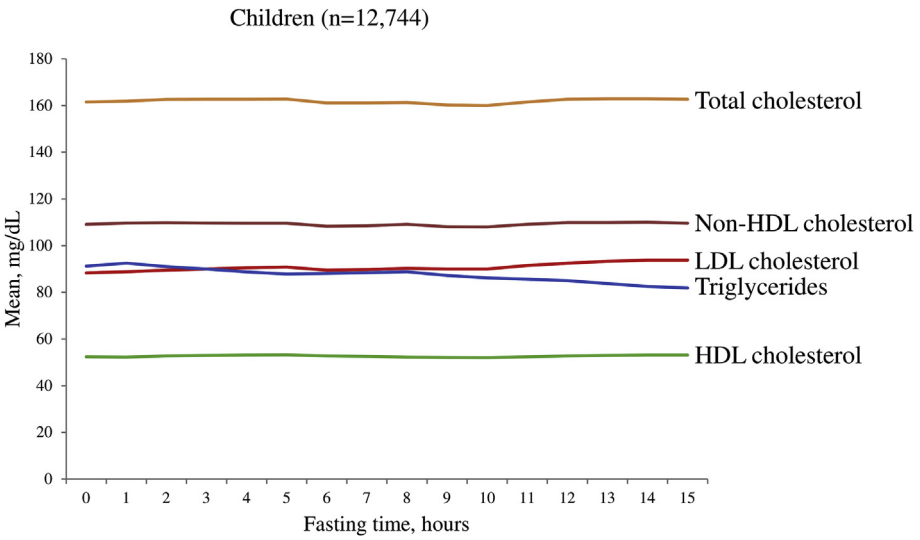

Fig. 7 Mean concentrations of lipids and lipoproteins as a function of the period of fasting following the last meal in children in the US general population. The last meal simply represents what the individual chose to eat on that day before blood sampling, with no information or requirement on amount or type of food eaten. Based on 12,744 children from the National Health and Nutrition Examination Survey. HDL, high-density lipoprotein; LDL, low-density lipoprotein. Reprinted with permission from *Eur Heart J* (Nordestgaard *et al.*).[8]

