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This is the podcast from *Clinical Chemistry*. I am Bob Barrett. Requesting that subjects fast before obtaining a blood sample for a laboratory analysis is one of the most common instructions and one that is often ignored, because serum triglyceride concentrations and therefore calculated LDL-cholesterol concentrations can be affected by short-term changes in diet. Some laboratorians have considered the so-called "direct measurements" of LDL cholesterol as preferable.

The May issue of the journal *Clinical Chemistry* published a report that may challenge that thinking. Dr. Samia Mora was lead author of that paper. She is an Internist and Cardiologist at the Division of Cardiovascular Disease at the Brigham and Women's Hospital, and is an Assistant Professor of Medicine at Harvard Medical School.

The same issue of *Clinical Chemistry* had an editorial on the topic by Dr. Børge Nordestgaard, Chief Physician in Clinical Chemistry at Copenhagen University Hospital and as Professor of Genetic Epidemiology at the University of Copenhagen. They are both our guests on this podcast.

Dr. Nordestgaard, everybody knows that you have to fast before a lipid profile measurement. Now, why is that the standard practice today? Is it normal for people to be in a fasting state?

Dr. Børge Nordestgaard:

Well, first of all, let me tell you that the fasting state is usually defined as somebody that hasn't eaten within the last eight hours. So if you think in 24-hour cycle, most people they would actually be in the nonfasting state, most of the time; maybe only a few hours before breakfast they will be real fasting.

So why do we usually measure it in the fasting state, then you could ask. The best reason I can come up with is because that's the way we have always done it. It has become standard practice without really having very good evidence for it.

Usually the arguments people come up with is that triglyceride would increase during a fat tolerance test. If you eat a lot of fat, triglycerides would increase, and therefore it could confuse things, and then also, when you want to calculate the LDL using the feasible equation, then you need to have fasting lipid samples. That's what it says in the textbooks anyway.

Host: So what different lipids are measured as part of lipid profile? Do all components measured need fasting prior to blood sampling?

Dr. Børge Nordestgaard: Well, a standard lipid profile is, you measure total cholesterol, total triglycerides, and HDL cholesterol, and based on these three you either calculate LDL cholesterol or you can choose to measure LDL cholesterol directly.

Should there be fasting or not? Well, if you read guidelines, for example, the National Cholesterol Education Program from the United States, they say that you don't need to measure cholesterol and HDL nonfasting, but you should measure triglycerides and LDL nonfasting.

Then there are other lipids that you can measure like non-HDL cholesterol, and then the apolipoproteins, apo B and apo A1, and they generally should not be nonfasting.

This is really confusing, because you read this in one guideline and when you read things otherwise, maybe in articles, then some people claim one thing, and some claim the other one.

Two very important papers: one, something that came out last fall in *Circulation*, the first one from the Women's Health Study with Dr. Mora, who you are also interviewing here, September 2 issue. Then we had one based on the Copenhagen City Heart Study that was published in November 11, 2008. Both these papers showed that if people eat normal food, just whatever they usually would have for breakfast or lunch, then the lipids don't really change very much in response to that normal food intake. So this is really challenging whether you need to fast prior to lipid measurements.

Also, these two papers together suggested that for most lipids, nonfasting sample were equally good as fasting sample in predicting cardiovascular risk.

Host: LDL cholesterol of course is very important measurement. Tell us how it can be calculated and measured directly.

Dr. Børge Nordestgaard: Well, first of all, originally how you measured the LDL was by way of ultracentrifugation. This is a very laborious technique and is expensive. You have to

also centrifuge for 24 hours almost before you can measure LDL.

Then in 1972, Friedewald and coworkers, they came up with a simple way of calculating LDL. You measure total cholesterol in plasma, you measure HDL cholesterol in plasma, and then you can subtract VLDL cholesterol, and VLDL cholesterol is estimated as your triglycerides number divided by five; if in milligram per deciliter, and the triglyceride divided by 2.2, if all values are in millimoles per liter, and then you get a calculated LDL cholesterol. So that's the standard practice today.

But then different companies started producing kits that could measure LDL directly, either after blocking or solubilizing other lipid proteins.

So these are really the three different ways you can measure LDL. By ultracentrifugation, you don't do it anymore; it's the golden standard, Friedewald calculation or directly measured in plasma.

Host:

Okay. Well, let's turn to you Dr. Mora. You recently published an article that used both Friedewald calculations and direct LDL measurement to predict cardiovascular disease. What were the main findings of the study?

(00:05:01)

Dr. Samia Mora:

Well, we took 27,000 healthy women with no prior history of cardiovascular disease, and these women were enrolled in the Women's Health Study. They have triglycerides less than 400 milligram per deciliter, and we measured LDL cholesterol directly using a Roche homogeneous assay. In addition, we also calculated their Friedewald LDL cholesterol.

These women were then followed for 11 years, and over that time period nearly 1,000 first cardiovascular events occurred. These included myocardial infarction, stroke, revascularization, and cardiovascular death.

