**Linking Fasting to Health and the Gut Microbiome**

Gut microbes effect humans in many ways, including altering the gastrointestinal tract’s function and influencing a person’s body weight, and the nutrients that people ingest can affect the microbiome. Researchers now ask how popular weight loss strategies involving calorie restriction change the bugs in our guts and human health overall.

Welcome to *The Scientist* Speaks, a podcast produced by *The Scientist’*sCreative Services Team. Our podcast is by scientists and for scientists. Once a month, we bring you the stories behind news-worthy molecular biology research.

In this episode, Niki Spahich from *The Scientist* spoke with Alex Mohr, a postdoctoral fellow in the Biodesign Center for Health Through Microbiomes at Arizona State University, about his work comparing the effects of calorie restriction versus intermittent fasting with protein pacing on the gut microbiome, weight loss, and other health indicators.

**How nutrition can influence the gut microbiome and overall health**

Narrator:

From digesting nutrients to modulating intestinal inflammation, human gut microbes have an influence on weight and overall health. Certain microbial communities can make people more susceptible to infections, inflammatory bowel disorders, and other digestive symptoms including gas, constipation, and diarrhea. A popular way to attempt to alter the gut microbiome is by ingesting probiotics—products containing “good bacteria” that can have beneficial effects once inside the body. However, probiotic efficacy is up for debate, and Alex Mohr thinks that nutrition is a better way to shift the gut microbiome toward a healthy state.

Alex Mohr:

The reason that I'm so interested in looking at nutrition as a lever for not only health, but also modulating the microbiome, once you're in adulthood, the microbiome is actually pretty stable. The alimentary canal and the mucosa, if we were to map it out, looking at its surface area, we're talking about 200 square meters, in square feet that's over 2000. So, you're looking at the blueprint of a family house, basically. The very small amount of biomass that you're taking in through probiotics probably isn't going to be doing very much. Whereas with diet, most everyone's eating on a constant basis. All those nutrients are coming into contact with not only the mucosa, where about 70% of our immune system resides, but also all the microbes that are there. If someone is in a compromised health state, we want to reconfigure their microbiome to be more in line with what a health associated microbiota might be producing beneficial metabolites, quelling things like inflammation, improving the mucus that's surrounding the epithelial layer, offering a protective barrier.

As I was going throughout my PhD, I honed in on looking at the effects of different types of dietary factors, different types of diets, and how they're interacting with the microbiome and how that microbiome is interacting with the host. So, it's kind of a two way street, forming a three way axis.

I'm interested in looking at the preventative side and how we can parlay the data that we generate into helping individuals make better choices through nutrition, to set them up for a better health trajectory throughout their life.

**The Effect of Protein Pacing**

Narrator:

Mohr embarked on a project looking at the changes in the microbiome and gut health due in part to fasting. While intermittent fasting is currently a popular modern weight loss strategy, humans have fasted throughout history for reasons including religious purposes and recovery from illnesses. Fasting’s effect on the gut microbiome has been unknown, but now with advanced sequencing methods, Mohr can analyze changes in this population over time.

The project kicked off when Paul Arciero, a human nutrition researcher at Skidmore College and senior investigator of the study, notified Mohr that he was running a trial that aligned with Mohr’s interests, comparing the microbiome and metabolome of participants engaging in two different diets: calorie restriction or intermittent fasting with protein pacing. Arciero pioneered protein pacing, a nutrition strategy that spreads out the amount of protein ingested throughout the day, and the thought of combining that with intermittent fasting seemed advantageous.

Alex Mohr:

If you look at dietary patterns, I'm using the United States, but protein is really backloaded. Most people if they are consuming breakfast very low protein, lunch is moderate to low, and then everything is kind of pushed back to dinner. And that's not really an optimal setup in terms of optimizing metabolism, particularly if that is backloaded very late in the evening, concurrent when you're getting ready to go to sleep.

If you're in a calorically restricted state, for metabolic health, the important consideration here is to make sure that you're having the appropriate triggers for muscle protein synthesis. Branched chain amino acids are very, very important, particularly leucine. To really pull that metabolic trigger, you need several grams of leucine and if you're consuming, say, for breakfast, five or 10 grams of protein, you're really not having the branched chain amino acid load to do that. So, protein pacing is really a great way to preserve lean body mass and metabolism when you're in these types of pretty restricted calorie scenarios.

