Dr. Helen Raybould is here from the Vet School at UC Davis, and what brings her here is in the spring, February and March there was a Northern California American Society Microbiology meeting in Pleasanton and she gave a talk with a similar title at that meeting and some of our microbiology students were at that meeting and were impressed with her research talk and suggested her as a potential seminar speaker. So I'm happy that she accepted the invitation to come up to Chico. And she's going to be talking on how our gut microbiota effects behavior, and so potentially we have an excuse for criminal activity with our gut microbes. So she did her graduate work at the University of Liverpool in England and then came to the United States and did a post doc at UCLA and stayed on their to do research at UCLA and stayed there for 14 years before taking a position at UC Davis where she's been for the past 14 years. So her research interest really are in nutrition, so ingested nutrients, and the impact of our nutrients and the processing of those nutrients have on the neurophysiology of the gut as well as our gut microbes and factors that they produce and how that impacts neurophysiology. She’s published a good bit of work on how nutrition and neurophysiology of the gut impacts obesity and other disease stakes. But she's going to be talking about the microbe side of her work today. So join me in welcoming Dr. Raybould.

Dr. Raybould: Well thank you very much and I'm delighted to be here. And I would like to thank Dr. Cline for the invitation to come and speak today. And I'm just, I thought I would be talking to about 10 people, it's Friday, 4 o'clock, so it's great to see such a big crowd and I certainly wouldn't be talking to this
many vet students if this was 4 o'clock on Friday in Davis, all the vet students would be gone by now. So it's a pleasure to be here. So first of all I should say you know, my name is Helen Raybould and I'm not a microbiologist. I'm really a physiologist, I apologize to those in the audience who probably know an awful lot more about microbiology than I do. But what I am interested in is that sort of intersection between the microbiology and the host physiology. And not in terms of pathogens or the kind of host pathogen interaction, but how these microbes actually may be influencing our overall health and behavior.
Dr. Raybould: So just a little bit on G.I. physiology because that's really who I am, I'm basically a G.I. physiologist. And so you know here is a picture of the gastrointestinal tract in a dog divided into different areas that have different functions but we all know what the gut does right. Basically it's involved in the intake, digestion, absorption of nutrients and water. And what we don't often think about is another couple of characteristics of the gut that I think are important in our understanding of what else the gut might be doing. It is actually the largest immune and endocrine organ of the body. So not only does the presence of nutrients in the gut generate signals that will regulate physiology within the gut itself. So when we eat a meal or a cookie like some of us just have, that will begin to be digested and those nutrients within that meal will start to stimulate hormone release and activation of neurons within the gut and send that information to the brain. So that we know the quality and quantity of what we've just eaten and this turns out to be extremely important in the regulation of food intake and also in the regulation of glucose homeostasis.

And what I always like to remind my students is that the gut is basically, even though we think about it as being inside our body because the lumen of the gut is actually the outside world. And so the gut is the biggest interface between us, our insides, and the outside world, other than the skin but even the gut has a bigger surface area than the skin. And of course, so it has this sort of dual function of having to maintain that barrier between the outside world and the inside world and it also has to be able to absorb things. So it has to sort of
maintain that barrier and also fulfill its physiological function of being able to absorb the nutrients in the water and the food.

So here you can just see where a lot of that action happens. This is a scanning EM of the duodenum so this is part of the small intestine so you can see here the muscular wall of the tubular gut and then you can see the submucosa which is this area here which contains the blood vessels and the nerves. And then it's really characteristic in the small intestine to see these, fingerlike projections or villi which cause a huge increase in surface area over which digestion and absorption of nutrients occur.
And so here you can see this in a histological section, you can see the muscle layers, here is the submucosa, and then here are the villi these fingerlike projections that project out into the lumen of the gut. So a lot of the work that I have done over for the past 20-25 years is really to try and understand the role of the gut as a sensory organ. So how does it respond to these nutrients that we take in food, how do the endocrine cells, these are specialized cells in the wall of the gut, respond to different nutrients and how is that information transmitted to the rest of the body. So I spent a lot of time on these cells here in the wall of the gut. So here, and this is basically a blowup of the single epithelial layer that lines the gut so it's like a single epithelial layer, it's a columnar epithelium. So here you see the columnar epithelial cells. These are the centrally located nucleus, this is the basement membrane down here. And here is the apical projection of these cells which is sort of whether digestion and absorption occurs.