We found that LDL cholesterol obtained by the two methods was highly correlated. However, LDL cholesterol concentration measured by the direct method was lower than the Friedewald LDL cholesterol by about 5-10 milligrams per deciliter.

This lower LDL cholesterol concentration, by the direct assay, resulted in misclassifying approximately

one in five women, or 20% of the women, into an incorrect NCEP cholesterol category. If the direct method was used instead of the Friedewald calculation, with most of these women misclassified into a lower risk category by the direct LDL.

We also looked to see how the two methods compared in predicting future cardiovascular events. In order to do this we divided the study population to two groups, according to fasting or nonfasting state.

Approximately three-quarters of the studied population were women whose last meal was eight hours or more from their blood drawn, and we considered these the fasting group. In this group of fasting women, LDL cholesterol by the two methods was nearly identical for predicting future cardiovascular events.

Now, the women who had eaten within eight hours of their blood drawn were considered the nonfasting group, and these represented a quarter of the women. In these nonfasting women, neither the direct LDL nor the Friedewald LDL predicted future cardiovascular events.

Host: Are these the results you were expecting or did you get some surprises?

Dr. Samia Mora: Well, yes, actually there were two surprises. First, we were quite surprised to find that the direct LDL cholesterol measurement was not any better than the Friedewald calculations.

Now, it's been believed that one of the advantages to a direct assay is that it may be used in nonfasting samples. We did not find this to be the case. Indeed, we found that LDL cholesterol in nonfasting samples did not predict future CVD risk, neither when measured by the direct assay, nor by Friedewald calculation.

So the direct assay did not provide any advantages over Friedewald calculation, plus it adds an extra cost. While the Friedewald LDL can be obtained free of charge, so to speak, if one's ordering a standard lipid panel.

Second, we were also surprised that one in five women was misclassified. Mostly these were misclassified into a lower NCEP risk category by the direct assay compared with the Friedewald calculation. This of course has clinical implications for

classification and treatment of these women, especially for drug therapies.

Host: So what's the bottom line for clinicians and laboratories based on the results of your study?

Dr. Samia Mora: Well, many labs across the nation currently use a direct assay to measure LDL cholesterol. The bottom line from this study is that direct assays may not have as much clinical utility as previously thought, and until there is clear evidence that these assays are superior to Friedewald calculations, labs and clinicians should use the Friedewald calculation instead, as long as the triglycerides are below 400 milligrams per deciliter.

The distributors of the direct assays promote that fasting is not required, and this was one of the primary reasons for developing direct LDL cholesterol assays.

Our study found that this direct assay did not predict future CVD in nonfasting samples, and may mislead clinicians for initiating treatment calls even on fasting samples. This should prompt further studies to examine not just the correlations of these direct assay results with the Friedewald calculation, but more importantly for the value of these assays in predicting future cardiovascular events, both in fasting and nonfasting states.

Host: Dr. Nordestgaard, what did you find the most important message of the Mora study?

Dr. Børge Nordestgaard: Well, first of all, I would like to say, I think it's a very timely and very important paper. Dr. Mora has told you all the details of what they found, but what I would like to focus is two things. I think the first and important thing is that this paper is really challenging, should we have all lipid measurements in the fasting state and ask questions whether would nonfasting be equally good, that's one important thing.

Another important thing is that it challenges whether we need to measure LDL directly in all patients. It seems from Dr. Mora's results that one would suggest that you would use the Friedewald calculations up to that triglycerides of 400 milligrams per deciliter, which is 4.5 millimoles per liter, and only if triglycerides were higher then you would measure LDL directly. So these are the most important things from my point of view.

(00:09:59)

Host: Okay. Dr. Mora, your study found that nonfasting LDL cholesterol by either method did not really predict cardiovascular disease. While we have traditionally measured lipids in the fasting states, some are now proposing we measure nonfasting lipids. In your opinion, how would this change affect the measurement of LDL cholesterol?

Dr. Samia Mora: Well, in the same group of women, we recently examined also whether fasting state affects lipids other than LDL cholesterol. We published these findings in September 2 issue of *Circulation*. We found that triglycerides, HDL cholesterol, and the total over HDL cholesterol ratio were just as useful for predicting future CVD events, whether measures fasting or nonfasting.

Also, in the Copenhagen City Heart Study, they also found that these lipids predict CVD in both men and women when measured nonfasting.

So if we take the results from these two large studies together, both the Copenhagen City Heart Study and the Women's Health Study, we can say that the nonfasting lipid panel is very useful if we pay particular attention to the triglycerides, HDL cholesterol, and the total over HDL cholesterol ratio.

Certainly, this could simplify clinical practice quite a bit, since it would be very practical to measure a lipid panel at the time of the patient visit, without the need for asking the patient to come back again at a later time when they are fasting, which would require another visit for another blood test.

We need more studies of course to examine whether LDL cholesterol in the nonfasting state may be as useful, but certainly a nonfasting lipid panel seems to be just as useful for predicting CVD if we focus our attention on certain lipids, in particular triglycerides HDL cholesterol, and the total over HDL cholesterol ratio, which in our study were found to be even more predictive than LDL cholesterol.

