Han Le: Hello, and welcome everyone. My name is Han, I'm a second year student at Nebraska Wesleyan University. And today, I will present a project that I did over the summer of 2019 at the University of Iowa, at the Abel lab and under the mentorship of Dr. Hinton. The project was about how insulin affects OPA-1 dependent mercs with states for mitochondria ER contact sites, in human and murine skeletal muscle cells. So, it has been shown that these proteins over here, they are called mitochondria associated membrane or MAMs proteins. MAMs is another name for MERCs. A miscommunication between these proteins was studied and suggested that this miscommunication can be associated with type two diabetes.

The proteins that I'm interested in is, okay, right here, is called OPA-1. OPA-1 is located on the crystal which is in the membrane of mitochondria. And again, I'd like to point out at ER stand for endoplasmic reticulum. So, another proteins that I will mention later on is MFN2. These proteins are actually located in between ER and mitochondria. So, notably, MERCs only happens when we have contact sites between the mitochondria and ER, with both rough and smooth ER. This figure over, this figure three over here show that disruption in mercs decrease insulins effects in human mouth tubes. So, these are some critical observations, the first observation is from a study para 2014. And what they showed was that after three hours of insulin stimulation, we see an increase in mitochondrial fusion and the morphology for mitochondrial fusion is an increase in area. However, they are decreases in similarity index in mitochondrial numbers. It's also an increase in crystae, and an increase in density.

So, I mentioned NFM2 earlier, our study has shown that NFM2 level also increase with insulin stimulation after four hours. Lastly, these are slides from the software and with OPA-1 knockout, we see an increase in MERCs. So, it suggests that MERCs increase without OPA-1. So, the question here is, how does insulin stimulates an OPA-1 mitochondrial stress, this stress is represented as MERCs. The aim is to determine if insulin simulation changes mitochondrial dynamics or more specifically fusion in human cells. And also to see if insulin simulation alters MERCs formation in human and merged cells.

So, the method was used mainly focusing on TEM or transmission electron microscopy analysis, so, more specifically we count the number of mitochondria, measure mitochondria area, square the index perimeter. We, for MERCs, we look at MERCs distance, so distance between the mitochondria and the ER, and also the length of their contact sites. We also use seahorse, essentially seahorse is to measure respiration, after mitochondria representing as oxygen consumption rates under stress and of course Western Blots.

So, for our first figure, this is when we decided to repeat the experiments on paper para 2014. And we measure OPA-1 level with insulin stimulation and after quantification we see an increase of OPA-1 after we treated the cells with insulin. We also see an increase in FAK2 level or bigger E F and G we don't have a significant, statistically significant results. These are the proteins for mitochondria IO tracker markers. And the Fs are the proteins these are the proteins that we saw earlier murines proteins. But these specifically are, were showed to be associated with fusion, fission of mitochondria. And lastly these are the proteins in oxidative phosphorylation, oxidative phosphorylation.

So with seahorse, so we ran seahorse and what we saw was that, after two hours there is a small increase. After four hours, there is a dramatic increase. And after six hours and is actually a decrease. So, what happened here, these experiments suggested that because one mitochondrion is under stress, a single mitochondrion decided to fuse together to make longer
mitochondria to accommodate for the stress, and that explained the increase in respiration. However, the further the mitochondria got damaged, at some point the low mitochondria also crash, that's why we see a decrease right here. So moving on, because we know a, we have a hypothesis on a relationship between insulin stimulation, OPA-1 and mitochondrial fusion we want to see if the relationship is actually true. So in order to do that, we first knock out insulin receptor, and insulins growth factor receptor, just to see if OPA-1 can stimulate mitochondrial fusion independently of insulin. So this is to show that this is to show, that we have knocked out insulin's receptor. So when we look over here, with GFP, we see fusion of mitochondria. Without GFP, I mean sorry, without insulin receptor, we don't see fusion anymore. So, again, fusion did not happen without insulin.

So next we knock out OPA-1. Now, what is interesting is after we knocked out OPA-1, right after GFP fusion happened, even though without OPA-1, fusion still happens. This is actually really interesting, because before we believe that OPA-1 was responsible for mitochondrial fusion but without OPA-1 fusion still happens. What could essentially happen here, if we go back to before, where I mentioned, where I mentioned MFN2 so back here, we saw an increase in MFN2 with insulin stimulation as well. In MFN2 it's actually in between the ER and the mitochondria, which means that the ER can play a role in mitochondrial fusion. So that is why we decided to measure MERCs, because if we see an increase in MERCs, that means that MFN2 might play a role in mitochondrial fusion and also the ER. Well, what the result show us was we saw a decrease in MERCs actually. So, again, at this point in order to investigate whether MFN2 actually responsible for mitochondrial fusion, we would have to knock out MFN2. And if knockout of MFN2 doesn't show fusion, that would help us understand that MFN2 is actually responsible for mitochondrial fusion.

So, we decided to repeat an experiment using human cells for higher application level, what we saw was an increase in OPA-1 level after two hours, instead of four hours. Our PAKT increased after four hours. These are confocal imaging and as you can see a fusion happens with insulin stimulation. These are just some more TEM images to show fusion. And again, we see a increase in number, increase in volume, increase in area increase in cristae, and cristae surface area, and cristae volume. Though this is not very significant. So, what we can imply here is perhaps OPA-1 is not responsible for mitochondrial fusion, but instead responsible for cristae formation, or cristae, increased cristae numbers. What is interesting here is that we see an increase in MERCs in human cells however. So that puts us back to the question whether MFN2 is actually responsible for mitochondrial fusion.

In conclusion, as I mentioned before, insulin increase mitochondrial fusion in both human and murine skeletal muscle cells. However, for more formation in murine cells, we have a decrease, and in human cells, we have an increase. So, for our future direction, we can either test MFN2 experiments or gather more data on MERCs formation under under insulin stimulation. So, I would like to acknowledge Dr Abel, Dr. Pereira, Dr. Hinton for their guidance. I also like to say thank you to my lab mates Jake, Margaret, Innes and Yahang, Sherif for helping me to get these data. And also, NIH, NWU, LSAMP organization, Iowa Biosciences Academy and BW fund. So if you have any question, please feel free to email me or any of my mentors. And thank you for your attention.