And then there are other kinds of cells in this epithelium. These are goblet cells that look sort of empty because in processing of the tissue the mucus that these cells secrete is removed so these are the goblet cells. But these are the cells that we've been interested in and these are the endocrine cells. So here you can see they have an apical projection and then the basolateral foot of the cell is it contains lots and lots of dense core vesicles which is where actually the hormones and peptides are released in response to stimulation at the apical membrane, will cause the release of these hormones and peptides from these endocrine cells. So these are sort of the chemo-sensing cells in the gut.
and we know that they respond to both nutrients and non-nutrients and make up part of this sort of concept that the gut is a sensory organ.
So here is an EM of one of these endocrine cells. Here you can see the apical projection and one of the important things to note is that all of the cells, these of absorptive endocrines, the columnar epithelial cells they have this microvilli brushed water. And that's important for digestion and absorption also. But here's an endocrine cell, you can see the dense core of vesicles at the basolateral foot of the cell. And you can see that it has these long of microvilli which we sort of think that doing this sort of sensing it, because they have contact with the lumen of the gut. And here is an image of chemistry, so we've used double labeling in this section of the gut. So here we are sort of looking down on to, we cut sort of through the villi and we're looking down towards the submucosa. So this is actually the lumen of the gut and it's surrounded by these epithelial cell layer. And here you can see we stained this cell for 5 hydroxytryptaminels serotonin, you can see this cell is in a positive for serotonin. Kind of have this flatfoot that abuts up against the basolateral membrane. And then we've stained for the receptor for 5-ht, the 5-ht-3 receptor and that's the green very punctate labeling in this neurofiber that's innovating this part of the gut. So you can see that stimulation in response to glucose for example in the lumen of the gut, stimulate the release of 5-ht which will then activate neurons that innovate and terminate very close to where these cells are.
So if we look at what happens to this information. So here we can see a diagrammatic representation of this, what I just showed you, an emnociteal chemical section so here the epithelial cells. So these are the sort of workhorses of the gut wall, they express many, many, different kinds of transporters that are involved in the absorption of the different nutrients, glucose, amino acids, and that kind of thing. And then there are the enteroendocrine cells, these chemo-sensing cells that I just showed you. So we think about these as the luminal chemo-senses, they release their products. Now of course several things can happen once they release, they can get into the bloodstream and produce effects and that would be a true endocrine action right. So the definition of something that endocrine is, is it has to be mediated through the bloodstream and so undoubtedly many of these hormones do it. The products released from these cells act as true hormones.

But we are sort of interested in the way that they activate these neurons that innovate the gut. And in particular we've been very interested in these neurons that innovate the gut that actually send information to the brain. And so this is actually one of the 10th cranial nerve that innovates a lot of the gut wall. And we're interested in these vagal afferent neurons and they innovate the mucosa and they are responsive to some of these products and these enteroendocrine cells. So the information can be transmitted to the central nervous system about what's going on in the gut lumen. And there is two things that happens to this information once it gets to the brain. In the brain stem it generates reflexes and changes parasympathetic efferent outflow and so that results in changes
in pancreatic secretions, smooth muscle function along the wall of the gut to help regulate gastrointestinal function in the postprandial period after a meal. But this information is also fed forward to higher brain centers that are involved in the regulation of behavior and in particular the hypothalamus which results in changes in food intake and also the stress response. And I'm mainly going to be talking today about changes in food intake.
So where does the gut microbiota come in. Well of course that's in the lumen of the gut, and much of this information has come sort of through some serendipitous findings. So the gut microbiota will determine anxiety type behavior. So I don't know whether there are any people working behavior in the audience, but if there are I'm sure you recognize what this is. So this is an elevated plus maze and essentially because rats and mice are prey species they will tend to like enclosed dark spaces, but of course they have to come out of a closed dark space in order to seek food. But they will always prefer to be in the elevated part of this maze which has walls and is dark rather than be in the open arm of the maze. And so you can measure how much time the rodents will spend coming out into this open arm, and it's taken as a measure of anxiety sort of behavior. So this is not work from my group this is work from a group in Canada, and what they showed is if you take a mouse that's germ-free. So this is a mouse that has never been exposed to any kind of microbes, it was born in a germ-free facility to a mother that was germ-free. Versus an animal that's been born in under normal sort of specific pathogen free environment which is how we normally keep are rodents.

So that you can see that this is the time spent in the open arm and you can see that the animal will SPF. A normal rodent will spend one or two forays out into the open arm sort of every minute and that diminishes as time goes on. But if the animal is germ-free it doesn't seem to care so much about it being in an open environment and this response doesn't fade it just spends much more time out in the open arm, and this is taken to be that the animals are less
anxious. And this also changes in the brain neurochemistry in these germ-free animals. So here if you look at the hippocampus here and in particular at the dentate gyrus which is an area of the brain associated with anxiety type behavior, here it is autoradiography for BDNF a neural growth factor. And you can see that there's a difference in the expression of BDNF between these two animals. And so here it's actually quantified so SPF animals, excuse me the germ-free animals have an increase in BDNF expression within the dentate gyrus of the hippocampus. So just having no microbiota ever being exposed to microbes in these animals lives makes them more anxious and changes their brain neurochemistry.
So this same group went on to do an even more elegant experiment and essentially what they did, they showed that this was a transmissible phenotype. So they took different mouse strains and so they took NIH Swiss mouse which has a different gut microbiota from another strain of mouse called BALB/c. And so this is the diagram here just showing the results and sequencing, looking at the different, the relative microbes that are in the BALB/c in blue versus the NIH Swiss in red. So you can see there’s a difference of signature of gut microbes in these animals. These two different strains also exhibit different kinds of behavior which is inherent in the strain and can be recapitulated in many different labs. So they looked at a couple of behaviors of time spent in the lightbox. So you can see the NIH Swiss mice were much more likely to spend more time in the light side of the box, and this is latency to step down and you can see again the NIH Swiss mice are much more likely to step down into a novel environment. So they are much more likely to have a higher degree of exploratory behavior than the BALB/c mice.

