EXPLORING THE ORIGINS OF DISEASE

MANITOBA RESEARCHERS AIM TO SHED NEW LIGHT ON HOW LIFESTYLE FACTORS INTERACT WITH BACTERIA IN THE GUT TO INFLUENCE THE DEVELOPMENT OF CHRONIC CONDITIONS SUCH AS ASTHMA, DIABETES AND CARDIOVASCULAR DISEASES

By Bob Armstrong

Deep inside every human being, there exists a secret world known as the microbiome.

Located inside the intestines, this community is home to billions of microbes, including as many as 3,000 different types of bacteria.

Since the beginning of time, these microbes have been labouring in relative obscurity, efficiently and effectively supporting the body's digestive system, helping with the production of certain vitamins, and just generally making sure that everything is running as it should.

Then, about 15 years ago, that started to change. Scientists started to realize that the microbiota in our gut can affect the behaviour of certain genes, which, in turn, can promote or discourage the development of certain chronic diseases.

Now, a team of Manitoba researchers is planning to build on this knowledge through a new research project known as The Manitoba Personalized Lifestyle Research (TMPLR) program.

The study, which has received $1 million in funding from Research Manitoba, is led by Peter Jones, a professor in food sciences at the University of Manitoba and Director of the Richardson Centre for Functional Foods and Nutraceuticals.

Among other things, the study is expected to shed new light on how lifestyle factors – such as diet, exercise and sleep – interact with the bacteria in our gut, and our genes, to influence the development of chronic diseases, such as asthma, cardiovascular disease and diabetes. The study is also expected to show that it is possible to develop a personalized health profile for patients as well as individually

Peter Jones with the mobile lab that will be used in The Manitoba Personalized Lifestyle Research project.

tailored lifestyle recommendations that will enable people to reduce their risk of chronic diseases and generally lead healthier lives.

Jones says the study is the most comprehensive of its kind ever undertaken in Canada, and will help position Manitoba as a leader in the field of microbiome/genetic research.

"There have been community-based surveys that have been launched across Canada by different groups, but nothing, to our knowledge, this comprehensive," says Jones, who was recently named a Distinguished Professor by the University of Manitoba for his outstanding research work. "This is the first (study) that wraps all these different factors together."

The key to the study lies in the bacteria that make up an individual's microbiome in conjunction with their genetic architecture and how these two factors contribute to a person's health. Jones says the research project will identify certain biomarkers that clinicians can use to identify potential disease risks, not unlike the way a set of lights on a car dashboard signals potential engine trouble.

"We're after those very, very early warning indicators that will portend disease risk," he says. "That's where the genetics, that's where the microbiome comes in."

As Jones explains, a number of genes are known to directly cause certain types of disease, such as Huntington's or cystic fibrosis. But there are also a number of examples where a person's predisposition to disease, such as cardiovascular disease, can be affected more subtly by variations in their genetics. These slight differences in a person's predisposition to disease could also be influenced by a number of factors beyond genetics, including the makeup of a person's microbiome.

"The microbiome appears to be affected by just about any dietary shift," says Jones. "It is also affected by stress, by exercising, and even by sleep patterns. That is what we are endeavouring to study – whether we can associate a microbiome profile with a certain set of disease risk markers.

"In other words, if your cholesterol level is high because you are stressed or have the wrong diet, is that accompanied by a difference in the distribution of gut bacteria? And is that consistent across individuals, or can that be used as a barometer of risk (for disease)? And, finally, can that risk ultimately be reduced by changing the gut bacteria directly?"

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The study was launched officially this spring with a call to Manitobans who are interested in contributing to science while learning more about their own health.

Researchers are currently looking for 1,200 Manitobans aged 30 to 46 to volunteer for a total of four hours of testing and information gathering.

"We wanted to get a big enough representation, so basically one out of 1,000 (Manitobans)," he says. "What we are after is to be able to obtain a real sense of how healthy Manitobans are, what lifestyle drivers create better health, and what the associations are with specific microbiome distributions and with specific genetic variations."

Volunteers can go to the Richardson Centre for testing or to the mobile lab, which will travel to locations throughout Manitoba this summer.

Testing requires two, two-hour sessions. Each participant receives a $100 honorarium for taking part, although researchers note that, for many participants, getting a detailed look at their own health will be the more important payoff.