Host: Your study included only women. Do you think the results apply to both men and minorities, too?

Dr. Samia Mora: Well, our studies included only women, and these women were apparently healthy. Most were White, although we did have about 1,500 women who were

minorities in this study. We do not know if our results would apply to men or other ethnic populations, although we do not have any reason to suspect that they would not.

For example, on the Copenhagen City Heart Study which had both men and women, no significant sex differences were noted. Of course, more studies would be needed before this question can be answered definitively.

Host: Dr. Nordestgaard, your two papers based on the Copenhagen City Heart Study, published in JAMA, looked at nonfasting triglycerides for cardiovascular risk and mortality prediction. What were your findings?

Dr. Børge Nordestgaard: We studied 7,600 healthy women and 6,400 healthy men who had triglycerides measured nonfasting back in 1976–78. So this was a single measurement. Then we followed them from 28–31 years. During that period, 3,500 developed a myocardial infarction, 1,500 had an ischemic stroke, and 7,800 died, approximately half or even more than half.

What did we find then? We very clearly found that the higher the nonfasting triglycerides, the higher the content of remnant cholesterol plasma, and at the same time, the higher the risk of all these three endpoints: myocardial infarction, strokes, and early death.

To give an example of the results, if we compare those with triglycerides above 5 millimoles per liter, which is 440 milligram per deciliter, versus those with less than 1 millimoles per liter in triglycerides, which is equal to less than 98 milligram per deciliter, then for women, these women with the high triglycerides had 17-fold risk of myocardial infarction, fourfold risk of stroke, and fourfold risk of early death, which is an extraordinarily high number. The corresponding values in men were fivefold, threefold, and twofold.

Host: Based on the evidence we have discussed now, what is the standard practice today for lipid measurement in your home country, Denmark, fasting or nonfasting, and what about rest of the world, including the US?

Dr. Børge Nordestgaard: In Denmark actually, in several hospitals we have used a nonfasting standard for several years and we have now just had a general recommendation for the

entire country that the standard practice in Denmark should be to have a nonfasting lipid profile, which is cholesterol, triglyceride HDL, and LDL, and then only if triglycerides is above 4 millimoles per liter or approximately 400 milligrams per deciliter, then the clinician can offer the patient to have a fasting triglycerides, just in case the patient has eaten a lot of fat just prior to this one last sample. But the standard practice is nonfasting lipid profile.

In the US and the rest of the world, well, in theory still everybody is supposed to fast, but I wonder whether that happens. I think a lot of patients and maybe also medical doctors tell their patients they don't need to anyway. So that's why I am much in favor of, why not really make it a standard practice to do it nonfasting in the future.

Host: Well lastly, I would like to ask both of you, in your opinion, where will future research lead us on these issues, Dr. Nordestgaard?

(00:14:58)

Dr. Børge Nordestgaard: Well, first on the LDL issue, which is the main thing on Dr. Mora's paper, well, it's important to show whether the results she found will be the same for men.

Then another question that's very important to address is that, how does Friedewald and direct LDL cholesterol compare if triglycerides are above 400 milligrams per deciliter, which is about four-and-a-half millimoles per liter?

Then on the other issue, generally on nonfasting lipids, and it would be very important for us to understand the association between nonfasting triglycerides. Is that because remnant cholesterol is very good at predicting risk of cardiovascular disease and early death? Then what I would love to see for the future would be lots of randomized intervention trials that aim at reducing nonfasting triglyceride and remnant cholesterol to see if this will reduce cardiovascular disease substantially. So these studies are really needed.

Host: And Dr. Mora, same question?

Dr. Samia Mora: Well, in regards to direct LDL cholesterol assays, as our study suggests a very high correlation between a direct assay and Friedewald calculation is not sufficient proof that the two methods are equivalent.

More attention in my opinion is needed to evaluate the predictive value of these methods and not just the correlation.

We also learned from our study that a small bias, for example, lower direct LDL by even 5 milligrams per deciliter compared with the Friedewald calculation was enough to misclassify approximately 20% of the study population. Since current guidelines use consensus cut points based on clinical outcome trials, then it is very important to assess classification of patients into these NCEP categories based on this method.

Finally, the rise in healthcare cost is a critical issue that future research needs to address, and more discussion needs to focus on that and whether nonfasting lipids are more cost effective.

For example, if a nonfasting lipid panel consisting of HDL cholesterol, triglycerides, and total over HDL cholesterol ratio performs just as well as a fasting panel for assessing risk in a primary prevention setting, then this would be expected to reduce repeat visits, reduce inconvenience to patients and providers, and also overall health care cost.

Host:

Samia Mora is an Assistant Professor of Medicine at Harvard Medical School, and Børge Nordestgaard is Professor of Genetic Epidemiology at the University of Copenhagen. They have been our guest in this podcast from *Clinical Chemistry*. I am Bob Barrett. Thanks for listening.

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