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Appetite Grows When New Bacteria Take Over the Gut

It reads like a plot straight from the pages of a science fiction novel: Hordes of bacteria infect mice and cause the rodents to develop voracious appetites. The ill-fated mice grow fat, their troubles compounded by insulin resistance, high cholesterol, and high blood pressure. To make matters worse, the microscopic troublemakers can move from mouse to mouse, spreading poor health habits to any rodent unlucky enough to play unwitting host.

A study published online March 4, 2010, in *Science Express*, revealed just such a scenario in real life, suggesting that gut microbes might one day be grouped along with inadequate exercise and overeating as a cause of obesity and metabolic syndrome, both of which shorten life expectancy.

Rob Knight, a Howard Hughes Medical Institute Early Career Scientist at the University of Colorado, Boulder, conducted some of the complex analyses that led to the discovery that gut microbes may help alter the behavior of mice. "It could be the case that bacteria are involved in obesity in a way that's transmissible between people," Knight said.

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To study the impact of gut microbiota on obesity, researchers led by Matam Vijay-Kumar of Emory University created mice without a gene for an immune system protein called toll-like receptor 5. TLR5, normally prominent in the gut lining, recognizes the protein that forms the flagella of invading bacteria and helps target the pathogens for destruction.

Without the *TLR5* gene, mice develop metabolic syndrome, a group of obesity-related abnormalities that increase the risk for type 2 diabetes and heart disease. Metabolic syndrome is on the rise in the United States, and some estimates indicate that one in four Americans may be affected by it.

The mice without the *TLR5* gene ate 10 percent more than other mice. Those mice also had a more severe response to a high-fat diet, and showed signs of insulin resistance and metabolic syndrome -- even when their caloric intake was restricted. When researchers transferred the gut microbiota from the *TLR5*-deficient mice into the guts of normal mice, these mice also developed the signs of metabolic syndrome: overeating, insulin-resistance, high blood lipid levels, and elevated blood glucose.

The experiments showed that the behavioral and metabolic transformation in these animals came from within – driven by shifts in the composition of the bacteria population in their guts. When the mice were administered a broad-spectrum antibiotic, which wiped out much of their gut bacteria, their behavior and other symptoms returned to normal.

Knight's lab, which has developed computational methods to analyze microbial diversity and its impact on health, characterized the changing microbe populations in the mice in collaboration with Ruth Ley at Cornell University. Their strategy was to analyze the sequence of the DNA encoding 16S ribosomal RNA (rRNA) in the gut microbiota. Ribosomes are the protein-making machinery of the cell, and 16S rRNA is an essential component of that process in bacteria. The gene encoding it makes a handy measuring stick because parts of it evolve quickly and parts of it evolve slowly. Differences in the gene from one bacterial species to the next supply important information about the evolutionary relationship among the species.

An individual animal's gut can contain hundreds of species of bacteria, and many of these refuse to grow when removed from their favored environment. So rather than attempt to culture and characterize the thousands of bacteria in any given sample of mouse gut microbiota, Knight and his colleagues focused on the DNA. They amplified all the 16S-rRNA genes in the sample. Then each group of closely related 16S rRNA sequences discovered was treated as a surrogate for a unique species.

After amplifying the 16S rRNA, Knight used next-generation sequencing technology called pyrosequencing to identify each genetic sequence, tagging it with a "barcode" created by Micah Hamady, a graduate student in his lab. Using a software program called UniFrac, developed in Knight's lab by Catherine Lozupone, the researchers examined the differences between many versions of the 16S rRNA, assessing their evolutionary history and using their similarities and differences to compare microbial communities.

"This allows you to use the universal tree of life as a ruler and measure the differences between each community," Knight said. Their analysis indicated that the relative abundance of 116 different groups of closely related bacteria was altered significantly in the mice that overate compared to normal mice.

The next step in this research will be to determine whether gut microbes affect humans in a similar manner. Lay published work in *Nature* in 2006

showing a change in gut microbes accompanies obesity in humans -- but that research did not show a cause-and-effect relationship. She and Knight collaborated on work published in the *Proceedings of the National Academy of Science* in 2005 on a mouse model of obesity that exhibited a different type of microbe change. It will take further research to tell which model best applies to human obesity, Knight said.

Knight says the scientists hope to determine how often microbial activity manipulates behavior, as it did in the overweight mice. The phenomenon is already a well-documented side effect of infection with *Toxoplasma gondii*, the parasite that causes toxoplasmosis. Rats and mice infected with *T. gondii* show no sign of fear when they smell the urine of cats -- a deadly predator. When the rat's newly daring behavior turns it into cat food, the parasite has the new home it needs for its next life stage, which requires a carnivorous host.

The diversity of the microbiota in humans may also hold secrets about human variation. "We're 99 percent the same on a DNA level, but our microbiota are 80 to 90 percent different," Knight said.

Knight points to weight reduction studies that consistently show only modest average improvements, even though a few study members respond dramatically. Maybe microbes can help explain the difference, he said.

"The human body is an archipelago of completely distinct habitats," he said. "Everything we find about the microbiome is unexpected. What intuitively makes sense turns out not to be true."