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Circulating Ceramides and Sphingomyelins and Risk of Mortality: The Cardiovascular Health Study

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Amanda M Fretts, Paul N Jensen, Andrew N Hoofnagle, Barbara McKnight, Colleen M Sitlani, David S Siscovick, Irena B King, Bruce M Psaty, Nona Sotoodehnia, and Rozenn N Lemaitre.
Circulating Ceramides and Sphingomyelins and Risk of Mortality: The Cardiovascular Health Study Perspective
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Guest: Amanda Fretts is an Associate Professor at the University of Washington School of Public Health, Department of Epidemiology and the University of Washington Cardiovascular Health Research Unit. Dr. Rozenn Lemaitre is an Associate Research Professor in the Department of Medicine at the University of Washington.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett. There is considerable interest in the effects of circulating sphingolipids and blood on health outcomes. In particular, it is becoming increasingly apparent that ceramides and sphingomyelins with different saturated fatty acids have divergent biological activities and that the associations of ceramides and sphingomyelins with health outcomes differ by the fatty acid acylated to the sphingoloid backbone.

In humans, high concentrations of circulating ceramides with palmitic acid have been shown to be associated with development of cardiovascular diseases and adverse cardiac events. A paper appearing in the December 2021 issue of *Clinical Chemistry* examined the associations of various ceramides and sphingomyelins with mortality from data gathered from the Cardiovascular Health Study.

Two of the authors of that paper join us in this podcast. Dr. Amanda Fretts is an Associate Professor at the University of Washington School of Public Health Department of Epidemiology and the University of Washington Cardiovascular Health Research Unit. Her research interests include cardiovascular diseases, diabetes, obesity, nutrition, fatty acids, and minority health.

Dr. Rozenn Lemaitre is an Associate Research Professor in the Department of Medicine also at the University of Washington. Her research interests include lipidomics, genetics, diabetes and cardiovascular disease. Dr. Fretts, we will start with you. The study was conducted using plasma samples from the Cardiovascular Health Study, can you tell us just what is the Cardiovascular Health Study?

Amanda Fretts: Sure. A Cardiovascular Health Study or CHS, as it is often called, is a large epidemiological study of risk factors for cardiovascular diseases among elderly adults in the United States. The study comprises a random sample from four

communities in Forth County, North Carolina, Sacramento County, California, Washington County, Maryland and Pittsburgh, Pennsylvania.

In total, the study includes 5,201 adults who are aged 65 years or older, and the first cohort of participants was recruited in 1989 and then a smaller second cohort, primarily blacks, were recruited in 1992. And all participants underwent a clinic or in-person examination once per year for the first ten years of the study. And then they completed telephone surveys two times per year thereafter.

And the study examinations were very comprehensive. They included a standardized personal interview to assess demographics and medical history, a physical exam, and a complete laboratory workup. Since participants have been contacted twice per year for over 30 years and advance adjudication is still ongoing. The study has generated a wealth of information about risk factors for cardiovascular diseases and related disorders.

In fact, there is data available on a wide range of areas: everything from diet and physical activity, genetics, cardiovascular events such as stroke and coronary artery disease, to noble biomarkers like single lipids. And although many participants have passed away since the start of the study, since they were 65 years of age, 32 years ago. Surveillance of survivors is ongoing.

And the Cardiovascular Health Study is funded by the National Heart, Lung and Blood Institute at the National Institutes of Health.

Bob Barrett: Why is studying mortality important?

Amanda Fretts: So, the CHS was designed to evaluate risk factors for cardiovascular disease with the main outcomes being coronary heart disease, angina, heart failure, stroke, transient ischemic attack, claudication and mortality. These outcomes are common in older adults and for this project on sphingolipids, we focused on mortality for several reasons.

First, mortality is an important outcome. It's important to individuals, and it's important to their physicians, and it's important to public health. Second, there's been an increasing body of literature describing association of flex ceramides with risk of death. But the previous studies have focused almost exclusively on populations with underlying cardiovascular disease or on populations at high risk of cardiovascular disease.

And whether these associations are applicable to the general elderly population. On CHS, the average age was 77 years

at the time of the sphingolipids measure, that's unknown, that was unknown before our study. Also, many of the previous studies focus on composite endpoints.

So, for instance they might combine fatal and nonfatal cardiac events as a single outcome. In an epidemiology, this is often done to maximize study power, but it really makes it challenging to develop hypotheses about potential biological mechanisms that may explain the impact of sphingolipids on health outcomes when we're combining both fatal and nonfatal events.

Bob Barrett: Well, let's get into the meat of the study. Highlight some of the study results, please, and some of the limitations that you found in the study.

Amanda Fretts: Sure, I'd be happy to. Two findings to highlight: the first is that relationships of circulating ceramides and sphingomyelins with risk of death differ by the length of the attached saturated fatty acid. So, we found that high levels of ceramides in sphingomyelins carrying the saturated fatty acid palmitic acid, which has sixteen carbons, were each associated with an increased risk of mortality.

On the other hand, high levels of several ceramides and sphingomyelins species carrying longer chain saturated fatty acids with 20, 22 and 24 carbons were each associated with a decreased risk of mortality. And second results to highlight is that the direction and magnitude of the risk estimates are very consistent for analogous species of ceramides in sphingomyelins.

And this may suggest that the length of the saturated fatty acid so 16, 20, 22 and 24 that are attached to the ceramide or sphingomyelin may be driving the observed associations. As far as limitations, I think that the biggest limitation of our data is that this is an observational study. So, we cannot demonstrate cause and effect.