Whether at the mobile lab or the Richardson Centre, taking part in the study will involve answering questions in a health survey, providing blood and stool samples, completing a fitness assessment on an exercise bike, and having a body scan which will measure body composition (amount and location of fat and muscle) and bone density.

Jones says efforts have also been made to ensure that the research program includes Indigenous Manitobans. "Indigenous Manitobans make up a sizable portion of the population," he says. "We want to make sure Indigenous Manitobans are included in TMPLR."

To that end, TMPLR researchers are collaborating with members of the Sandy Bay First Nation on a study. "We're hoping to get TMPLR's mobile research unit out to communities such as Sandy Bay to bring the research directly to the community," says Jones.

The number and variety of tests involved in the study are a function of the interdisciplinary nature of the project.

Researchers are looking at the complicated interactions of many different factors in the health of adults.

Some aspects of the study, such as the benefits of exercise, are familiar to just about everybody. Others, such as the impact of gut microbiota

(including bacteria and fungi), aren't as often on people's minds. Questions about gut microbiota, however, are very much worth examining, since we depend on billions of these micro-organisms to obtain nutrients and energy from the food we eat.

"We live in peaceful harmony with our gut microbiota most of the time," notes Jones. But when that harmony is disturbed – by a case of food poisoning, for example – we realize how badly we need to keep our guts happy."

Adding to the complexity of the picture is that one factor can influence another. We can change the composition of the community of micro-organisms in our gastrointestinal tract by changing the food we eat or by exercising. Our earliest childhood experiences also influence the kind of gut microbiota we start off with. And underlying those factors are differences in genetics, which mean that some people react to exercise or to changes in diet or in gut microbiota differently.

As a result, the project will require a great deal of number-crunching and has the potential to lead to insights pointing toward many different applications.

"The mining of this data is just huge," says Jones. "The number of research questions we can ask… it's just phenomenal."

The use of the mobile research unit will allow the project to obtain a more complete picture of the health of Manitobans. Instead of just being limited to participants who live close to the U of M's Fort Garry campus, the study will be able to involve communities with differences in diet, lifestyle or genetics.

Program co-director Meghan Azad, who is also an assistant professor in the Department of Pediatrics and Child Health and research scientist at the Children's Hospital Research Institute of Manitoba, is a leading researcher in the effects of gut microbiota on human health, especially in children. She says she will be interested in seeing how the health of Manitobans in their 30s and 40s can be traced back to their earliest experiences.

"[In pediatrics] I study babies," she says. "I usually look forward as the child grows up, but here I'll be studying adults and looking backward to see if I can find any associations with their early life."

Participants in the study will be asked about their childhood health experiences, and, where possible, their mothers will also be surveyed. The researchers will also ask permission to access Manitoba health records for those born in the province in order to examine childhood encounters with the health-care system.

Surveying participants' mothers will allow for insights into how maternal health, such as whether they had gestational diabetes while pregnant, how the participants were born (vaginal or Caesarian birth) and how they were fed continue to affect health decades later.

"I've done research showing that infants born by Caesarian section have a different microbiome, and that breastfeeding also affects the microbiome," she says. "We know that these factors have a huge impact on the microbiome of infants, but now we'll see if the effects last until later in life."

This information will be combined with other data on their health as children, including whether or not they had a lot of viral infections early in life or had other illnesses. Researchers will also examine stress in early childhood, as "there's evidence that stress in early life can impact your long-term health." Other questions will ask about allergies, which Azad describes as "the first chronic disease that presents in life."

Azad compares the study to some famous major studies conducted in the U.S., which have yielded important

Top: Peter Eck is leading the genetic research stream for TMPLR.
Middle: A selection of cell cultures in Peter Eck's lab.
Bottom: Meghan Azad is a leader in microbiome research.

insights into health, lifestyle and various treatments.

Surveying mothers of participants, for example, was part of the Nurses' Health Study, which surveyed approximately 120,000 U.S. nurses beginning in 1976 and over time led to greater understanding of links between smoking, red meat consumption, alcohol use and illnesses later in life. TMPLR team has modified the survey used in the Nurses' Health Study.

Such "observational studies" are the first step in improving efforts to prevent illness, Azad says.