**The Calorie Restriction Versus Intermittent Fasting Protein Pacing Diet Trial**

Narrator:

With Mohr on board to handle the omics analyses, the researchers enrolled 41 middle-aged individuals with BMIs in the overweight or obese range to participate in the eight-week study. The participants were randomly assigned to the two diet groups. In the calorie restricted diet group, women ingested 1200 calories every day, while men ingested 1500 through three heart-healthy meals and snacks, consisting mainly of fresh vegetables, fruits, nuts, and legumes. The intermittent fasting protein pacing group ate both whole foods and meal replacement shakes. This group was divided into two, with people intermittent fasting for one or two days, followed by protein pacing for the rest of the week. On average, participants in either fasting group consumed an average of 1350-1500 calories per day for women and 1700-1850 calories for men, while intaking only 350-550 calories per fasting day. All diets in this trial resulted in significant calorie restriction for the participants—the United States Department of Agriculture recommends 1600-2400 calories per day for adult women and 2000-3000 for adult men.

Alex Mohr:

I want to highlight how much these participants in this study were calorically restricting. 40% is very difficult for anyone to adhere to. That actually was one of the reasons that this was a little bit of a shorter trial. Again, it was eight weeks long. When we talk about these types of designs, a lay person, one of the reasons that intermittent fasting might be attractive for them as it makes compliance a little bit easier.

The intermittent fasting protein pacing group did receive meals from a study sponsor. These were in the forms of liquid meals, basically like nutritional shakes, which we find have a pretty high level of compliance once you find the right flavor profile for a particular individual. And then they were guided on what to eat for their full food meals at dinner. The calorie restricted group, they received a stipend and they worked closely with a registered dietician who formed menus to really help guide them through. And hats off to the registered dietitians that work on these types of studies because they're receiving text messages, emails all day long, really trying to coach individuals through studies.

**Collecting Fecal Samples for Multiomic Analyses**

Narrator:

At the beginning, middle, and end of the trial, the participants filled out detailed questionnaires about their gastrointestinal symptoms and provided blood and fecal samples for multiomic analyses, including metabolomic studies using mass spectrometry and 16S ribosomal sequencing to identify microbiota community members. Getting good fecal samples was key to assessing changes in the microbiome over the course of the study.

Alex Mohr:

We wanted to make sure we are doing our due diligence, so we had a commode kit. This is a specialized bowl that you place ~~at~~ in the comfort of your home. They basically have an expression, we would collect the whole stool, freeze it immediately, and then send it back to the lab. So, this is where my lab work got a little, you know, it is what it is, obviously had full PPE, but we basically make fecal slurries to make sure that the sample is fully homogenized and we're getting a representative sample when we take aliquots for metabolomics, various assays like lipid polysaccharide binding proteins, inflammatory markers, and DNA extractions to do sequencing work to profile the microbes. It's a lot of work. But when you want to really get a good handle on the sample that you're dealing with, that's really the way to go.

**Intermittent Fasting Protein Pacing Improves Gut Health Measures and Alters the Microbiome**

Narrator:

At the end of the trial, according to the symptomology questionnaire, individuals in the intermittent fasting protein pacing group had greater improvements in symptoms such as bloating, diarrhea, and cramps. Further analyses showed that this group had significant increases in cytokines linked to lipolysis, weight loss, inflammation, and the immune response. People in both diet groups lost significant amounts of weight, with the intermittent fasting protein pacing group losing on average about 8.5% of their initial weight and the calorie restricted group losing 5.5%. Notably, this weight loss came mostly from visceral adipose tissue, the fat that sits underneath the abdominal sheet. This type of fat is concerning because it is pro-inflammatory and can contribute to metabolic syndrome, which is associated with cardiovascular disease and type 2 diabetes.

When looking at the composition and nature of the microbiota in the fecal samples, Mohr found that the overall biomass did not change for participants over the course of the study in either group, which highlighted the stability of the microbial community. However, he did find one notable change in the intermittent fasting protein pacing group.

