To fast or not to fast? Fat is the question

Anne Paxton

October 2020—For nearly five decades, clinicians and laboratories aiming to screen LDL cholesterol (LDL-C) in adults to assess cardiovascular disease risk have contended with a problem generally beyond their control: lack of assurance that patients told to fast before a blood specimen is collected for lipid testing have indeed fasted.

Even as evidence to the contrary began to emerge, fasting was long believed to be necessary for the accurate assessment of LDL-C without the need for expensive and labor-intensive ultracentrifugation of plasma.

Patient compliance, however, often fell short of the ideal. Although many patients could be trusted to fast, others forgot to fast, tried to but failed, thought they had fasted but overlooked food intake, or opted not to be tested because they didn't want to fast. "People would say, 'Oh, yeah—I fasted,' forgetting the doughnut they had," says James Otvos, PhD, strategic director, NMR Diagnostics, LabCorp.

European recommendations published in 2016 supported nonfasting LDL testing and a U.S. guideline concurred two years later.

Now the struggle over fasting may be over for good—not because patients have mended their ways, but because a new formula for calculating LDL-C, developed by the National Institutes of Health and published this year, can be used with nonfasting lipid testing to produce results that for most purposes are just as good as fasting lipid testing (Sampson M, et al. *JAMA Cardiol.* 2020;5[5]:540–548).

With validation complete, the new NIH formula steps in to replace the Friedewald equation, which debuted in 1972. An improved competitor equation, developed by Seth Martin, MD, of Johns Hopkins University, was introduced in 2013. However, that equation did not fully edge out the Friedewald equation, in part because the Martin-Hopkins equation was proprietary and required a royalty for commercial use. For the Friedewald equation, it was taken as a given that lipid panel blood samples provide more accurate results from patients who were fasting.

More than 250,000 patient samples from LabCorp and other clinical laboratories were analyzed to validate the new NIH equation. The analysis showed that the equation calculates LDL-C more accurately than does the Friedewald equation, says Dorothy M. Adcock, MD, LabCorp's chief medical officer, who is leading the adaptation of the company's testing panels to the new NIH equation.

Dr. Adcock

Measuring LDL-C directly adds cost and in the past was tedious, Dr. Adcock notes, which is why the Friedewald equation was introduced long ago. It calculates LDL-C using this formula: Total cholesterol – HDL cholesterol – (Triglycerides/5). "With the Friedewald equation, most clinicians recommended that patients fast because we knew that triglycerides carried in chylomicrons, which for almost all of us increase after a meal, can significantly interfere with the calculation of LDL cholesterol when they are elevated," Dr. Adcock says.

A guideline on the management of blood cholesterol put forth by multiple organizations and reported online in 2018 by the American College of Cardiology and American Heart Association established that lipid panels could be obtained in either a fasting or nonfasting state (Grundy SM, et al. *J Am Coll Cardiol.* 2019;73[24]:3168–3209). Despite that, most clinicians, trained that they will get a more accurate result with a fasting sample, have opted to

play it safe—by recommending fasting—because results could be inaccurate when triglycerides are elevated.

“But even the 2018 guidelines state that you can collect samples in a fasting or nonfasting patient for initial testing, although if you identify a significant abnormality on the screen and want to repeat the test, you should repeat it fasting,” Dr. Adcock says. “That would be to confirm hypertriglyceridemia in those with pancreatitis or suspected pancreatitis, if you are screening for early-onset heart disease, and if you are screening for familial hyper-cholesterolemia.”

The biggest problem with the Friedewald equation is the way it estimates very-low-density lipoprotein cholesterol (VLDL-C), she notes. “It makes the assumption that VLDL-C is equivalent to total triglycerides divided by five.” But as triglycerides become elevated, their relationship with VLDL-C changes, which leads to significant underestimates of LDL cholesterol, particularly when LDL levels are very low, Dr. Adcock says.

In the 1970s and 1980s, treatment with statins or with the newer PCSK9 inhibitors was not available, so the Friedewald equation was more appropriate, she says. “But now we see low cholesterol levels in individuals on statins or PCSK9 inhibitor therapy—meaning that sometimes the Friedewald equation can lead to falsely low and sometimes nonsensically negative LDL-C levels.”

The new NIH equation is more accurate when triglycerides are elevated or LDL-C is low (below 70 mg/dL), Dr. Adcock says. “The guidelines state that if LDL-C is below 70, you should consider measuring direct LDL-C. But this will no longer be necessary if you use the NIH equation.”

