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Paola Ramos, Sarah M Jenkins, Leslie J Donato, Stacy J Hartman, Amy Saenger, Nikola A Baumann, Darci R Block, Allan S Jaffe, and Jeffrey W Meeusen.
The Biological Variability of Plasma Ceramides in Healthy Subjects.
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Guests: Dr. Paola Ramos is a senior clinical chemistry fellow in the Clinical Specialty Laboratory at Mayo Clinic and will shortly be directing the Biorepository Laboratory at Mayo Clinic. Dr. Jeffrey Meeusen serves as co-director of the Cardiovascular Laboratory Medicine program, the Clinical Specialty Laboratory, and Laboratory Services at Mayo Clinic.

Randye Kaye: Hello and welcome to this edition of *JALM* Talk from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye. Ceramides are bioactive lipid species that mediate numerous cell-signaling events. Ceramides circulate in the plasma but under normal conditions, their concentrations are very low. However, multiple studies have demonstrated that elevated plasma ceramides are associated with several cardiovascular disease risk factors. Further, ceramides can be altered by lipid-lowering therapies. While plasma ceramides are not widely measured in clinical laboratories currently, understanding their biological variability could inform their use as biomarkers.

The July 2022 issue of *JALM* includes an article describing a study that measured the intra- and inter- biological variability of plasma ceramides in a healthy reference population at different time intervals. The results of the study have the potential to contribute to the use of ceramide variability as a biomarker for conditions such as cardiovascular disease, renal impairment, diabetes, and liver disease.

Today, we're joined by two authors of the study, Dr. Paola Ramos and Dr. Jeffrey Meeusen. Dr. Ramos is a Senior Clinical Chemistry Fellow in the Clinical Specialty Laboratory at Mayo Clinic and will shortly be directing the Biorepository Laboratory at Mayo Clinic. Dr. Meeusen serves as Co-director of the Cardiovascular Laboratory Medicine Program, the Clinical Specialty Laboratory, and Laboratory Services at Mayo Clinic. Welcome, doctors. Let's start with the basics. What are ceramides?

Paola Ramos: Yes, a great question. Ceramides are lipids or fats, more familiar example is cholesterol. Just like cholesterol, ceramides are metabolites, which have a very important bioactive role in the body. And like cholesterol, ceramides are synthesized, or created, by every cell.

Ceramides have a bit more variation in the physical properties such as high hydrophobicity that can modify the permeability of membrane and ceramides are precursors of the sphingolipid synthesis and have been associated with cellular processes such as cell proliferation, which is cell growth, and apoptosis, which is cell death.

Randye Kaye: Thank you. We know that there is good cholesterol and there's bad cholesterol. Are ceramides good or bad?

Jeffrey Meeusen: I'll jump in for a quick moment on that one. We were very fortunate, I was bringing up this test from the perspective of a clinical chemist and at the same time, Paola, Dr. Ramos was completing her graduate studies on the topic of ceramides, so then we were able to invite her into the lab to participate in some projects that led to the publication we're talking about, but I'll let her talk more about how ceramides can be both good and bad.

Paola Ramos: Yes. When we think about ceramides under normal conditions, ceramides concentrations are very low. However, they have the ability to quickly be generated in response to any sort of stress or stimuli and their concentration can increase about tenfold very quickly. It is important to mention that ceramides productions are regulated by different enzymes and each of these enzymes have a unique fatty acid length preference.

Unlike cholesterol, which is a very specific molecule, there are many types of ceramide molecules. In the clinical aspect of it, measuring ceramides, we do look at different molecules with different acyl chain length. There is convincing evidence from our lab and others that elevation of mainly three specific circulating ceramide species can be used as predictors for atherosclerosis and cardiovascular diseases.

Randye Kaye: Predictors are extremely important. Now, current clinical guidelines and public health resources for cardiovascular disease management, they recommend measurement of cholesterols, but not ceramide. Why should we consider measuring ceramides in the clinical lab?

Paola Ramos: That is true. Cholesterol and more specifically low-density lipoproteins or LDL cholesterol is measured to look at the person's risk for atherosclerosis, which is the underlying cause for cardiovascular diseases. Now, circulating ceramides are also increased in cardiovascular diseases, however, they serve as biomarkers as well with the very important but nuanced distinction. While LDL cholesterol predicts atherosclerosis, ceramides can predict cardiovascular events.

Randye Kaye: Question, can you explain the difference between cardiovascular events and atherosclerosis?

Paola Ramos: Yes, atherosclerosis is a build-up of lipids inside the artery wall and this process is very slow. It can take up to decades, which is why cardiovascular disease risk increases with age.

Typically, we do not experience symptoms as the plaque grows. However, once we see the first symptom is whenever the plaque has already thickened and it reduces the blood flow. A worst-case, the plaque breaks into the artery forming a clot and it deforms the artery wall causing an aneurysm, for example.

Now, these disruptions in blood flow are cardiovascular events and they can cause either a heart attack or a stroke, depending on the artery that was affected.

Randye Kaye: Wow. We certainly don't want to wait until it's too late. How are ceramides able to predict these cardiovascular events?

Paola Ramos: Well, this test is new and really the basic science is still being worked out, but it has to do with the dysregulation of the lipid metabolome. The clinical use of ceramides as biomarkers is really based on the outcomes of studies. Ceramides were measured in patients who were then followed for several years for any cardiovascular events. So, those were recorded.

