In the Lab

A Fishy Tale

Why scientists find fish so fascinating

IN THE BASEMENT OF THE BIOLOGICAL Sciences Research Building, behind the heavy metal door with the combination lock, it's always a tropical 82 degrees. The rooms are filled from floor to ceiling with racks of clear plastic tanks. The only sound is the quiet bubbling of oxygen through water.

At first, the tanks appear to be empty, but move closer and you'll see quick flashes of silver as hundreds of inch-long striped fish dart around. These are zebrafish — more formally known as *Danio rerio*.

Native to streams and marshes of India, Pakistan and Southeast Asia, they were originally imported for aquariums. Now they have a new home in biomedical research laboratories worldwide.

According to the U-M's animal census, there were nearly 24,000 zebrafish on campus in 2009. Over the last two decades, the U-M's fish population has grown as more scientists switch some, or all, of their research from mice to fish. Zebrafish have special advantages that make them particularly valuable for biomedical research.

FOR EXAMPLE:

- It takes 19-20 days for a female mouse to produce a litter, but a female zebrafish can lay hundreds of eggs that turn into embryos just one to two days after fertilization.
- Zebrafish embryos are transparent and develop outside the mother's body, so it's easy to see internal organs as they develop.
- There's no need to inject zebrafish with experimental drugs. Just add the drug to tank water and the fish will quickly absorb or swallow it.
- Even a small lab can afford its own fish colony and most scientists are happy to donate "starter" fish to colleagues.
- Fish are vertebrates with a backbone and all vertebrates evolved from a common ancestor, so zebrafish and people have a lot in common. Both progress as embryos through the same developmental stages and share similar genes.

Daniel Goldman, Ph.D., a neuroscientist, professor of biological chemistry and research professor in the Molecular & Behavioral Neuroscience Institute, studies genes and signaling pathways that control development of the central nervous system, especially the optic nerve and retina. He was one of the first Medical School scientists to be hooked by zebrafish.

One thing Goldman finds fascinating about zebrafish is their ability to regenerate damaged organs. If you injure the retina of a mouse, the cells die and the mouse goes blind. Injure the retina of a zebrafish and the fish will just grow a new one.

"No one knows why zebrafish retained the ability to regenerate organs or why mammals lost that ability," says Goldman. "We know that zebrafish have the same retinal cells as mammals, and the genes that regulate regeneration in fish are still present in mammals." Understanding how regeneration works in zebrafish could be the first step toward finding a way to restore vision in people with damaged retinas.

Goldman has persuaded many U-M scientists to become fish enthusiasts. James Dowling, M.D., Ph.D., assistant

Zebrafish in Dan Goldman's lab

SCOTT SODERBERG, U-M PHOTO SERVICES

professor of pediatrics and communicable diseases and of neurology, is one of them. "I started with 10 fish that Dan gave me," says Dowling.

Dowling uses zebrafish for research on myotubular myopathy and Duchenne muscular dystrophy. For Dowling, the big advantage of working with fish is that they are almost all muscle. And it's easy to identify fish with defective muscle tissue.

"Fish have to swim to live; they get their oxygen and food by moving around," Dowling explains. "Fish that can't swim normally don't live more than a couple weeks. Mice, on the other hand, can have a lot of muscle weakness and it doesn't trouble them that much."

Mark W. Russell, M.D. (Fellowship 1996), the Aaron Stern Professor of Pediatric Cardiology, uses zebrafish to study a protein called obscurin that organizes and supports skeletal and cardiac muscle fibers as muscle develops in an embryo. "Zebrafish are an excellent model of muscle growth and development," he says. "Access to the embryos allows you to take cells from one fish and put them in another. You can add or remove genes and then watch the embryos develop to see what happens."