The NIH equation performs equally well in fasting and nonfasting individuals, she says, “except for those few cases where you still need to obtain a fasting lipid panel. Using this new equation will allow the vast majority of patients to get their lipids drawn in a nonfasting state.”

LabCorp is introducing the NIH equation into its panels that include calculated LDL-C, and it is working closely with clients about the potential need to change their systems to accept the testing. LabCorp is also educating clinicians, “so they will be aware of the increased accuracy using the NIH equation in those samples with low LDL cholesterol values as well as in calculating LDL-C when triglycerides are elevated—above 400 mg/dL.”

The NIH equation is likely to encourage patient compliance with clinicians’ orders for testing, in Dr. Adcock’s view. “Some patients just don’t want to fast. Some don’t want the hassle of fasting and going to get their blood drawn first thing in the morning—that’s typically when the draw stations are the busiest.” While some other tests do require fasting, “nonfasting testing will make it more convenient for people to have the lipid panel drawn during the day when it may be more convenient for them and the draw site is not as busy.”

“I hope that those patients who may have been forgoing lipid testing because of the hassle of fasting will get their lipids tested,” she says, “because LDL-C is critical to determining cardiovascular risk.”

A key development pushing the U.S. consensus toward nonfasting lipid testing was the 2016 consensus statement of the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine, with Børge Nordestgaard as senior author, says coauthor Alan T. Remaley, MD, PhD (*Clin Chem.* 2016;62[7]:930–946). Dr. Nordestgaard has been a proponent of nonfasting lipid testing—now widely accepted and practiced in Europe—for many years, explains Dr. Remaley, director of immunoassay and special chemistry, National Heart, Lung and Blood Institute, and section chief of the lipoprotein metabolism laboratory, Cardiovascular and Pulmonary Branch, NHLBI.

As the European statement notes, nonfasting is essentially the norm; most people consume several meals during the day and some consume snacks between meals. “The postprandial state therefore predominates over a 24-hour period,” the authors wrote.

In the U.S., the American Board of Pediatrics had made a recommendation in 2011 that fasting wasn’t necessary for children. But it wasn’t until 2018 that the American Heart Association and American College of Cardiology reported its guideline that nonfasting samples are sufficient for the initial screen for cardiovascular disease.

“For the most part,” Dr. Remaley says, “the lipid parameters do not change that much in the postprandial state. The only thing that significantly changes is triglycerides, and this is used in calculating LDL cholesterol by the Friedewald equation. Because of that, people recommended that you fast, since as triglycerides go up, the equation doesn’t work as well.” The inaccuracy starts at around 150 or 200 mg/dL, he says, “a range of TG that can readily occur in the postprandial state even in normal subjects.”

“Almost every lab and university immediately switched to direct HDL-C tests when they first became available in the late 1990s because HDL-C tests can be done on the automated instruments. Direct LDL-C tests became available shortly after that, but they’re still not widely used, although they are catching on. The direct LDL-C assays, unless the triglycerides are very high, are usually fairly accurate,” Dr. Remaley says.

Data from the Women’s Health Study showed that postprandial lipids were more predictive of cardiovascular events than fasting lipids. Earlier, Dr. Nordestgaard and many others had also shown that the lipoproteins that accumulate in the postprandial state are also atherogenic or at least are associated with cardiovascular disease risk, Dr. Remaley says. “Because most people aren’t in a fasting state—many people eat around the clock—it’s thought that the exposure of lipoproteins that you have after you eat may be more reflective of your risk. That seemed to hold true in the Women’s Health Study. So people started asking, ‘Well, why are we fasting?’”

Dr. Remaley

Dr. Remaley’s group developed the new NIH equation for calculating LDL cholesterol. It is accurate up to a TG of at least 800 mg/dL, he says. “So it’s not really necessary to fast to get an accurate LDL cholesterol any longer. You can either do it by a direct measurement or by this new calculation.”

The benefits of switching to nonfasting samples go beyond convenience and more lipid testing, one of which is safety. “There are reports of deaths, mostly from people who have diabetes and then go into hypoglycemic shock from fasting, leading in some cases to motor vehicle accidents,” Dr. Remaley says.

It’s also difficult to know how well patients comply with a fasting order, but Dr. Remaley suspects quite a few fail to do so. “Clinicians may not be aware of the current guidelines and may still tell their patients to fast, but laboratories often do not capture this information. When you look carefully, many people aren’t properly fasting.”

Although statistically, he notes, most lipids do not show a major change in the postprandial state, “there are some people, depending on what they ate last or maybe based on their metabolism, who will have a significant change in their HDL or LDL cholesterol. But they are the minority. For most people, the change, except for TG, is minor or clinically insignificant, when using this information for primary prevention or establishing what you should do to reduce cardiovascular risk.”