So what these investigators did was they took a germ-free mouse, they took the microbiota from Swiss mice, and microbiota from BALB/c mice, and they conventionalized the germ-free mice with either microbiota. And what they found was that when they did this regardless of whether this was a Swiss mouse or a BALB/c mouse it was the microflora that determined the behavioral phenotype. So if you put the microflora from the Swiss mouse into a germ-free BALB/c mouse it would look like a Swiss mouse and vice versa with a BALB/c mice, and you can see that here. So these are NIH Swiss recipient mice and
you put in the sequel content from a BALB/c mice, a germ-free BALB/c mice, you can see that there's difference in the phenotype both in the Swiss mice and in the BALB/c mice. And they also see this difference in the brain neurochemistry as shown previously. So this is a transmissible phenotype and it's dependent upon the gut microbiota.
And which was very interesting to us when this work came out, that this pathway that we're really interested in, that's going from the gut to the brain. This vagal afferent pathway, if they section that in these mice they completely abolished this difference in the behavior. So it seemed that it was dependent on this neural pathway.
So let's go back and talk a little bit about what we know now about the gut microbiota. And of course what we know versus what we knew sort of 10 years ago is because we now have these high throughput sequencing techniques whereas previously we had to rely on a culture techniques to know who was there and how much of who was there in the gut. And of course many of the guts, many of the microbes in the gut are anaerobes in the difficult culture. But with these high throughput sequencing techniques we have a huge amount of information and most of this work has come out of two labs at least in the USA. There is a big lab in Europe that has also done theirs, and it's been funded by the NIH, the Human Microbiome Project. And so a lot of this work has come out of the lab of Jeff Gordon at St. Louis WashU and also Rob Knight at University of Colorado in Boulder.