It may seem like a long way from fish tank to clinic, but U-M scientists say it could be closer than you think. They believe zebrafish have the potential to accelerate the pace of biomedical research and bring about faster, better treatments for human diseases. That makes little *Danio rerio* a very big fish on campus. —Sally Pobojewski

MORE ON THE WEB

Other U-M scientists using zebrafish for research

MASTERFILE.COM

Engineering a Better Hand

MANY VETERANS ARE RETURNING FROM WARS IN IRAQ AND AFGHANISTAN with amputations. They need prosthetic devices that can restore as much normal function as possible, but most existing robotic devices have limited motor control, no sensory feedback and can be uncomfortable to wear.

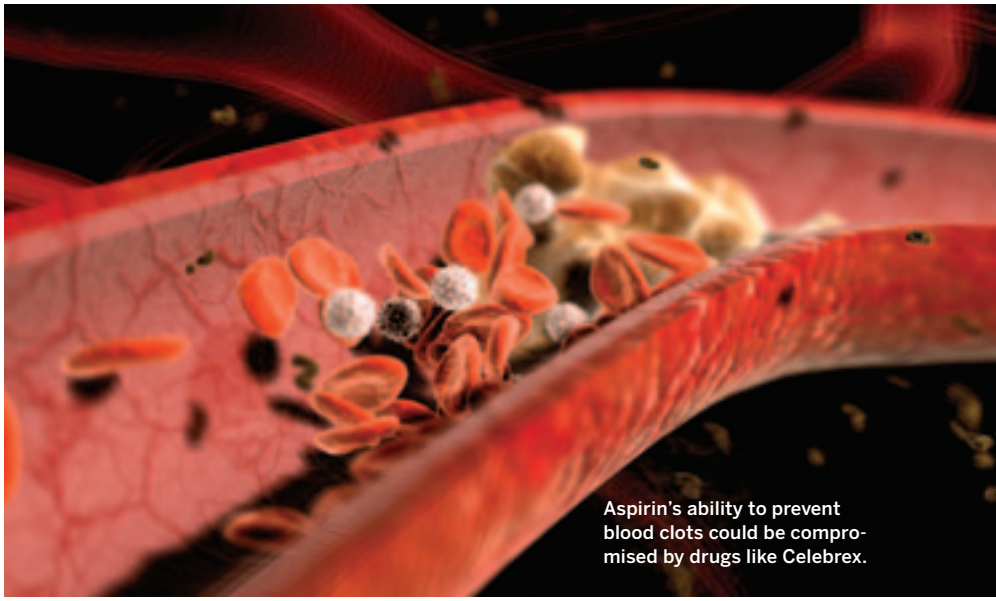
The U.S. Department of Defense is funding U-M research to develop a bioengineered nerve interface that could improve the function of prosthetic hands and restore the sense of touch for injured veterans. "Current prosthetic designs were developed decades ago," says Paul S. Cederna (M.D. 1989, Residency 1997), associate professor of surgery. "We want to make a prosthesis that moves like a normal hand and has the ability to provide sensory feedback."

Using tissue engineering technology, researchers developed an "artificial neuromuscular junction" made of muscle cells and an electroconductive polymer on a biological scaffold. Initial studies with rats showed the interface relayed motor and sensory impulses and helped nerve endings grow and connect properly. Researchers hope to begin testing the bioengineered scaffold in people within the next few years. —SP

MORE ON THE WEB

In the Lab

Celebrex, Low-dose Aspirin Don't Mix



Aspirin's ability to prevent blood clots could be compromised by drugs like Celebrex.

MILLIONS OF AMERICANS WHO take Celebrex for arthritis or pain control also take a low-dose (81 mg) aspirin every day. Now, Medical School scientists have discovered that drugs like Celebrex interfere with low-dose aspirin's life-saving ability to prevent blood clots and reduce the risk of heart attack and stroke.

Using X-ray crystallography, researchers found that Celebrex binds to COX-1, an enzyme that promotes clotting, which prevents aspirin's COX-1-blocking action. In animal studies, researchers found more clumping of platelets — the initial stage of clotting — in blood from animals given Celebrex and low-dose aspirin than in animals given low-dose aspirin alone.