The biologic variability of most of the lipids is less than 10 percent, Dr. Remaley says. “But for triglycerides, it is very high—at least 20 percent, based on fasting studies. It could easily be larger than that in the postprandial state. That’s why if you’re using triglyceride, which is now the new recommendation as a risk-enhancer test, I think one should not use it based on the result of one sample. Previous recommendations stressed the importance of having multiple samples over a several-week period, maybe as many as five before making a decision on therapy. Ideally you should make a decision based on at least two or three samples, but even that, I think, most people don’t follow.”

Seldom are lipid results medical emergencies, Dr. Remaley notes, but laboratorians should flag some critical lipid values. “For example, when triglycerides are over 1,000 mg/dL, you start worrying about pancreatitis. I also study

a lot of rare genetic disorders and I often see patients with very low HDL—many labs would say 'undetectable.' But you should probably consider flagging these results, too, so that these patients are referred to a lipidologist, because there are now new therapies for some of these rare genetic diseases."

Abetalipoproteinemia is one of those rare genetic diseases. "They [patients] have undetectable LDL, and clinicians all the time say, 'Oh, you're going to live forever.' But these patients may develop fat-soluble vitamin deficiencies and they can go blind and develop a neuropathy. Clinicians often just think of lipids in terms of cardiovascular disease, not in terms of the risk associated with pancreatitis or other conditions that can occur with rare genetic disease. Sometimes it takes 10 or 20 years until these patients get the correct diagnoses." In his laboratory, he and colleagues have recommendations of their own as to what lipid values they should flag.

Dr. Remaley hopes to get the word out that the NIH equation, which is freely available, is a more accurate way to measure LDL. (To download it, go to https://doi.org/10.35092/yhjc.11903274.) "We evaluated it on over a quarter of a million people in our study, working with LabCorp and Mayo Clinic Laboratories. It can even save labs money from having to do direct LDL testing on high-triglyceride samples." LabCorp has already made the switch to the new equation, and Mayo Clinic Laboratories is in the process of implementing it and plans to go live in December.

Mayo makes every effort to provide early morning phlebotomy appointments when patients are asked to fast before blood draws, says Jeffrey W. Meeusen, PhD, clinical chemist and co-director of cardiovascular laboratory medicine at Mayo. "Historically, fasting patients reported in the mornings and were accommodated regardless of their actual phlebotomy appointment time," he says.

But during the pandemic, patients are asked to report at their scheduled time to reduce crowding in lobbies and waiting areas. "The ability to accurately assess LDL-C without a need for fasting is one of the strongest drivers for adoption of the new equation at Mayo Clinic," Dr. Meeusen says.

When Dr. Otvos started a company called LipoScience in the late 1990s, the plan was that its nuclear magnetic resonance clinical analyzer could be introduced into the clinical laboratory workflow and used to produce an LDL particle number with minimal difficulty. The goal was to have a turnkey NMR analyzer on which a medical technologist could load 200 samples, push a button, and walk away, Dr. Otvos says.

LabCorp acquired LipoScience in 2014 and ended up keeping all the NMR analyzers for use at LabCorp, with the exception of one—the analyzer sitting in Dr. Remaley's lab at NHLBI under a cooperative research agreement. "We are continuing to discover interesting things besides lipids and lipoproteins that can be extracted from the same NMR scan, such as glucose, different amino acids, and a biologically stable signal called GlycA that serves as a very good measure of systemic inflammation," Dr. Otvos says. "So in essence, NMR is sort of analytic gravy; there's no incremental analytic cost for measuring more than one thing."

Specifically with lipids, an NMR scan that can give an LDL particle number also can give high-quality traditional lipid panel information as well as apoB. At LipoScience, Dr. Otvos says, "we had left it to other laboratories to measure the lipids. We were all about measuring what they couldn't, which is the lipoprotein particles that contain the lipids. But we then came to the realization at LabCorp that it might be beneficial if the same measurement could serve many purposes—obtaining total cholesterol, HDL cholesterol, triglycerides, and apoB from the same spectrum with no reagents. And the analytic efficiencies associated with that could potentially allow it to be offered at lower cost, which has been the barrier to widespread adoption of apoB." LabCorp received FDA clearance for this extended lipid panel assay in 2018, he says.

LDL particle number, like apoB, is unaffected by fasting and nonfasting, Dr. Otvos says. "It's really only LDL cholesterol that suffers from the nonfasting calculation problem. So if we were using apoB or LDL particles as an alternative to LDL cholesterol for clinical decision-making about whether LDL-lowering is sufficient in a given patient, there would not be a worry about fasting versus nonfasting."