Alex Mohr:

We did see global changes in the microbiome at the family level. Normally with dietary interventions we're seeing changes at the species level, perhaps even the genus level. But it was really remarkable that we found changes at the family level, and particularly we found a family called *Christensenellaceae* increase substantially in individuals in the intermittent fasting protein pacing group, a two fold change. This microbe is associated with decreases in visceral adiposity, something that we actually found in the clinical study, individuals actually reduced their visceral adiposity by over 30%, which is quite remarkable. It's also associated with increased protein consumption. Looking through the literature and seeing what might be potentially going on here, protein, along with the reduction in calories, might have been supplying the colon with more substrates to increase or cause blooms of *Christensenellaceae*. This can be viewed as a steward of the microbiome, at least for lean phenotype, not only increasing its own relative abundance, but increasing relative abundance of other associated microbes. We also saw some reflections in the inflammation markers that we measured in plasma that cohere with what we're seeing with *Christensenellaceae.* Specifically, increases in interleukin 4, 6, 8, and 13, which have been shown to be anti-inflammatory**.**

**Case Study: Continuing the Intermittent Fasting Protein Pacing Program**

Narrator:

One individual lost 15% of their body weight while on the intermittent fasting protein pacing diet, and they were motivated to continue beyond the two-month study period. The scientists secured additional funding to support this effort and have now tracked this person’s progress with rigorous clinical supervision for two years. After half a year, they lost 100 pounds. Often, after significant weight loss, individuals may fall prey to the yo-yo effect, where they gain back much of what they lost. However, this study participant kept off the weight. During this time, Mohr continued to collect samples and analyze their microbiome over time.

Alex Mohr:

What we found was a complete restructuring of their gut microbiome that kind of stuck with that person as they progress throughout the year. This paralleled improvements in weight status, but also other health markers. This is what we're getting at. If we're looking at the microbiome, we want to get that person out of a metabolically compromised state or disease associated state and move them into a health associated state. The changes that we saw in the microbiome paralleled that particularly with some notable microbes, like *Akkermansia muciniphila*, which is a very popular microbe these days in the research setting. There's even a probiotic supplement available. This microbe is very interesting in that it resides in the mucous layers in the gut and acts like a lawnmower. It trims old mucus, encourages goblet cells to produce more mucus, keep that layer healthy, which of course helps limit a lot of the inflammatory exposure that we might get from the gut, principally driven by endotoxins, for instance. So, having that present and increased in this individual was a pretty interesting finding, particularly to have microbes like that persist over the entire year.

**Perspectives and Future Directions**

Narrator:

In this study, both the calorie restriction and intermittent fasting protein pacing groups showed improvements in their health measures. However, intermittent fasting may be easier for some people to stick with, due to how calories are consumed throughout the week. While what works best is different for every individual, Mohr hopes that his current and future work highlights several general takeaways that can help people live healthier lives.

Alex Mohr:

Depending on the health status of the person, some dietary patterns might be more appropriate. Over-consuming energy is obviously going to cause major issues. From microbiome perspective, we know that the types of molecules, like different dietary fibers, polyphenols, we want to actually have a diet that's really rich in those because that's what's going into lower regions of our gastrointestinal tract to really promote healthy community. So, one thing that I do promote to everyone from a dietary perspective is to make sure that you're eating a diverse diet. And a lot of that actually comes down to different plant foods, which we know contain a lot of these fibers and these dietary phenols. Simple things like eating the rainbow, we know that things like that are very beneficial for our health.

I'm very interested in better understanding the interaction of nutrients, it's effects on the microbiome, and then globally in the host. My hope is to deeply phenotype somebody, going from the genome down to the metabolome, and then bridging that with the microbiome, and doing cohesive analyses, which aren't really available right now. It's going to take some time, but that's really what I want to pursue, because not only from a diagnostics aspect with medicine, but also on the preventative side, when we're doing different profiles of people that's really going to be the way for the better understanding health trajectories and getting someone healthy.

Outro:Thank you for listening to *The Scientist* Speaks! This episode was produced by the Creative Services Team for *The Scientist* and narrated by Niki Spahich*.* Please join us again in September, as we explore the role of autophagy in infectious disease. To keep up to date with this podcast, follow *The Scientist* on social media, and subscribe wherever you get your podcasts.