And basically what we know now is that there's 1 X 10^{13} to 1 X 10^{14} microorganisms in our gut. And so that basically means if you want to be existential about it that we are actually 10 times more microbe than we are human, okay. And it gets worse because there is a hundred far more genes in our gut microbiota than actually we have in our own cells. And so, and we know that this is a very diverse microbiota so we have over 1000 species and 7000 strains. And I think I made the slide a few months ago and I think it's probably gone up since then.
And if we look at the phyla level, so this is sort of the first level at which we can look at. So this is work out of Rob Knight's lab, and basically he took ABCD represent for individuals who, young, male adults and you can see that this analysis at the phyla level shows that there can be quite a lot of difference in the phyla level. So this is bacteroidetes, so there's more bacterial oddities in this person than in these two people are very much less than this person. And then the firmicutes, the yellow, and so minimal firmicutes here and particularly in this person. But basically what they've showed in this analysis that's been done hundred and many thousand, and in fact you can actually send your poop to this lab and you can get a snapshot of your gut microbiota as part of this human microbiome project. So but basically what this tells us is that even though there's quite a lot of individual variability that the most numerous are actually in the bacterial oddities phyla and the firmicutes phyla, and then the other phyla are represented but less abundantly.
So the bacteroidetes and firmicutes are the two predominant bacterial phylotypes. And the actinobacteria, fusobacteria, proteobacteria, and verrucomicrobia are present in relatively low abundance. I think it's interesting and I think it's helpful to think about it like this. But I think it's also dangerous to play the numbers game because there may be species of very low abundance that may be very important in determining changes in physiology of the host. And really, so most of these bacteria we live very happily with, and in fact we need them, we need the genes that they represent to help us function normally. So it's very important in educating our immune system and also our indigestion. And so we view this relationship between microbes and the host as being mutualistic so it actually benefits both parties.
So this is another way of looking at the microbiota at a finer resolution, and again this is work out of Knight's lab. And so he really addresses the diversity and so here you can see that these are the ever expanding outward rings of this circle represents going from kingdom to phyla through to the species and strains of bacteria that are found in the microbiota. And so you can just see that this portion which the firmicutes is blown up here so you can see it with a bit more detail. So they took these four individuals and they sequenced the RNA from the bacteria from these four individuals. They all had representation from the dominant phyla, there was a high degree of variability at the phylum level. And if you look just at the firmicutes here you can see that when you see red it's one individual, individual B. Where you see blue it's individual C, and you can see that they each have their own species that is not shared by the other individual although there is some overlap in some of the species.
So there's been a lot of work done over the last 8 to 10 years through the human microbiome project on studying the diversity not only within healthy people but also in a number of different diseases. And so just, just as a different way of thinking about it rather than looking at who's there and how much of who is there, it's what genes is actually encoded in this microbial population. And so this is a metagenomic analysis from the tongue, and from the feces of one individual. And so here you can see the proportion of the different phyla of bacteria in these two different regions, the tongue and the feces, and you can see it, it actually looks very different. So the feces has lots of bacteriodetes and very little in the level of the tongue whereas there is more firmicutes in the tongue. But if you look at the genes that these bacteria encode, even though there's a lot of difference at the phyla level and probably at the species level. Actually that doesn't seem to be so much different in terms of the sort of metagenomic of these. So even though there's tremendous species diversity that could be very similar functions that occur because of these different bacterium.
I'm in a vet school so everybody asks me what's been done in companion animals, and of course there is less information on companion animals. But there are actually very interesting differences. So dogs they also have a predominance of firmicutes and bacteriodetes but far more use of bacteria then we do. And cats have a lot more firmicutes, very few bacteriodetes, and a few more proteobacteria, and of course cats are far more carnivores, were as a dog is always considered to be an omnivore. So that maybe something to do with it too.
So the gut microbiota plays a well-established role in education and development of the adaptive and innate immune system. We know that it plays a role in the regulation about the barrier between the inside and the outside world. We also know that it breaks down indigestible fibers you know carbohydrates that we don't have the enzymes to break down. We know that colonization is a postnatal event, it commences at birth with vaginal delivery which exposes the infant to that complex microbiota, and that will develop over time and at about two years of age it looks more like an adult microbiota. But this is a very plastic period and there's a lot of research going on around how that very important. The initial exposure to a microbiota and how that microbiota is sort of taken care of by the food that an infant takes can actually make a difference to the adult signature. And we know that this is a norm, we want to have a balanced signature but we know that also gut microbiota which is sometimes referred to as dysbiosis, can be associated with disease. And so something that we might know, sort of antibiotics of course is going to cause a dysbiosis at least temporarily, infection a gastrointestinal infection, and of course diet has a very profound effect.
One way of looking at it is like attending your lawn. Okay so you want your lawn to be nice and healthy like your gut microbes. And so here we have a healthy meadow if you like with grasses, lots of different species, and if we give it antibiotics then we kind of wipe everything out indiscriminately. If there is change in the diet or inflammation we can get the predominance of one species over another. And so one way of attending this meadow, our gut microbes is to see the good microbes, to feed the good microbes with prebiotics, or to transplant. And so fecal transplantation is becoming, its FDA approved at least for the treatment of some antibiotic resistant gastrointestinal infections. And so we want to keep our microbes healthy.
So why am I interested in this as a physiologist who's interested in the gut brain access in those neurons that innovate the gut. Well I'm really, because I'm a physiologist I'm really interested in homeostatic mechanisms. What keeps us healthy, what keeps us able to respond in a normal way to our environment, and what happens when we have changes in that homeostatic pathways, like an altered gut microbiota, and intestinal permeability. And consequently this barrier between the outside world and the inside world being broken down.
And there's plenty of information that this occurs in inflammatory bowel disease, irritable bowel syndrome, and what I'm interested in is obesity and metabolic disease. But there's also some interesting data coming out on autism, depression, and stress associated with changes in the gut microbes.
Why Maintain a Healthy Gut Microbiota?

- Altered gut microbiota
- Increase intestinal permeability
- Passage microbial factors across epithelium

Raybould lab – focus on role of gut microbiota in regulation of feeding behavior and body weight.