In Dr. Otvos' perfect world, apoB would be adopted as the alternative to LDL cholesterol for guiding LDL treatment decision-making, as European guidelines have recommended, he says. But a major impediment to doing so, he

notes, “is that people have been doing things based on LDL cholesterol for 50 years. So you’d have to re-educate people to a certain extent. And then there’s the added analytic cost of apoB, which is typically measured using immunoassays.”

The Johns Hopkins researchers who introduced the Martin-Hopkins equation called attention to the Friedewald formula’s inaccuracy at low values, Dr. Otvos says. “It didn’t used to be the case that very many people had very low LDL cholesterol because there weren’t drugs to make that happen. Pharmacology has totally changed the landscape in the last five years.”

“It really is kind of a big deal because laboratories had to decide, for many years after the Martin equation was introduced, did they want to undergo the added cost of a royalty to Hopkins for the ability to report LDL-C calculated by an improved method. Most opted not to, and that was LabCorp’s decision.”

Now, however, the NIH equation has come along. “It is at least as good as the Hopkins equation and there is no need for a royalty to be paid,” Dr. Otvos says. He sees no real barrier to universal adoption of the NIH formula.

Dr. Otvos

Dr. Otvos was never a fan of nonfasting lipid panels because, he says, “there is other information in a fasting sample that’s useful clinically.” He and others are interested in assessing diabetes risk concurrent with assessing cardiovascular risk, for example.

“Insulin resistance is something that leads to diabetes, and having a higher fasting triglyceride level is associated with insulin resistance. So if your triglyceride is 200 mg/dL fasting, there is something to be made of that clinically, if you choose to do so. If your focus is only on LDL cholesterol and LDL management, then fasting or nonfasting, it doesn’t matter.”

But fasting status should be indicated on laboratory reports, he believes. “So at least you will have a heads-up if the specimen was nonfasting and the triglyceride is 500 mg/dL. That would normally raise eyebrows because at a minimum the patient might be at risk for pancreatitis, and you’d worry about lowering the triglyceride therapeutically.” However, “If you had eaten a big fatty meal two hours before giving the specimen, your actual fasting triglyceride could well be 150 and not 500. The difference can be that big. So you don’t want to be inferring that a nonfasting triglyceride means anything clinically. You would want to order a fasting specimen for the clinical purpose of making something of the triglyceride information.”

Many clinicians may not have mixed purposes when they order a lipid panel. “But the fasting hypertriglyceridemia associated with pancreatitis risk is an independent clinical concern that’s well known to people. A lot of doctors are using the ratio of triglycerides to HDL cholesterol as an indirect measure of insulin resistance. It’s coming for free from the lipid panel, so you get a heads-up that here’s a patient for whom you might want to worry about diabetes risk.” As an aside, Dr. Otvos notes, LabCorp has biomarkers from the NMR scan that are much more effective than the triglyceride-to-HDL cholesterol ratio in assessing risk of diabetes—“one more fringe benefit of using an NMR machine to generate the lipid panel.”

Even before the new NIH formula became available, Dr. Otvos says, he found it fascinating that papers were being written about there being no need to fast. “That was without replacement of the Friedewald formula. So people were accepting the inaccuracy of LDL cholesterol that would occur with nonfasting samples from individual patients, because of the notion there wouldn’t be so many of those, and in the great majority of people there wouldn’t be a clinically significant difference. So the merits of convenience and the other drivers of nonfasting

would take precedence over the accuracy of clinical decision-making on individual people."

He found that bothersome. "No estimation is going to overcome completely a certain inaccuracy with a sample that is taken at a suboptimal time, when the fact that you just ate has caused a big change in what the lipoproteins look like."

Dr. Otvos cautions against thinking that this better calculation of LDL cholesterol has eliminated all the downsides of LDL cholesterol as a marker of LDL-related risk. "The reality is, it absolutely does not. Even the most accurate direct measurement of LDL cholesterol is going to produce a value that will be discrepant with apoB in many, many patients, mostly when LDL cholesterol is low; then apoB will be higher. So this new NIH equation isn't going to solve the world's problems."

"But now we have a calculation of LDL cholesterol that's more robust and less sensitive to nonfasting. There is greater acceptance of nonfasting now that the Friedewald part of the reason for not going there is gone," Dr. Otvos says. The national reference labs are offering it, "and there's no reason," he says, "why other laboratories should not be taking advantage of the added convenience to people."

Anne Paxton is a writer and attorney in Seattle.