As part of the study, TMPLR researchers working with the newly gathered data will be able to detect associations between certain kinds of gut microbiota, genetic profiles, activities, or childhood experiences and positive health outcomes. Then, in the next step, researchers will be able to design experiments to see how to help people achieve that benefit. If, for example, one particular gut microbe shows up in the data as associated with better health, the next step might be to design a functional food [a food modified for health effects] that encourages the growth of that microbe in the gut.

A key player in the microbiome research for TMPLR is Ehsan Khafipour. An assistant professor of gastrointestinal microbiology in the Department of Animal Science and Department of Medical Microbiology at the University of Manitoba and a research scientist at Children Hospital Research Institute of Manitoba, his job involves analyzing the composition of the microbiome through a process known as high-throughput sequencing.

He says advances in technology over the years have enabled scientists to get deeper insights into the bacteria found within the microbiome and how they influence our genes.

"Twenty years ago, our technology was limited to conventional

MAKING A CONTRIBUTION TO SCIENCE

BY BOB ARMSTRONG

What does it take to make a contribution to science?

I decided to find out recently by taking a few of the tests that are being done as part of The Manitoba Personalized Lifestyle Research (TMPLR) program.

Once I boarded the program's mobile testing lab, I was put through my paces.

First, a technician positions me on the DEXA (Dual Energy X-Ray Absorptiometry) machine so that it can carry out scans that will determine my body composition, including both the amount and placement of my fat, and my bone density.

I lie on a flat surface and the scanning arm slowly passes over my face and down to my feet. The technician places a foam block under my knees in order to change my position so that the machine can scan my spine. After that, she repositions my legs, turning them a little bit awkwardly to one side, in order to take a scan of my hip.

By measuring bone density, the scans will help to predict my risk of osteoporosis and fractures later in life. The pictures of my fat will reveal whether I carry it largely below the belt – the healthier place to have it – or above the belt. They'll also show if my fat is largely spread around under my skin (subcutaneous fat) or positioned around my internal organs (visceral fat), which is associated with worse health.

The scan is only part of the process of being one of the 1,200 research volunteers in TMPLR.

I also take a turn on an exercise bike, breathing into a snorkel-like mouthpiece connected to a tube that leads to a machine that measures carbon dioxide. The exercise bike test involves first spending about 10 minutes just sitting on the bike in order to give researchers a measure of my resting respiratory quotient. My respiratory quotient is the ratio of carbon dioxide released in my breath to oxygen consumed, and reflects the type of fuel my body is using.

Bob Armstrong undergoes a test in the lab.

Then, once the test begins and pedalling starts, the resistance on the bike is increased every three or four minutes until my pulse reaches 85 per cent of my maximum heart rate (maximum heart rate is calculated by subtracting your age from 220, so that at age 55 your maximum rate is 165 beats per minute). At the 85 per cent heart rate, the machine measures my oxygen consumption. Oxygen consumption is a good measure of cardiovascular fitness, with higher oxygen consumption associated with greater fitness, explains Peter Jones, Director of TMPLR.

These two tests are only part of the process. Before the session can begin, a participant must first give consent, a process which involves going over various documents with staff. Next, staff will take the participant's blood pressure and a sample of blood, which is tested for substances like cholesterol and blood sugar and also used in the genetic tests that are an important part of TMPLR.

After giving a sample of blood, the participants go through a questionnaire in which they're asked about a wide range of health and lifestyle subjects, ranging from their childhood experiences to their current sleep habits.

The next stage in the process takes place at home. Since an important part of TMPLR project is the study of the participants' gut micro-organisms, researchers need a stool sample. Over 1,000 different species of bacteria and other organisms live in our digestive tract – some beneficial, some not – so the little plastic specimen bottles in the lab's storage fridge hold scientific gold. I'm too old to be a participant in the study (the team is looking for men and women aged 30 to 46), so thankfully I get to skip this stage.

HOW HEALTHY ARE YOU?

Researchers are looking for 1,200 Manitobans aged 30 to 46 to participate in the Manitoba Personalized Lifestyle Research Program.

The study is designed to shed new light on how lifestyle factors – such as diet, exercise and sleep – interact with our genes and the bacteria in our gut to influence the development of chronic diseases, such as asthma, cardiovascular disease and diabetes. It's also expected to show that it is possible to develop a personalized health profile for patients as well as individually tailored lifestyle recommendations that will enable people to reduce their risk of chronic diseases and generally lead healthier lives.

