Study could lead to ACL injury prevention for female athletes

In recent years, health care professionals have seen a trend with knee injuries; more female athletes injure their knees than males. What could be the cause of this trend? **James R. Slauterbeck, M.D.** aims to find out.

"I think there are many factors that cause female athletes to tear their anterior cruciate ligament (ACL) more than males, and many of them will likely interplay together to be the actual reason why females are more at risk," he said.

Dr. Slauterbeck, currently an associate professor at the University of Vermont, first saw this trend while completing a two-year sports medicine fellowship at the University of California, Los Angeles.

"During those two years, we were beginning to identify that our female soccer players were tearing their ACLs at a much higher rate than players from the male team."

Wondering if this phenomenon held true in other sports programs, Dr. Slauterbeck conducted an informal survey across the National Collegiate Athletic Association (NCAA) to determine the incidence of ACL tears among females. The survey results identified a sex difference in ACL injury among some college teams.

At that time a formal study conducted by **Elizabeth Arendt, M.D.**, director of sports medicine at the University of Minnesota, Minneapolis, and **Randall Dick**, associate director of research for the NCAA, showed similar results.

"The results of these studies made us question why this occurs," said Dr. Slauterbeck. With a Research Grant he received in 1999 and the Clinician Scientist Award he received in 2004, both funded through OREF, he decided to conduct further research on this issue.

Dr. Slauterbeck was one of three clinician scientists to receive an OREF Clinician Scientist Award in 2004. Funded by the **Dr. Dane** and **Mrs. Mary Louise Miller** Endowment Fund, Dr. Slauterbeck's award is providing him with a $100,000 per year salary stipend for three years.

"There are so many clinical demands on a surgeon or on a clinician today to support the medical school, to support the clinics and to support the running of the departments," he said. "This award has very effectively reimbursed the school for my academic time so that I can actually go to the lab and be freed up from clinical responsibilities."

This will allow him to continue investigating why injuries occur more often to female ACLs than male.

According to Dr. Slauterbeck, both extrinsic factors, such as coaching differences, playing surface differences, and fatigue, as well as intrinsic factors, including knee position, sex hormones and neuromuscular characteristics, probably share the responsibility for the higher incidence of ACL tears among female athletes. Dr. Slauterbeck's study will focus on the intrinsic aspects.

"One of the theories I'm investigating is that there are differences between males and females in the way ligaments remodel," he said.

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Remodeling, Dr. Slauterbeck says, occurs throughout everyday life as well as in response to injury. For ligaments, remodeling is a delicate balance of building up and breaking down collagen.

To study how this differs between females and males, Dr. Slauterbeck, along with **Daniel Hardy, Ph.D.,** an associate professor of cell biology and biochemistry at Texas Tech University with whom Dr. Slauterbeck has an NIH grant, has begun to perform experiments to find out whether the genes that encode for synthesis and breakdown of collagen are up-regulated (increased) or down regulated (decreased) in females.

LifeGift, a not-for-profit organ procurement organization dedicated to recovering organs and tissue for individuals needing transplants in West Texas and much of the United States, permits Drs. Slauterbeck and Hardy to

Leave Your Legacy - Endow a Named OREF Research Award

The goal of the 50th Anniversary Endowment Campaign is to permanently endow several research awards. This chart illustrates the amounts needed to permanently endow some of our research awards and grants. For more information on how you can endow OREF grants and awards, please contact **Gene Wurth** at wurth@oref.org or (847) 384-4362.

If you don't have the means to endow an award at this time, please consider a contribution to OREF's 50th Anniversary Campaign by completing the form on the following page.

Research Grant/Award	Description	Annual Amount Awarded	Funding Commitment to Endow
OREF Clinician Scientist Award	This award encourages young orthopaedic surgeons to pursue a career as a clinician scientist, someone who wants to pursue a career in research while remaining in clinical practice.	$100,000 for each of three years	$2 Million
OREF Career Development Award	This award encourages a multi-year commitment to scientific research in orthopaedic surgery.	$100,000 for each of three years	$2 Million
OREF Prospective Clinical Research Grant	This award funds promising prospective clinical research projects.	$50,000 for each of three years	$1 Million
OREF Research Grant	This award recognizes outstanding clinical research related to musculoskeletal disease or injury.	$50,000 for each of two years	$1 Million
OREF Resident Research Award	This award encourages development of an interest in research for residents and orthopaedic fellows.	$25,000 for one year	$500,000

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In contrast, quadriceps pulls the leg forward, which increases the force on the anterior cruciate ligament."

This, he believes, is possibly another reason why females injure their ACLs at a higher rate than males, and these findings encouraged Dr. Hewett to develop strength training programs that teach female athletes to jump and land properly, decreasing their risk of injury.