Adapted from Gryam and Citrun, 2012 Nature Reviews Neuroscience

[Silence]
So obesity, so if you take obese vs lean rodents or humans they have a different signature gut microbiota. Similar to that other transplantation experiment I was telling you about. If you take the microbiota from the lean mouse or the obese mouse, put it into a germ-free mouse you can recapitulate the original phenotypes, so again this is transmissible. Of interest to people who work in animal science and to any of us that eat meat, so some therapeutic exposure to antibiotics in young mice leads to adult-onset obesity. So if you do change that microbiota at that very vulnerable point where it's becoming established. You can alter the phenotype of the adult and so this is interesting particularly because I come from a vet school, I talk about this because of course in food animals they use these low-dose antibiotics as to promote growth. So they don't quite understand why they promote growth, maybe because they changed the microbiota and they use antibiotics so.
So here you can see you take a lean mouse put it on a high-fat diet you get an obese mouse and you get a difference in the microbiota. Interestingly if you take a germ-free mouse and you put it on a high-fat diet it remains lean.
If you take a germ-free mouse, take lean microflora it remains lean regardless of what kind of diet you put it on.
And if you take an obese microflora and put it in the mouse it will become obese. So again this is a transmissible phenotype.
So why are we interested in this, well you know if David, if Michelangelo choose David as a model right now this is probably what it would've looked like.
And of course we're doing this to our companion animals and of course this is, you know there is high incidence of type II diabetes in cats. And so this is an interest on not only in human medicine but also in veterinary medicine.
So what is the microbiota doing when we give animals a high-fat diet and this is just a model of obesity that's very convenient for us to use because it sort of represents an abundance of high-fat high calorie diets. So this is an experiment done by a Kay London in North Carolina. What she did was she took mice that have a fluorescent reporter for NF-kB, so wherever you see green fluorescence in these pieces of gut which are aligned up here you see, you can see that NF-kB has been activated. So one of the things that has sort of become of interest in their field of obesity is that it's sort of an inflammatory condition, right. So once a person or an animal is obese the increase in adiposity, so this increase of number of cell adipose sites in the tissue. And also they're more pro-inflammatory so they're releasing cytokines. And so it was considered that this inflammatory state was all about the adipose tissue but about four years ago we began to think about, well okay that's fine once a person has a higher level of adiposity then you can understand, you get this increase in cytokines from the tissue, from the adipose tissue. But what's actually driving that change, why, if you believe in physiology and homeostasis, right, we should be able to control how much we eat right and we should be able to have feedback mechanisms to regulate food intake.

So we started to think what was going on at the level of the gut. And so basically what this experiment very simply shows is that there's animals on a low-fat diet and there's animals on a high-fat diet for two weeks, six weeks, and 16 weeks. And what you see is that very early on in the response to high-fat diet within two weeks you're getting inflammation at the level of the gut.
This is way ahead of any change in the adipose tissue, adipose tissues not even beginning to expand at this point and certainty not releasing cytokines. So the initial inflammatory response seems to be coming from the gut.
And then this is just sort of the other work that went alongside those images. So here you can see conventional animals on a high-fat diet you can see they gain weight. Whereas germ-free animals there's no difference between low-fat and high-fat, here's the amount of adiposity. The conventional animals on a high-fat diet put on weight but the germ-free, put on adiposity excuse me, but the germ-free animals don't. And then here if we look at expression of the inflammatory cytokine TNFalpha, you can see in the high-fat animals there are high levels of TNFalpha in the gut 2, 6, and 16 weeks, where there's no effect in germ-free animals. So the gut microbes are determining this response to the high-fat diet.
So we wanted to understand what it was about the change that is occurring at the level of the gut. A change in the availability of nutrients and maybe signaling across this gut epithelium. And this low grade inflammatory response that's occurring to high-fat diet and so we started to think about what's happening to these neurons that we're interested in. Now one thing about these neurons is that they express a lot of receptors and if you activate these receptors. So for one of the very common of the hormones that we know a lot about cholecystokinin, is that CCK is released in response to lipid and protein in the meal. It activates action potentials in these neurons and so will produce reflex changes in gastrointestinal function, and also will inhibit food intake. But it doesn't only induce action potentials, over longer time course it will actually induce plasticity changes in the phenotype of these neurons innovating the gut.
Gut-Brain Axis: Regulation of Food intake and Body Weight

3. Plasticity – change in neuronal phenotype

1. Chemosensing

NUTRIENTS

Receptors:
CCK₁
5HT₃
CB₁
Orexin
Leptin (Ob1)
Ghrelin (GHSR)
Y₂
GLP₁/₂

[Silent]
And the interesting thing about these neurons is that if you think about almost any hormone that's release from the gut or any neurotransmitter and this is just a very partial list of cholecystokinin, serotonin, cannabinoid, orexin, leptin, ghrelin, PYY, and glucagon-like peptide one and two. These neurons express receptors for those mediators. So we began to ask question well what happens to this pathway when we eat a high-fat diet, what happens to the signaling in this pathway, and is it, is this plasticity being altered.
So this is work of a graduate student in my lab Claire de la Serre is now a faculty in the University of Georgia in Athens. And basically what we did was we took rats and this is a non-congenital strain of rat so they're not identical genetically and littermates will vary on the propensity to how much weight they put on on a high-fat diet. So we have low-fat fed animals and we have high-fat fed animals and some of the animals will remain lean on the high-fat diet which is what we all want to do right, and others will become obese on the high-fat diet. And what we found was that the resistant animal, the obese animals has an increase in body weight, increase in adiposity, they're eating much more, and there's an increase in plasma levels of leptin. And if we sort of look at the integrity of this gut brain access.
So if we activate this gut brain access with giving CCK so we can mimic the effects of food in the gut, we give CCK which will activate these neurons and inhibit food intake.
You can see that this is present. This inhibition of food intake in response to CCK is present in low-fat fed animals, is also present in animals fed a high-fat diet but remain lean, but is completely abolished in the obese animals.
We looked at the gut microbiota in these animals, we looked at total 16S RNA, and you can see that there’s not a whole lot of difference between the animals that are resistance or prone to the high-fat diet. Although there is a statistically significant decrease in diversity in response to the high-fat diet. If you look at the cluster dialysis which is part of family in the firmicutes phyla that putting animals on a high-fat diet will increase the number of the abundance of this family. And but it doesn't depend on whether they get fat or not, it's the same. So this is a diet driven effect but it's got nothing to do with the expression of the obese phenotype.