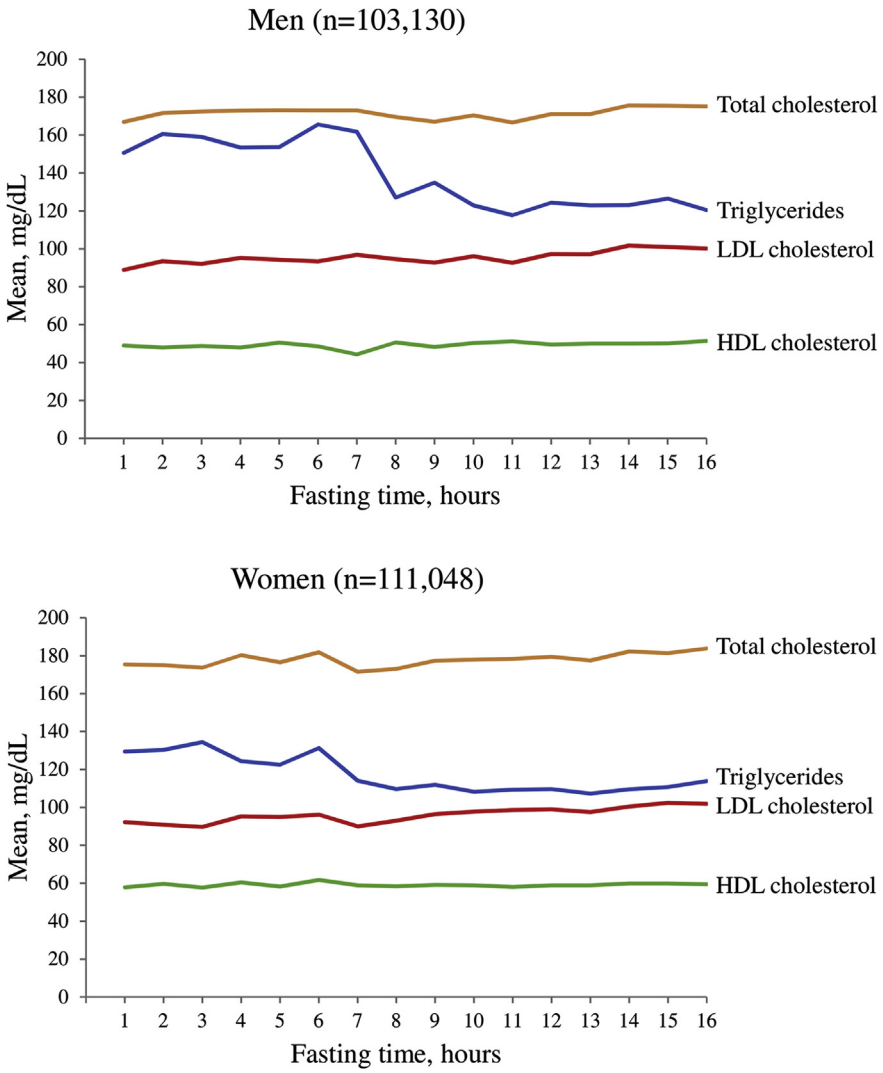

Fig. 8 Mean concentrations of lipids and lipoproteins as a function of the period of fasting following the last meal in men and women from the Canadian general population. The last meal simply represents what the individual chose to eat on that day before blood sampling, with no information or requirement on amount or type of food eaten. Based on 209,180 men and women from Calgary Laboratory Services. HDL, high-density lipoprotein; LDL, low-density lipoprotein. Reprinted with permission from *Eur Heart J* (Nordestgaard *et al.*).[8]

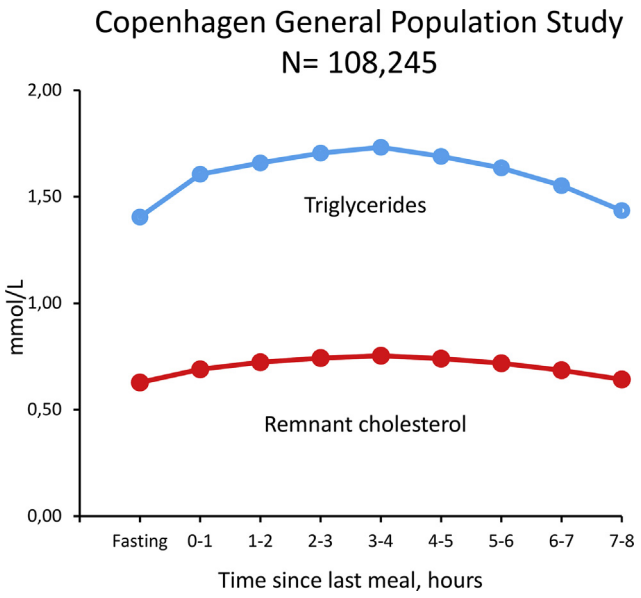

Fig. 9 Plasma triglycerides and remnant cholesterol as a function of time since last habitual meal in individuals in the general population. The mean maximal increase in triglycerides of 0.3 mmol/L (26 mg/dL) and in remnant cholesterol of 0.13 mmol/L (8 mg/dL), compared with fasting levels, occurs 3–4 hours after the last meal. Reprinted from *J Am Coll Cardiol* (Nordestgaard)[48] with permission from Elsevier.