We found that the elevations of three specific ceramides were linked to an increase [in] events within one to five years. In fact, some studies include patients with known cardiovascular disease already, and based on the history of a heart attack or imaging of atherosclerosis, while other patients were completely healthy. Now, in both cases, elevated ceramides were able to predict the risk of heart attacks and strokes.

Randye Kaye: All right. Thank you. So, now, we've learned that elevations in ceramides are predictive of adverse events and there are multiple types of ceramides. What does it mean if some of the ceramides are at normal levels and the others are elevated?

Paola Ramos: Good catch. Biomarkers like ceramides are important tools that describe biological processes in the body and they provide a non-invasive means to look for disease or any advised intervention strategies. Multiple biomarkers strategy, like measuring different ceramides, has a potential to capture multiple pathological processes. However, there is also the possibility for confusion if the results are discordant.

In the case of ceramides, there have been so many studies, all replicating the same findings, that ceramides can -- we can have a risk score that, that way we can base it on many patients, over 10,000 patients and incorporates four different ceramides that measured and a scale that goes from zero being at a low risk, to 12, that is the high risk.

Randye Kaye: Thanks. Once a patient and their health care provider, once they learn that ceramide score, what can be done about it?

Paola Ramos: Like cholesterol, like we mentioned, fortunately studies have found that ceramides are modifiable and they can be lowered with the same strategies that we already know. So, increasing exercise, following a healthy diet, and even some of the lipid lowering medicines like statins or PCSK9 inhibitors are able to reduce ceramides and lower an individual's risk for cardiovascular events.

Randye Kaye: Are there any specific ceramide levels that the patients are supposed to strive to achieve.

Paola Ramos: This is where our study came in. Ceramides are a relatively new biomarker and we're still learning about this molecule. In order to know if we're moving things in the right direction, we needed to know how much the ceramides measurements vary, so how different are ceramides between individuals, but also how much the ceramides fluctuate within a given person.

We evaluated the variability of ceramides and we're able to calculate what changing concentrations reflect an actual change due to an intervention versus just regular normal variation. To make it a little bit more complicated, we also needed to look at the analytical variation or in other words, what results change depending on the day that the serum result was given, so whether the instrument and handling differences.

And so, we're talking about mainly three variables. We are looking at the analytical aspect, the biological variability between subjects, and within subjects. For example, to put it into context, the high-density lipoprotein, so HDL cholesterol, is very stable and it doesn't really change within a given person. However, your HDL and my HDL are likely to be very different.

Now, compare this to triglycerides, which tend to change a lot tremendously within an individual depending on what you had to eat and how recently you ate it. These variations are very important to keep in mind when we're using these biomarkers to monitor and to diagnose.

Randye Kaye: How did your team determine variations in ceramides?

Paola Ramos: For this study, we looked at fasting samples for 24 healthy subjects, for daily variation, for five days. We also looked at weekly variation for four weeks, and monthly variation for seven months. For our methodology, we use mass spectrometry and in terms of ceramides concentrations, the previously published risk score that I was talking about incorporates the values of ceramide 16, 18, 24-1, and all of these three ceramides are normalized to ceramide 24.

This ceramide is an abundant ceramide that is not correlated to disease. We looked at coefficient of variation, that is also known as the relative standard deviation, to tell us the dispersion of data points around the mean, usually shown as a percentage. Now, we also refer to a critical difference, also known as the reference change value. Critical difference is a very useful parameter in this kind of studies since calculating the critical difference tell us the needed change in results beyond the expected variability for us to consider significant.

Randye Kaye: Thank you. So, finally, what would you say are the overall findings and the takeaway messages from your study?

Paola Ramos: We found from this study that the analytical variation is lower than the biological variation, which is really good and it tells us that the assay is reliable for its intended clinical use. We also learned that the within individual variation was smaller than the between individual variation.

Most importantly, the critical difference was lower than the difference within individuals, meaning that a change of more than 10% is consistent with the true difference. Now, it's worth mentioning that our only limitation was that our cohort included only healthy and relatively young individuals. This might not be representative for the entire population.

Overall, the take-home message is that ceramides, they have very stable concentrations overall and this is beneficial for assembling of a biomarker. This says that any difference detected can be attributed to a lifestyle change or an intervention. And so, overall, we concluded that ceramide vary very minimally within an individual over time, which can allow us to measure patients in an annual basis.

Randye Kaye: Dr. Ramos, thank you so much. Dr. Meeusen, is there anything you want to add?

Jeffrey Meeusen: Just wanted to say that we are hoping that this marker will be able to be picked up more and more by the general medicine community and we're grateful for the work that Dr. Ramos has done on this in her time here in our lab at Mayo Clinic.

Randye Kaye: All right. Well, thank you both so much for joining us today.

Paola Ramos: Thank you, Dr. Meeusen and thank you, Randye.

Randye Kaye: That was Drs. Paola Ramos and Jeffrey Meeusen from Mayo Clinic describing the *JALM* article, "The Biological Variability of Plasma Ceramides in Healthy Subjects." Thanks for tuning into this episode of *JALM* Talk. See you next time and don't forget to submit something for us to talk about.