What does happen in the fat animals is that there’s a bloom in this proteobacteria, the enterobacteriales family. Which is associated with an inflammatory response. And then we also see a low level a very low level of LPS in the plasma. So this is a breakdown product from the cell walls of the bacteria which increases in the obese animals but not in the resistant animals that can remain, that remain lean on the high-fat diet.

So basically high-fat feeding is associated with an awful decrease in the number of bacteria, decrease in diversity. And an increase in clostridiales with the firmicutes phyla. But obesity is associated with this bloom in enterobacteriales, an increase in intestinal permeability, an inflammatory response in the gut which I haven't shown the data for that and this low grade what is called metabolic endotoxemia. So this is something like you get with sepsis, right. Where you get a bacterial infection, huge amounts of LPS in the.
plasma, overwhelming anything else and that will. This is a very low level of LPS in the plasma but its chronic this is happening over weeks. Whereas the sepsis is only going to happen over hopefully a matter of 24 to 48 hours, but this is happening over much longer time period.
So what we showed was that this high-fat diet changes the microbiota. But then there's this sort of vicious cycle that goes on when you get a low-grade inflammation in the gut, a bloom in the proteobacteria, an increase in LPS in the plasma, activation of the receptor for lipopolysaccharide that TLR4 the receptor 4. Which we think is changing tight junctions and changing credibility. So really now the question we can ask is well how does this actually change the activation of our vagal afferent pathway.
So we, the first experiment we did was to say when does this actually happen and where does it happen. Is it throughout the gut, is it the small intestine, and is it the large intestine. So we put animals on a high-fat diet and we killed them at 1, 3, or 6 weeks. You can see they gain weight as anticipated and they gain adiposity and there is also this gradual increase in the plasma levels of LPS as we have shown before. This is a different strain of animals and so they all get fat.
We looked at intestinal permeability at different regions along the gut so in the jejunum, ilium, the caecum, and the colon. And we looked at both of paracellular permeability so that's the ability of molecules to go between those epithelial cells. And we also looked at transcellular permeability which is the ability of the large molecules to be endocytosis through the transcellular pathway.

And what we found was really quite interesting because very early on after you put the animals on a high-fat diet at one week there’s a very significant increase in paracellular permeability. So change in tight junction function in the small intestine and nothing is really happening that took to transcellular permeability all to anything in the colon. But later on at three and six weeks in the caecum and the colon we see this large increase in the flocks of large molecules across the gut wall because LPS is a large molecule and so we think this is probably the mechanism by which LPS might be getting across the gut wall to cause that metabolic endotoxemia.
We took the contents from the gut at these different time points. I'm not sure whether you can see it, I'll just, see if you can see that. So basically there are different colored dots that represent the principal component analysis of the abundance of the different bacteria in the different treatment groups. So here's high-fat, here's the control or low-fat fed animals, this is the ileums microbiota and you can see it's changed by the high-fat diet. Here's the cecum microbiota which is significantly different from that in the small intestine, and here can see it's in the cecum. Here's the phyla level abundance in the ileum and the cecum. And then we did what is called lepsy analysis which is the way of being able to pull out what's driving this principal component analysis at the species level.
And essentially what we found was that this is the ileum and so where you see red that means it's higher in the chow fed or low-fat fed animals. And where you see green it means it's higher in the high-fat fed animals. So we saw an increase in lactococcus in the high-fat fed animals and an increase in the actinobacteria and then a decrease in all of these others in the ileum. And then you can see that actually in the cecum whereas most of the change in ileum was a disappearance of microbes. In the large intestine actually it was an appearance in the high-fat fed animals so all the way you see green particularly in the bacterial oddities. You saw a lot of different appearance of different bacteroidetes, again we also saw an increase in lactococcus, increase in clostridiaceae, and also an increase in bacteria proteobacteria which we seen before.