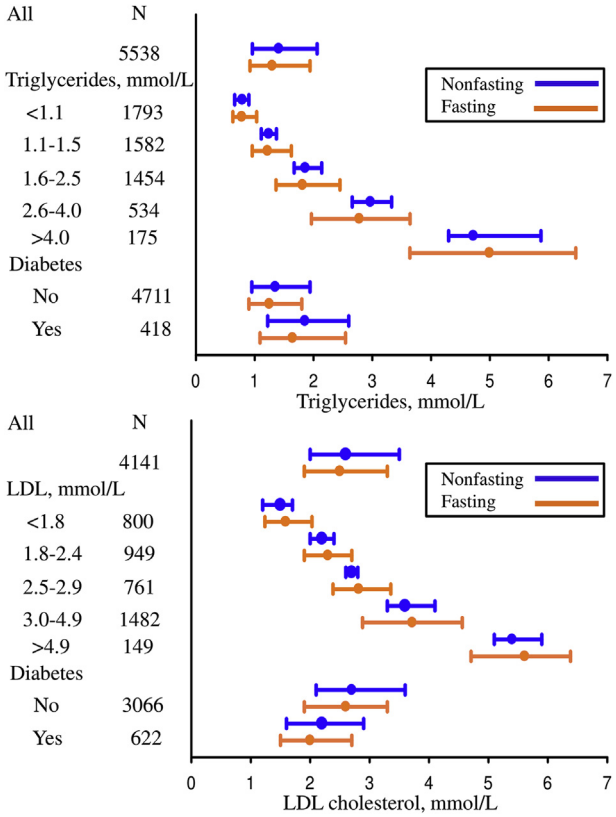

Fig. 10 Comparison of concentrations of plasma triglycerides and low-density lipoprotein (LDL) cholesterol measured in the nonfasting and fasting states in the same patients. Diabetes was determined as a haemoglobin A1c of 7.1% (of all 5538 patients with both fasting and nonfasting triglyceride measurements, 371 did not have a haemoglobin A1c measurement). Values are medians and interquartile ranges; in strata of plasma triglycerides, the interquartile ranges are larger for fasting than for nonfasting values, which is explained by regression dilution bias as the groups were defined initially based on the nonfasting measurements. Based on unpublished data on patients from Herlev and Gentofte Hospital, Copenhagen University Hospital, in the period 2011–2015. Reprinted with permission from *Eur Heart J* (Nordestgaard *et al.*).[8]

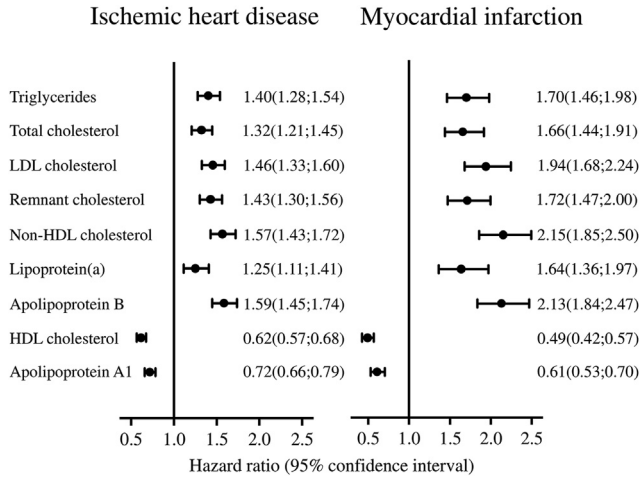

Fig. 11 Risk of ischaemic heart disease and myocardial infarction for highest vs lowest quintile of random nonfasting lipids, lipoproteins, and apolipoproteins as part of standard and expanded lipid profiles in individuals in the general population. Hazard ratios were adjusted for age, sex, smoking, hypertension, diabetes, and use of statins. Based on 108,602 individuals from the Copenhagen General Population Study recruited in 2003–2014. HDL, high-density lipoprotein; LDL, low-density lipoprotein. Reprinted with permission from *Eur Heart J* (Nordestgaard *et al.*).[8]

cardiovascular mortality.[66] The Emerging Risk Factor Collaboration (ERFC) examined 302,430 individuals without vascular disease at baseline from 68 long-term prospective studies in Europe and North America and found that for non-HDL cholesterol the hazard ratio for coronary heart disease was 1.72 (95% CI, 1.51–1.95) for nonfasting individuals versus 1.41 (1.30–1.53) for fasting individuals.[14] For triglycerides and HDL cholesterol the predictive values were similar for fasting versus nonfasting individuals. A meta-analysis involving 262,525 individuals with 10,158 cardiovascular disease events from 29 prospective studies found no major differences in the strength of associations between triglycerides and coronary heart disease in studies including fasting participants compared with studies including nonfasting participants.[67] In 13,956 participants from the Copenhagen City Heart Study including 1529 incident ischaemic strokes there was a stepwise increase in risk with increasing levels of nonfasting

Fig. 12 Historical development of endorsement of random, nonfasting lipid profiles by societies, guidelines, and statements. Particularly from 2016 and onwards, the use of nonfasting lipid profiles has been endorsed widely. Reprinted from *J Am Coll Cardiol* (Nordestgaard)[48] with permission from Elsevier.

triglycerides.[68] Also, in 116,550 individuals from the Copenhagen general population it was shown that nonfasting triglycerides from 2 mmol/L (177 mg/dL) and above were associated with high risk of acute pancreatitis.[59] Finally, for prospective studies, we examined the risk of ischaemic heart disease and myocardial infarction in 108,602 individuals from the Copenhagen General Population Study recruited in 2003 through 2014. For random nonfasting individuals, the highest versus the lowest quartile of triglycerides, total cholesterol, LDL cholesterol, remnant cholesterol, non-HDL cholesterol, lipoprotein(a), and apoB were associated with higher risk of both endpoints (Fig. 11). Also, some lipid-lowering trials such as the Anglo-Scandinavian Cardiac Outcomes Trial – Lipid Lowering arm,[69] the Heart Protection Study,[70] and the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine,[71] used nonfasting blood samples for assessment of lipid levels and all found that reducing levels of nonfasting lipids reduced the risk of cardiovascular disease. Collectively, this vast amount of evidence highlights the scientific arguments for measuring lipids, lipoproteins, and apolipoproteins in the nonfasting state. Additionally, this would simplify blood sampling for patients, laboratories, and clinicians worldwide.