Study participants will be asked to answer questions in a health survey, provide blood, urine and stool samples, undergo a fitness assessment on an exercise bike, and have a body scan that will measure body composition (amount and location of fat and muscle) and bone density.

Each participant receives a $100 honorarium for taking part, although researchers note that, for many participants, getting a detailed look at their own health will be the more important payoff.

For more information, visit www.tmplr.ca, or contact a TMPLR team member at TMPLRtrial@umanitoba.ca or 204-298-5483.

culturing or molecular methods targeting a specific microbe," says Khafipour. "We could not study the whole microbiome, or the whole community of microbes in the gut. Now, with the use of high-throughput sequencing technologies, we can look at the community as a whole, identify what is there, understand the composition of the microbes and predict their function."

This allows scientists to get a better understanding of how the bacteria in the microbiome interact with genes in the human body. "The microbes within a person co-evolve with the genetic characteristics of that person," says Khafipour. "You can also say that the microbes have modified the genetic characteristics of that person. So it is a co-evolution between the microbes and the host throughout that person's lifetime. It is very interesting for us to see what type of genetics and what type of microbiome are associated with each other and whether they are a predictor of a chronic condition."

The genetic stream of TMPLR, led by Peter Eck of the U of M's Human Nutritional Sciences and the Children's Hospital Research Institute of Manitoba, will look at genetic variations that have impacts on health that can be modified with dietary interventions. For the study, he will focus on genetic analysis involving measurement of telomeres, protective structures at the end of chromosomes. The length of telomeres has been linked to aging, mortality and chronic conditions including cardiovascular disease, obesity and diabetes.

Another of Eck's research interests is genetic differences in the ability of people to obtain vitamin C from their food. Two specific proteins in the body are involved in transporting vitamin C across cell membranes. If genetic variation means that this transportation process doesn't work as well for some people, they would be prone to have low levels of the vitamin, which can be linked to some cancers, cardiovascular disease, diabetes, infections and other illnesses.

If ability to obtain vitamin C varies because of genetic differences, standard dietary advice about the required portions of fruit or the need for supplements may not apply to everybody. Knowledge of an individual's genetics may allow for more personalized recommendations for vitamin C and for any other nutrients where similar variation exists.

Leading the physical activity portion of TMPLR is Todd Duhamel, associate dean (research) at the U of M's Faculty of Kinesiology and Recreation Studies. He hopes the research can lead to more specific activity prescriptions tailored to an individual's genetics and other factors. The questions around activity go far beyond the simple fact that moving your body is good for you. By analyzing volunteers' activity along with gut microbiota and genetics, the researchers hope to learn what kind of activity works best for certain people.

"So the exercise prescription you get is different from the one I get, based on our individual genetics," he says.

Ehsan Khafipour, TMPLR lead in gut microbiome research, will use high-throughput sequencing to anlayze the microbiome of study participants.

The connection between activity and gut microbiota is also one with potential for improving health. "There's a lot of emergent science that we want to exploit in the study," he says.

When you exercise, you actually change the composition of the community of micro-organisms in your digestive tract, encouraging growth of the beneficial ones, he notes.

The researchers also hope to learn more about the specific kinds of activity that help specific people to stay healthy. Again, applying the "personalized" part of the project's name, the team members want information that can allow for advice that's more specific than simply "try to get 10,000 steps a day."

In the physical activity portion of the study, researchers will conduct a survey of participants' activity, measure their fitness level (including heart rate and oxygen consumption) while they pedal an exercise bike, and assess their actual daily activity by sending them home wearing an accelerometer. The accelerometer is described as "a pedometer on steroids." Like the more familiar pedometer, it measures the amount an individual moves, but it also measures the intensity of movement or the speed at which they move.

Measuring the intensity of activity is important in understanding the effect of activity on health because of the growing understanding that intense activity is particularly beneficial. "There's evidence that if you do more high-intensity activity, you don't need to do as much," says Duhamel, adding that "we know that no matter who you are, aerobic capacity is key."

As the research project continues, chances are we are going to learn quite a bit more about how lifestyle factors such as exercise affect our health. In so doing, these researchers will not only help individuals learn how they can live healthier lives, says Jones. They will also help "reduce the burden of chronic disease on our province."

Bob Armstrong is a Winnipeg writer.