The Epigenetic Patterns of Exercise

Methylation analysis and RNA-Seq are helping researchers understand how endurance training makes epigenetic changes to the human genome.

Introduction

It's been said that "Human bodies are designed for regular physical activity."[1] And rightly so. Over the past decade, hundreds of studies have been published showing that exercise offers benefits to overall well-being—regardless of age, sex, or disease status. Just 30 minutes of brisk walking, 5 times a week, or 75 minutes of intense aerobic exercise a week have been shown to prevent and ameliorate various conditions, including cardiovascular disease, metabolic disease, osteoporosis, cancer, and depression. But while it's clear that regular physical activity is important to healthy living, what's not clear is how it confers its multitude of benefits. Carl Johan Sundberg, MD, PhD, a professor of molecular and applied exercise physiology at Sweden's renowned Karolinska Institutet, has spent his career trying to understand how exercise makes positive changes to the human body.

"Our goal is to understand something that is massively beneficial to the way that overall health is regulated," Dr. Sundberg said. "By investigating the body's reaction to exercise at the molecular level and linking that reaction to any functional changes, we hope to shed some light on what drives those benefits."

Dr. Sundberg and his team use the Infinium® HumanMethylation450K BeadChip and RNA sequencing (RNA-Seq) on the HiSeq® System to understand how physical activity alters the epigenome, the chemical signals that tell genes when, where, and how to express their associated proteins. More importantly, they hope to understand how those alterations might trigger the myriad of physiological processes that result in health gains linked with exercise.

Dr. Carl Johan Sundberg led a team of researchers, including Dr. Francesco Marabita, in performing a unique epigenetics research study that measured the epigenetic signatures of exercised and non-exercised leg muscles in 23 volunteers.

A Unique Experimental Paradigm

Studying human physical performance isn't always easy. Most studies attempt to control as many factors as possible—including age, diet, and health status—to make sure that any changes can be directly attributed to differences in particular exercise regimens. As Dr. Sundberg and colleagues began to study epigenetic alterations, they were faced with a unique challenge: how could they control for the diversity of the human genome? It's not a simple task. Genes, gene expression, and epigenetic patterns differ dramatically from person to person. Their solution was to recruit study participants who could act as their own controls, thus ensuring that biological and environmental factors outside the experiment would be the same between the samples being compared."[2]

"We wanted to improve the understanding of the functional changes behind the benefits of exercise and look at the regulation of these adaptation processes in the skeletal muscle," Dr. Sundberg said. "To date, there have been only a few studies looking at DNA methylation and exercise because it's not easy to do. What we did was to build in a control situation by exercising only one leg in each of our participants and using the contralateral leg as the control."

The team recruited 23 volunteers, individuals who were only moderately active, to come into the lab and perform supervised endurance training for 45 minutes, 4 times per week across three months. But it was a special kind of exercise—the one-legged kind. Dr. Sundberg likens the homemade exercise device that they used to a customized stationary bicycle—yet one where participants trained only one leg in a circular kick-like motion.

The modified exercise bike used in the study allowed participants to exercise 1 leg, while the other leg remained stationary. It enabled each individual to be a study subject and act as their own control.

"It's a bit artificial, of course, for experimental purposes," Dr. Sundberg stated. "Participants did this one-legged kicking for 45 minutes, and it was an endurance aerobic exercise session. Many of our participants talked or read magazines during the work-out, but you could see that it wasn't easy for them. By training one leg, we could control for genetics, as well as other environmental factors. This way, we knew that the trained and untrained legs were exposed to the same diet, stress, alcohol intake, and sleep schedule over time. We were confident that any changes we saw were linked to the training."

To perform the epigenetic analysis, Dr. Sundberg and colleagues collected biopsies from the vastus lateralis, the outer part of the quadriceps muscle. They took tissue from both legs at the beginning of the study, and then again after participants completed the three-month training period. They also measured the performance capacity and enzyme markers for the muscle energy metabolism capacity of each leg.

"After three months, they became aerobically fit in the trained leg," Dr. Sundberg added. "However, the trained legs didn't change in strength or muscle volume because that's not what results from this kind of training."

A great many changes occurred beneath the surface—ones that Dr. Sundberg and his colleagues measured when methylation analysis and RNA-Seq were performed on the biopsy samples.

Exercise and the Epigenome

Francesco Marabita, PhD, a post-doctoral fellow at the Karolinska Computational Medicine Unit, says that the group selected the HumanMethylation450K BeadChip because it is so cost-effective. "The HumanMethylation450K BeadChip really is the platform of choice," Dr. Marabita said. "We obtained good methylation data with it at a good cost. And, it delivers the raw data quickly. We have also systematically compared bioinformatics pipelines for the analysis of 450K data, examining the reduction of technical variability with a data-driven approach."[3]

He was also happy with how easy it was to perform RNA-Seq. "Using the HiSeq 2000 System and the TruSeq® RNA Library Prep Kit gave us the benefit of reproducibility," Dr. Marabita said. "The kit has all the reagents you need in one box. You have all the enzymes so you don't have to bother buying separate lots." The array and sequencing data were obtained from the Karolinska Institutet's Bioinformatics and Expression Analysis (BEA) core facility and the Science for Life Laboratory (SciLifeLab) center, respectively.