"The greatest risk is for people who take aspirin for cardiovascular problems that are known to be mitigated by aspirin, including patients with unstable angina or those at risk for a second heart attack," says William L. Smith, Ph.D., the Minor J. Coon Professor of Biological Chemistry. If clinical studies confirm the same effect in people, Smith says it will be important to find a balance in dose or regimens so aspirin and Celebrex can both be effective. —SP

MORE ON THE WEB

Diet-Disease Link Intensifies with Age

GOOD NUTRITION IS EVEN MORE IMPORTANT FOR preventing disease in people over 50 than it is for younger adults, according to results from a study directed by Bruce Richardson, M.D., Ph.D. (Residency 1979), professor of internal medicine. The study showed that inadequate amounts of two important dietary nutrients — folate, found in leafy greens, and methionine, found in fish and nuts — led to abnormal gene changes in cultures of immune cells from older adults.

Richardson's findings suggest that the timing of age-related diseases like heart disease and lupus is connected to an increase in a chemical reaction called DNA demethylation. This process alters gene activity and can "unlock" genes that have been silenced by a related process called DNA methylation. Activating silenced genes at the wrong time can lead to abnormal changes in cells.

To test the effects of nutrient deficiencies on methylation, Richardson and colleagues removed immune cells from blood samples of healthy adults, ages 22 to 81, and cultured the cells with low levels of folate and methionine. Immune cells from adults over age 50 showed abnormal gene changes, while cells from younger adults were not affected.

Maintaining a balance between the dual processes of methylation and demethylation is essential for normal cell function. In previous research, Richardson discovered that DNA methylation decreases with aging, while demethylation increases. He also found that demethylation in immune cells called T cells can contribute to the development of autoimmune diseases like lupus.

Richardson hopes his research will call attention to the importance of a healthy diet for the elderly, as well as lead to clinical trials of folate and methionine supplementation. —CLE

Gene Therapy for Failing Hearts?

THE DREAM OF USING GENE THERAPY to treat people with heart failure is one step closer to reality, thanks to a recent study by scientists at the U-M and the University of Minnesota. Todd J. Herron, Ph.D., a research assistant professor of molecular and integrative physiology, and others on the research team used an adenovirus to transport genes that regulate muscle contractions into cardiac muscle cells from failing human and rabbit hearts. One of these genes, the fast myosin motor gene, improved contractions of damaged heart muscle cells in the study. —SP

MORE ON THE WEB

Cancer's Inflammatory Connection

U-M SCIENTISTS HAVE DISCOVERED an important connection between inflammation and breast cancer: a receptor called CXCR1 that triggers the division of cancer stem cells in response to inflammation and tissue damage. In a recent study, scientists found that mice treated with repertaxin — an organ transplant rejection drug that blocks the receptor — had fewer breast cancer stem cells and metastases than mice treated with chemotherapy alone.

"Anti-inflammatory drugs like repertaxin could be a new treatment strategy for targeting cancer stem cells," says Max S. Wicha, M.D., Distinguished Professor of Oncology. Clinical trials based on this work are being planned. —SP

MORE ON THE WEB

Putting the Brakes on COPD

CHRONIC OBSTRUCTIVE PULMONARY DISEASE, OR COPD, IS A progressive, debilitating lung disease that affects more than 12 million Americans — mostly smokers or former smokers. The disease causes wheezing, shortness of breath and chest tightening. Breathing becomes more difficult as the disease progresses and there are few effective treatments.

Lung damage begins long before people with COPD are aware of symptoms. By the time they seek medical help, the destructive forces of chronic lung inflammation have taken a heavy toll.

To find ways to detect and treat the disease early, when patients might benefit most, researchers need to understand the immune system's role in COPD, says Jeffrey L. Curtis, M.D., professor of internal medicine at the Medical School and chief of pulmonary and critical care medicine at the VA Ann Arbor Healthcare System.