So this will help us sort of be able to say what's happening at these different sites. Try and sort of say what is happening to permeability and inflammation responses in the gut at those different time points and see if we can begin to pull out what the bacteria might be doing and which different families or species might be influencing our response.
And so we did a little bit more of analysis on this. And so we saw a number of different patterns of prevotella for example, present in low-fat fed animals, disappeared after week one on a high-fat diet and never reappeared. And then back to bacteroides for example was present at low levels in low-fat, increased in high-fat and remained high-fat. But we also saw different patents too so we saw in the bacteroidales order a gradual increase with time on the high-fat diet. And in blautia we saw a gradual decrease. And then there was other unclassified, lachnospiraceae that increased in the first week and then went back to control. So we're very interested in this because this sort of correlates with that initial change in small intestinal permeability.
I'm going to skip that in the interest in time. So now what we've done is we've gone on to say okay we put animals on a high-fat diet, we get these gradual changes in intestinal permeability and appearance of these bacterial products in the plasma, in the interstitial fluid that in the gut. And these changes in permeability and changes in neuronal function as determined by the plasticity of the vagal afferent neurons. So we thought well if we can try and solve this dispyosis can we actually reverse the effects of the high-fat diet. So we are interested in looking at synbiotic bifidobacteria which is a probiotic bacteria which will, is defined as a live microorganism that may confer a health benefit on the host. And these milk oligosaccharides which are prebiotics which we can't digest but actually feed the bifidobacteria. So these are complex oligosaccharides that are contained within milk.
We know that beneficial bacteria can have an effect on the gut brain access. So here again we go back to our open arm maze and here you can see that stress will increase levels of corticosterone in response to being out in the open arm. And if you give these animals a probiotic you can decrease this response to stress. And similar to another stress model for swim test where you see a decrease which is abolished in the hippocampal expression of BDNF which you can reverse by the addition of a probiotic.
We looked at milk oligosaccharides on permeability in the gut and weight gain. We find that if we put these bovine milk oligosaccharides into a high-fat diet we can actually reduce the weight gain. And not only that but we completely reverse these harmful effects on the increase in paracellular and transcellular permeability in the gut just by addition of these bovine milk oligosaccharides to the high-fat diet.
We also changed the microbiota. So here you can see the phyla level of analysis in the western diet in the cecum and then with the BMO, this is in the colon. You can see that we decrease the proteobacteria by the addition of the BMO.
We can look again at PCoA analysis. Here's the representation of the gut microbes in a healthy animal.
Bovine Milk
Oligosaccharides Restore a Healthy Gut Microbiota

[Silent]
Changed by western diet and in most individuals reverse towards a more normal phenotype by the addition of these prebiotics. There's one exception which actually may provide some interesting information.
We’ve looked again by lepsy analysis to see exactly what were changes are. So caecum is red, colon is green, control western diet, and then western diet plus BMO. So there is a bloom in the firmicutes. So these are the clostridiales which we can decrease when we put the animals with a high-fat diet with bovine milk oligosaccharides. Lactobacillus which is a known probiotic which will very nicely eat up these up milk oligosaccharides. We can increase them if we put animals on a BMO and so this may be why we’re getting this beneficial effect. But then there is other microbes which we can also increase by BMO which is this akkermansia which is actually able to eat the mucus produced by the gut and may actually stimulate mucus production to help maintain a backup barrier.
So basically what I tried to show you today is how our gut microbiota is really part of this gut brain access. And along with things like nutrients that can be sensed in the gut can activate mechanisms, sensory mechanisms to change permeability. Allow for the passage of microbial products that may well influence these neurons which are then going to change not only reflects function but also changes in higher brain functions such as food intake behavior, anxiety, and exploratory behavior. So what we're spending a lot of time working on now is exactly understanding how these neurons change in response to the gut microbes. And so we're looking very actively at the set of receptors that are expressed by these neurons in response to the microbiota. And how these changes in the microbiota may alter the metabolites that these vagal afferent neurons are seeing in the different physiological response.
[Silence]
Dr. Raybould: So finally I would just like to thank the people that actually did the work. Particularly Will de Lartigue, Kristie Hamilton, and Charlotte Ronveaux. For my colleagues in the foods for health initiative in the milk bioactives group at UC Davis, and also I like to thank the people who fund this work. Thank you for your attention.

Audience Member: [Inaudible]

Dr. Raybould: No they weren't. So the question was about the balance of the fat in the diet, what kind of fat we were using. This is really, these are usually lard in the diet so it's all animal fat. So we haven't addressed you know the different kinds of fats but I think it would be a really interesting experiment to do. It would, yeah. Yes.

Audience Member: So you mentioned how the development of the gut where having a vaginal birth is where they would first get exposed to this. But with increasing numbers of women getting C-sections for various reasons, how does that effect the initial growth and development of that essential gut flora.

Dr. Raybould: So it alters it a lot, and so, I mean this is you know just work that I seen presented at various meetings. And so what happens is that vaginal birth, so they get inoculated with the fecal bacteria and also the vaginal bacteria from the mother. If it's cesarean they tend to get colonized by skin so if you take a baby that's been born by cesarean section and within a short you
know a period after birth of weeks and months their microbiota in the gut has the same signature as the skin. Now it will change with time but that's the initial colonization.

**Audience Member:** And then you were talking about fecal transplants. Do you know how that is done and with the fat mice versus the skinny mice can you significantly alter the community composition of your gut flora by having like a fecal transplant done. And so.