With these tools, the research team analyzed the biopsy samples. They specifically examined markers for skeletal muscle metabolism, as well as the methylation status of nearly 500,000 sites in the genome. In addition, they tracked the activity of over 20,000 genes. Taken together, the group discovered there were strong associations between epigenetic methylation (the addition of a methyl group to specific nucleotides), as well as gene expression in over 4000 different genes. What was more interesting, were the strong associations the team found between the epigenetic alterations and the activity in genes implicated in metabolism and inflammation. Specifically, the tissue in the trained legs showed an increase in methylation in genes related to muscle adaptation and metabolism, and a decrease in methylation in genes linked to inflammation.

"We had a hunch we would see this at the expression level because of previous microarray studies," Dr. Sundberg said. "But RNA-Seq allows you to go deeper—so you can find genes that are expressed to a very low degree. That's why we saw about 4000 genes that were differentially regulated. We obtained more solid numbers and a more consistent read-out with RNA-Seq, which allowed us to detect more genes altered by the training. The increased methylation seemed to be related to remodeling of the tissue and metabolism, while decreased methylation was related to inflammation in various ways. This might explain why exercise has such a broad range of benefits."

The results, however, weren't totally without surprise. Dr. Sundberg and his colleagues discovered that most of the epigenetic alterations occurred on enhancer sites, short regions of DNA, which activate gene transcription from a distance, as opposed to promotors, transcription initiators located close to those genes.

"Differential methylation in enhancers seemed to be possibly more, at least statistically, important for the changes in gene activity," Dr. Sundberg explained. "We had the notion that maybe the promotor regions would be most directly related to those changes. But in hindsight, new research has shown that enhancers seem to play a large role in gene transcription in various cancer tumor tissues. Because we see similar gene regulation with training, it suggests that various types of tumor tissues might 'hi-jack' adaptation mechanisms from normal physiology. That might be an important discovery."

Understanding Epigenetic Changes in Muscle and Beyond

Dr. Sundberg argues that human muscle tissue provides good insight into how exercise might foster epigenetic alterations in other body tissues. "Human muscle is a good model for adaptation in many tissues," Dr. Sundberg said. "All tissues are affected by physical training. Yet, if we look at the parallel processes happening in various tissues after training, many are specific to that tissue. For example, bone has different signals than muscle, and the brain will have different signals, too."

Dozens of studies have demonstrated that physical activity improves mood, cognition, and mental health. Animal models also suggest that aerobic exercise results in neurogenesis, or the growth and development of new brain cells.[4] "We might find some interesting clues in the muscle that help us understand the adaptations in the brain," Dr. Sundberg added. "The changes in metabolism, for example, probably have similar adaptations in the brain to the ones we see in muscle."

Muscle is also easier to analyze than other tissues. "With muscle tissue, we can direct, supervise, and monitor what people do and don't do," Dr. Sundberg said. "It's a voluntary thing—you either activate your muscle or you don't. We can control it for experimental purposes, enabling very exact functionality testing to measure endurance capacity, aerobic capacity, and strength. The other benefit is that we can safely take muscle samples from humans. Few other human tissues lend themselves so well to biological investigation. By studying the impacts of exercise on muscle, I believe we can learn more about the general principles of tissue adaptation related to this lifestyle change."

Moving Forward

Dr. Sundberg and his team are currently using a similar paradigm to study the epigenetic modifications that occur in the muscle after interval training, an exercise program that involves differing rates of speed and effort.

"The participants go through a very, tough training over three weeks," Dr. Sundberg said. "We've taken biopsies just before training, 30 minutes after training, and 3 hours after training during these exercise sessions," he says. "It's a different challenge to the body. We're looking forward to seeing what happens to gene expression and methylation, both immediately and over the course of three weeks. The idea is to pinpoint any changes over time."

Future studies might also include strength training and other forms of exercise. Taken together, Dr. Sundberg says that this brand of research might help us better understand what work-outs are best for individual people.

"There is so much variation between people—some respond much more strongly than others to training," Dr. Sundberg added. For example, endurance training might improve performance but not reduce blood pressure in one person. Another person might show improvement in blood pressure and inflammation measures, but not performance. The why and how of these differences, are unknown.

"It's likely that if a person does not respond favorably and show certain health improvements with one type of exercise that there is another exercise type that will work," Dr. Sundberg said. "We need to investigate what kind of exercise activity will benefit each individual person. Maybe strength training will work, or maybe that person will only respond to interval training. We don't know yet. That is the importance of this and other colleagues' work. We might find new ways to optimize the way people conduct physical activity to gain the most health and function."

References

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4. Van Praag, H. Neurogenesis and Exercise: Past and Future Directions. *NeuroMolecular Medicine.* 2009;10(2):128-140.

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