Curtis and colleague Christine Freeman (Ph.D. 2006) recently found that excessive activity by dendritic cells — sentinel cells that activate the body's immune response — could be an important first step in the development of COPD. "If we could alter or stop the action of dendritic cells, perhaps we could stop the disease from progressing," says Curtis. —SP

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Jeffrey Curtis and Christine Freeman

AUSTIN THOMASON, U-M PHOTO SERVICES

In the Clinic

Anatomy of a Pandemic

H1N1 brought surprises, challenges to the Health System's response

Shon Dwyer

IT'S NOT THAT THE HEALTH System wasn't prepared for the H1N1 influenza pandemic. By the time the virus spread to the U.S. in April 2009, officials had spent years developing plans to handle major pandemics that would challenge all aspects of operations.

But when the first H1N1 patients arrived in May, they didn't present like seasonal flu patients. They didn't come only to the ER; they arrived as emergency transfers from other hospitals. And they weren't just sick; they were dying.

"We expected an overrun of mild to moderately sick people with some progressing to inpatient admission," says Shon Dwyer, R.N., the Health System's associate director of operations and ancillary services and incident commander for a team of physicians, nurses and experts charged with managing the response to the pandemic. "We were prepared for problems in ambulatory care clinics, the ER and inpatient areas. What we didn't expect were desperately ill people requiring long stays in the ICU."

The first patient arrived via Survival Flight and was admitted to intensive care with a diagnosis of severe acute respiratory distress syndrome (ARDS) from suspected bacterial pneumonia.

"At that point, no one knew that ARDS was a likely complication from H1N1," says Lena Napolitano, M.D., a professor of surgery who directs the surgical intensive care unit. "Everyone assumed there would be some serious complications, including respiratory failure, but we expected those in older patients."

After standard treatments didn't help, the ICU team put the patients on extracorporeal membrane oxygenation, or ECMO, a therapy developed by professor emeritus Robert Bartlett, M.D., in the 1960s for infants with acute respiratory failure. Similar to a heart-lung bypass machine, the device removes blood from the body, filters out carbon dioxide and adds oxygen before returning the blood to the patient's body. It's a life-saving, but risky, treatment reserved for desperately ill patients in heart or lung failure.

At the time of the first wave of the H1N1 pandemic, the U-M was the only hospital in Michigan with the equipment and ICU staff trained to care for adults with severe ARDS on ECMO. U-M is also a regional referral center for patients with ARDS. So it wasn't long before hospitals all over Michigan and Ohio started transferring more patients with severe ARDS to the U-M for critical care treatment.

"All of a sudden, we were dealing with this rapidly increasing adult population that needed ECMO," says Dwyer. "These were not traditional stays of five to seven days. These patients were on ECMO for three weeks at a time. They were consuming a lot of staff resources, and we found ourselves having difficulty meeting that need."

Back in the ICU, the team was beginning to realize that these patients were different from other ARDS patients. Their blood oxygen levels were lower and they had multiple organ failure. In spite of heavy doses of anti-coagulants, their blood kept clotting, yet their lungs had both bleeding and clotting. The rapid screening tests for the H1N1 virus were negative, but to be sure, the ICU team sent respiratory samples to the Centers for Disease Control for confir-

SCOTT GALVIN, U-M PHOTO SERVICES

mation. The tests came back positive for H1N1 influenza.

When the first ARDS patients arrived in intensive care, they weren't placed in isolation because no one knew they had H1N1. Now an entire team of physicians, residents, students, nurses, secretaries and clerks had been exposed to the virus. To protect them, the Health System gave them TamiFlu, a drug that helps prevent influenza infection.

"The first wave of H1N1 patients filled half of our ICU," says Napolitano. "At one point, we had four critically-ill patients on ECMO who required round-the-clock care. We were running out of infection control supplies and ECMO circuits." But, combined with the second wave which was to come, she adds, "We achieved a 70 percent survival rate in these severe ARDS patients."

In late summer, Shon Dwyer's job was to make sure the problems encountered with the first wave of H1N1 patients weren't repeated when the second wave hit in the fall. "In the time between wave one and wave two, we made some proactive investments including ICU staffing and ECMO, so we could care for everyone, even if we were full all the time," Dwyer says. "That strategy worked very well for us."