HISTORY OF IMPLEMENTING NONFASTING LIPID PROFILING

Up until 2009 most societies, guidelines, and statements recommended an 8–14 hour fast prior to blood sampling for a lipid profile. However, in 2009 the Danish Society of Clinical Biochemistry in Denmark was the first society in the world to made an official recommendation on measuring lipids in the nonfasting state for cardiovascular risk prediction (Fig. 12).[1] Following this, in 2011 the American Heart Association published a statement on triglycerides and cardiovascular disease stating that nonfasting triglyceride levels can be used to screen for high triglyceride levels.[2] The National Institute for Health and Care Excellence Guidance on Cardiovascular Disease, Risk Assessment and Reduction, including Lipid Modification from the UK published in 2014 stated that a fasting sample is not needed for a full lipid profile before starting lipid-lowering therapy for prevention of cardiovascular disease.[3] Also, in 2014 the Veterans Affairs and US Department of Defense made a recommendation that a nonfasting lipid profile is an accurate measure for risk calculation.[4] In Europe in 2016 the European Atherosclerosis Society and the European Federation of Clinical Chemistry and Laboratory Medicine published a consensus statement recommending using nonfasting samples for lipid profiling,[8] and also the most recent guidelines for the management of dyslipidaemias from the European Society of Cardiology and the European Atherosclerosis Society recommend nonfasting samples.[6] In Canada the Canadian Cardiovascular Society[5] and the Canadian Hypertension Education Program Guidelines Task Force[7] recommends nonfasting lipid determination as a suitable alternative to fasting levels. In Brazil the Society of Clinical Analyses, the Society of Clinical Pathology/Laboratory Medicine, the Society of Cardiology, the Society of Endocrinology and Metabolism, and the Society of Diabetes made a joint consensus statement in 2017 on using nonfasting samples for a lipid profile.[10] Finally, as the latest in 2017, the American Association of Clinical Endocrinologists and the American College of Endocrinology recommended using nonfasting lipid profiles for cardiovascular risk prediction.[9] Overall recommendations are spreading throughout the world and hopefully more and more countries will endorse these guidelines and use nonfasting samples to simplify blood sampling for patients, laboratories, and clinicians worldwide.

CONCLUSION

In a standard clinical setting, a standard lipid profile for cardiovascular risk prediction consists of triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol; in high risk patients it is relevant to measure the genetically determined lipoprotein(a) once. Further and without extra cost, calculation of remnant cholesterol and non-HDL cholesterol could (should) be included in the profile.

Previously, all guidelines recommended individuals to fast prior to blood sampling for a lipid profile. Not much scientific evidence exists to support this recommendation. One of the main arguments proposed in favour of fasting samples is that triglycerides are observed to increase during a fat tolerance test.[72,73] However, during a fat tolerance test one typically eats 1 g of fat per 1 kg of bodyweight, while during a normal meal, individuals most likely eat far less fat. Furthermore, as shown in 108,245 individuals from the Copenhagen General Population Study (Fig. 6), in 26,330 women from the Women's Health Study in the US, in 12,744 children from the National Health and Nutrition Examination Survey in the US (Fig. 7), and in 209,180 individuals from Calgary Laboratory Services in Canada (Fig. 8), lipids, lipoproteins, and apolipoproteins only changed minimally in response to normal food intake.[60,62–64] Maximal mean changes were +0.3 mmol/L (26 mg/dL) for triglycerides, −0.2 mmol/L (8 mg/dL) for total cholesterol, −0.2 mmol/L (8 mg/dL) for LDL cholesterol, and −0.1 mmol/L (4 mg/dL) for HDL cholesterol.

Nonfasting samples are a better option (1) for the patient, simplifying the procedure and enabling blood to be drawn at any time of day; (2) for individuals with diabetes mellitus, minimising the risk for hypoglycaemia in those using glucose-lowering medication; (3) for the laboratory, distributing the number of patients more evenly during the day and eliminating the need to ask patients to return for a new visit if not fasting. Taken together, shifting from fasting to nonfasting samples for lipid profiles would be health, time, and cost saving.

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