Dr. Slauterbeck said that he appreciates the Clinician Scientist Award because it will enable him to work more closely with Dr. Hardy to collect data that can be used to obtain another NIH grant to allow him to complete his research

"The opportunity to spend two full days a week in the lab is probably the most important thing that the Clinician Scientist Award has done for me," he said. "It's given me a much greater appreciation for my collaboration with Dan Hardy. His basic science knowledge and my surgical and sports medicine knowledge together have created a team more valuable than either of us working alone."

With this collaboration, perhaps the risk of knee injuries could be reduced for female athletes, allowing them to continue playing the sports they enjoy.

use ACLs from younger individuals. Gene expression analyses allow them to study the genes and the amount of protein being produced.

Using real-time polymerase chain reaction (PCR), a process by which Drs. Slauterbeck and Hardy are able to quantify the actual numbers of genes expressed in their ligament samples, has enabled them to find the genes expressed in uninjured male ligaments versus those present in uninjured female ligaments.

"What we actually do is quantify mRNA (messenger RNA) with real-time PCR to find the actual numbers of genes that are being expressed in each of our ligament samples," said Dr. Slauterbeck. "What we are finding is that genes that encode for collagen degradation are up-regulated, or increased in females and down-regulated or decreased in males."

They have also found that an enzyme, matrix metalloproteinase 3 (MMP3) — also called stromelysin — that is responsible for collagen breakdown is increased in females.

According to Dr. Slauterbeck, the mere presence of genes does not mean they are "turned-on," leading him to employ the Western Blot method to see if the proteins responsible for collagen synthesis are also present. He's found that there is a high correlation between the amount of genes encoding proteins and the proteins that they are specifically producing.

The next step will be to perform gene arrays. Because the human genome has been fully identified, unknown genes can be compared to known genes, allowing Drs. Slauterbeck and Hardy to select about 1,000 genes and map what those particular genes are doing in each sample.

"What that will do is allow us to assess many more genes for each experiment," said Dr. Slauterbeck. "And we'll most likely use the gene array technique to place ACL tissue in culture and manipulate hormonal situations. We'll be able to find out how the ligament responds to hormonal differences."

This, Dr. Slauterbeck hopes, will lead to a reasonable explanation for why female athletes tear their ACLs more often than males. Although it's too soon to tell how, Dr. Slauterbeck hopes to translate these findings to clinical cases.

"If you know what is happening at the cellular level, it is much easier to begin to explain how to treat or prevent ACL injuries," he said. "With this information you might be able to maximize how ligaments remodel, such as through diet or nutrition or some other way to prevent injuries, but we won't know until we find out what the problem is."

Dr. Slauterbeck is also studying other possible intrinsic causes for the higher rate of female ACL tears in collaboration with **Javad Hashemi, Ph.D.** at Texas Tech University Department of Mechanical Engineering. They are employing digital photography and 3-D reconstructions of actual ligaments of males and females of similar size and age to study size differences of the ACL. Although Dr. Slauterbeck says that more work needs to be completed in this area, his initial data show that female ligaments are smaller in area and volume than a similarly sized male's ACL.

In addition, Dr. Slauterbeck is working with **Tim Hewett, Ph.D.**, director of the Sports Medicine Biodynamics Center at Cincinnati Children's Hospital Medical Center, to develop a simple screening test to identify the sex differences in neuromuscular control between males and females at the time of puberty. They hope to use the screening test to identify which boys or girls might be at risk for sustaining a knee injury and then teach the athletes a neuromuscular training program to decrease the ACL injury risk.

"Females have different neuromuscular characteristics — they tend to fire their quadriceps when they land more than their hamstring muscles," Dr. Slauterbeck said. "The hamstrings protect the ACL because they're pulling the leg back, keeping it from moving forward.
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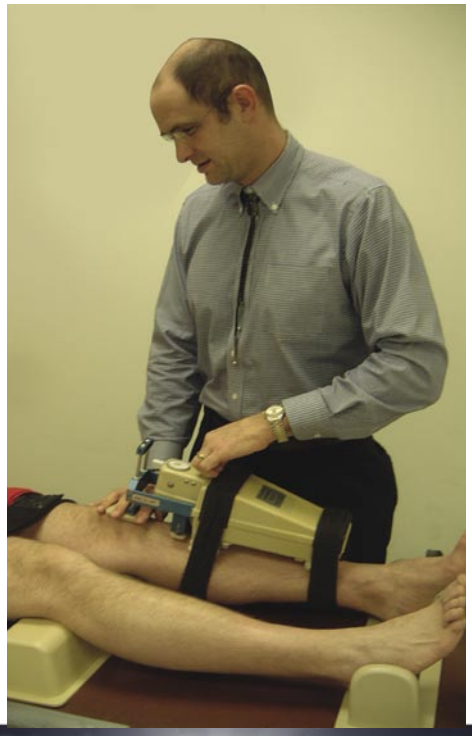

James R. Slauterbeck, M.D. measures knee laxity on a test patient