And also, although we accounted for many demographic, behavioral, and clinical factors in our analyses, this type of study design cannot rule out the possibility that unmeasured risk factors or factors that were measured imprecisely may account for some of our observed associations. On the other hand, measurement error in sphingolipids assays would reduce through associations.

Also, the CHS is a study of older adults. As I said, the average age being 77 years at the time of the sphingolipids measure. So, we don't know if our findings are generalizable to younger populations. Although our findings are very important since older adults are at the highest risk of mortality when compared to younger populations.

Bob Barrett: Well, thank you so much. So, Dr. Lemaitre, let's go to you. Can you tell us why you chose to relate ceramides and sphingomyelins to the risk of mortality and why these specific species of ceramides and sphingomyelin in particular?

Rozenn Lemaitre: Well, this is a good question. In particular, because ceramide and sphingomyelin belong to a very large family of sphingolipids. There are over 200 sphingolipids species in the circulation in humans. So why did we choose those eight? Those four ceramides and four sphingomyelins. We did because we had a hypothesis. This was based on previous observations in CHS based as well on experimental studies and it was based on basic biology.

So, if I may, I would like to walk through those three parts that made the hypothesis. So previous observations in CHS. The same cohort in which we measured the sphingolipids. Well, in features, we have also measured the overall fatty acid composition in plasma phospholipids. And we publish the saturated fatty acids with a very long carbon chain measured in plasma phospholipids are associated with lower total mortality.

So, we were talking about fatty acids with 22 and 24 carbons. Now plasma phospholipids are a broad mix of lipids. And the study does not tell us which lipids were associated with lower mortality. But it is well known that sphingolipids themselves carry very long fatty acids and one sphingolipid in particular, that is part of plasma phospholipid, it's part of the mix is sphingomyelin. So, we were interested in looking at sphingomyelins that carry very long-chain saturated fatty acid and that was part one of our hypothesis: that sphingomyelins with very long chain saturated fatty acids associated with lower mortality.

Now the other sphingolipid which is found in plasma and has gathered much attention is, of course, ceramide. So, ceramides have a very wide range of biological activities that might influence mortality. And one very well-known such activity of ceramide is apoptosis. Apoptosis means programmed cell death.

Well, it turns out that not all ceramide species promote apoptosis. Only very short chain ceramide that are cell permeable and ceramide with sixteen carbon fatty acid, palmitic acid promotes apoptosis reliably in cell animal experiments. And the other ceramides, those with a very long-chain saturated fatty acid don't have this effect and they even appear protective. So, this was part two of the hypothesis that ceramide with 16/0, which is palmitic acid, increased mortality, while ceramides with very long chain saturated fatty acid lowered mortality.

And then the last piece of the hypothesis was that ceramide and sphingomyelin are interrelated sphingolipids. During synthesis, during De Novo Synthesis, sphingomyelin is synthesized by the addition of a head group, a phosphocholine head group to ceramide. Then at a later stage, ceramide itself can be generated from sphingomyelin by action of sphingomyelinase.

And when they are generated from each other, ceramide and sphingomyelin carry the same fatty acid. So, it makes sense to examine analogous species of ceramide and sphingomyelin and we hypothesized that both sphingomyelin and ceramide with palmitic acid would be associated with higher mortality and the species with very long-chain saturated fatty acid would be associated with lower mortality. So, this was a very long-winded answer.

Bob Barrett: Yet very comprehensive, thank you. So finally, looking ahead, what are the implications of your study findings?

Rozenn Lemaitre: So, there are several implications that we can see with this study. And one implication might be the source of plasma ceramides. So, the ceramides that circulate in the blood may originate from the liver or the intestine by De Novo Synthesis or the ceramides can come from the sphingomyelin that circulates in the blood by the action of sphingomyelinase.

And we think that the parallel association of the sphingomyelin species and the corresponding ceramides species suggest -- it does not prove, but it suggests -- that the ceramide species that are associated with mortality might be derived from sphingomyelin rather than De Novo Synthesis. Similarly, it suggests that the sphingomyelin in the blood might be a source of ceramide and the sphingomyelin fatty acid composition might reflect the ceramide composition.

So that's one implication. And it also would suggest the importance of measuring sphingomyelin. And this is because sphingomyelin is the most abundant sphingolipid in plasma, in humans and it is much more abundant than ceramide. And in general, more abundant species are easier to measure which means less measurement error.

So, with similar association of sphingomyelin and ceramide. Sphingomyelin might be a better target than ceramide or at least we might consider measuring it concurrently. So, that's one implication. Another implication is really a caution regarding shutting off the synthesis of ceramide.

It may be that shutting the whole synthesis of ceramide has its application in specific diseases. But in general, we don't

want to shut down the synthesis of all ceramide. We want to preserve those species of ceramide of sphingomyelin that are associated with lower risk of mortality as well as lower risk of other diseases. So, that's another implication.

And then circling back to our search for lipids that contain very long-chain saturated fatty acids and are associated with lower mortality, we were correct in focusing on sphingomyelin and ceramides, but it doesn't exclude the possibility of other lipids. So that would be further studies, and in thinking of further studies of course, one important piece will be to figure out what we might do to promote increases in this court, good ceramide and sphingomyelin and decreasing in the others.

Bob Barrett: That was Dr. Rozenn Lemaitre from the University of Washington. She was joined by her colleague in Seattle, Dr. Amanda Fretts, in this podcast on circulating ceramides and sphingomyelins and risk of mortality. Their paper on that topic appears in the December 2021 issue of *Clinical Chemistry*. I'm Bob Barrett, thanks for listening.