**Dr. Raybould:** Right. Well so we kind of think that maybe you know increasing the intake of milk oligosaccharides or being able to take maybe some other prebiotics that can feed the good bugs and create a better balance as maybe a more palatable way of doing it. And we certainly shown that we can reproduce a biological effect and we change the microbiota. So at least in our rodent models it's possible. For fecal transplantation so it's used for the Cdifficile infections you know which people get in hospital when they are off and on, sort of high-dose long courses of antibiotics. And they're kind of you know can be a very acutely sick patients that have been using it. And basically what they do and I don't know the exact details but you know they basically take the microbes and they put it into a pill form. Yeah, so, but it's only FDA approved right now for Cdiff. Yeah, so.

But everybody kind of says okay you know what we should have done is actually saved our grandparent's microbiota because they were probably much
healthier than in terms of their gut microbes than we were. You know, so.

**Audience Member:** [Inaudible]

**Dr. Raybould:** I'm sorry.

**Audience Member:** If you take your grandma’s poop there is something wrong.

**Dr. Raybould:** Yeah exactly, so.

**Audience Member:** So the milk oligosaccharides are settling in the cow poop or is it in?

**Dr. Raybould:** No. So the milk bioactive group, initially we did some experiments with human milk oligosaccharides. So because they tend to be slightly more complex structures, so these are basically just like glucose and galactose and fucose molecules join together by different kind of bonds. And then they have sidechains and they sort of, glycobiologist talk about them being sort of decorated. And so they become very very complicated molecules that have sort of sialic acid fucose and very complex sort of treelike structures along the chain. And so the bacteria, and in fact one species of bifidobacteria that was isolated from infants, when they have grown on milk oligosaccharides it changes the genetic genes that are switched on in these bacteria. So that
they can break down those complicated molecules. So it can actually change the phenotype of the bacteria depending on what you grow the bacteria on.

So we initially did the human milk oligosaccharides but bovine milk oligosaccharides are now being characterized. And so we can use those because we can get them in higher quantities than human milk saccharides obviously. So they're not exactly the same molecules but there's a lot of similarities between bovine and human but they're not exactly the same for sure. But of course in milk, milk oligosaccharides are not that plentiful, I think it's a very very small amount of milk. So we, they end up in the way and so the way it goes is a waste product from cheese making and you know there's like, I don't know. Somebody once told me about how much cheese is made in California every day and its like metric tons of cheese being made. The way is a waste product and the way contains oligosaccharides. And so they're trying to work on methods to purify and capture that oligosaccharides stream because it could actually be a really good sort of nutrient source to help a variant number of diseases to sort of reestablish a healthy microbiota, but particularly in infants.

**Audience Member:** [Inaudible]

**Dr. Raybould:** So yogurts for example are, they are, if you read the label on some yogurt they put inulin in. Inulin again is an oligosaccharide so its lots of sugar molecules joined together with bonds that we mammalian enzymes can't
breakdown. But they are much simpler it's just a straight line, they don't have all these branches with the sialic acids and the fucose and stuff. So they are much simpler. Problem is that other bugs other than bifidobacteria and lactobacillus like to chew on inulin and what happens with those is they make [Inaudible] and they make short chain fatty acids. Now short chain fatty acids maybe beneficial in some respects but it creates a lot of gas. And so if you talk to anybody who has eats a lot of inulin in their diet it's very intolerable after a while because they feel very bloated, very uncomfortable. And so we're sort of trying to move beyond that sort of simple way of looking at a prebiotic. To reestablish a healthy microbiota.

**Audience Member:** [Inaudible]

**Dr. Raybould:** That's interesting. Were these wild or captive, do you know?

**Audience Member:** I think they were wild.

**Dr. Raybould:** Yeah that's interesting.

**Audience Member:** So if the mice are getting better bacteria from birth how exactly we're the mice germ-free?

**Dr. Raybould:** So the mothers are born into a germ-free environment and they remain in a germ-free environment. They become pregnant in a germ-free
environment and pups delivered in a germ-free environment and they are raised
germ-free. So it's a, it's a huge very expensive thing. Not many, there's more and
more places that have germ-free facilities. I mean up until about 5 years ago I think
it was St. Louis, North Carolina, and maybe one in Boston. And now there's far
more because it's such a powerful way of doing it. And then of course what you can
do and what you really want to do is because germ-free is a really artificial way
because we know that the microbes everywhere are going be really important for
educating the immune system. And so these animals are really abnormal in a lot of
ways. But what they're trying to do is actually sort of do them know biotech which
means you know the microbes they have. So you, they're born germ-free or they
are born to mothers where you kind of given them like a diverse microbes but you
know exactly which microbes so maybe 8 or 10 that seem to be able to induce more
of a normal phenotype. And then you can sort of manage that population a little bit
easier. So that's actually becoming a really popular way of studying this too.