Other issues weren't so easily resolved. The Health System had patients — and 19,000 faculty and staff members, especially direct health care providers — who needed to be vaccinated against H1N1, but there was no vaccine. They had ordered 220,000 doses, but not one vial had arrived.

"When vaccine finally started showing up, it came in dribbles," says Dwyer. "We spent every morning figuring out what shipments were coming in and making sure each one was allocated according to CDC guidelines to a high-priority group."

When it took more than a week to get the results of outsourced H1N1 tests, Dwyer's group helped Duane Newton, Ph.D., associate professor of pathology, obtain the funds, equipment and staff needed to test samples in-house. When health care workers started running out of masks, despite aggressive supply chain management, Dwyer's team developed rules for mask conservation.

As H1N1 cases started to spike in October, the team worked with Sandro Cinti, M.D., associate professor of internal medicine and president of the Washtenaw County Medical Association, to establish uniform visitor restrictions at all county hospitals. When the emergency room couldn't handle the volume of sick children, the team called upon Ambulatory Care Services to set up a pediatric pandemic care clinic to manage the overflow.

After six months of daily meetings and being on call 24/7, Dwyer and her team are finally able to reflect on how the Health System coped with the H1N1 flu pandemic. It was much more challenging than anyone anticipated, but they are proud of the response to the crisis.

"In the face of adversity, we always focused on doing the right thing for the patient," Dwyer says. "When we said 'This is what we need to do to take care of these patients,' it happened and it happened quickly. Every level of the organization stepped up and did what needed to be done." —SALLY POBOJEWSKI

Reaching Out to Haiti

AFTER A JANUARY 12 EARTHquake struck Haiti, Ora Hirsch Pescovitz, M.D., U-M executive vice president for medical affairs, couldn't stop thinking about the urgent needs of the survivors. She asked Tony Denton, chief operating officer, to organize Health System volunteers to offer medical and humanitarian support. The response was overwhelming.

Within three weeks of the earthquake, 33 pallets of medical supplies had been shipped to Haiti. The Student Nurses' Association and other student groups held fund-raisers and donated the proceeds to relief programs. On January 21, a Survival Flight airplane and crew transported two injured patients to the U-M for specialized medical care.

More than 925 health care providers volunteered to serve in Haiti, if needed. On February 15, the first team left Ann Arbor to care for earthquake victims on the U.S. Navy's ship Comfort. Visit www.umhaitirelief.org to learn more about continuing relief efforts in Haiti. —SP

MORE ON THE WEB

ALBERT CAMPBELL/DREAMSTIME

In the Clinic

A Blood Test for GVHD

U-M RESEARCHERS HAVE identified a biomarker for graft-versus-host disease of the skin — a serious, often fatal, complication of allogeneic bone marrow transplants. The biomarker is a protein called elafin, which is produced in the surface layer of skin cells in response to inflammatory proteins involved in GVHD. Elafin levels can be measured in a simple blood test, which U-M hopes to make available to clinicians soon.

Allogeneic transplants, in which the patient's bone marrow cells are replaced with bone marrow cells from a donor, are a life-saving treatment for people with sickle cell anemia, leukemia, lymphoma, myeloma and other blood diseases. Rashes often develop after a bone marrow transplant. Sometimes rashes signal the onset of acute GVHD, but they also are a common reaction to antibiotics.

Until now, a skin biopsy was the only reliable way to determine whether these rashes were caused by antibiotics or GVHD of the skin, according to James Ferrara, M.D., the Ruth Heyn Professor of Pediatrics and Communicable Diseases and director of the Blood and Marrow Transplantation Program.

Physicians often prescribe high-dose steroids to suppress GVHD in all patients with rashes, but steroids can weaken a patient's already compromised immune system. "The blood test will make it possible for physicians to adjust therapy to the degree of risk, rather than treating every patient in exactly the same way," says Ferrara.

The research team also found that bone marrow transplant patients with high levels of elafin were three times more likely to die of complications than people with low levels, says Sophie Paczesny, M.D., Ph.D., an assistant professor of pediatrics and communicable diseases, and the study's first author. —SP

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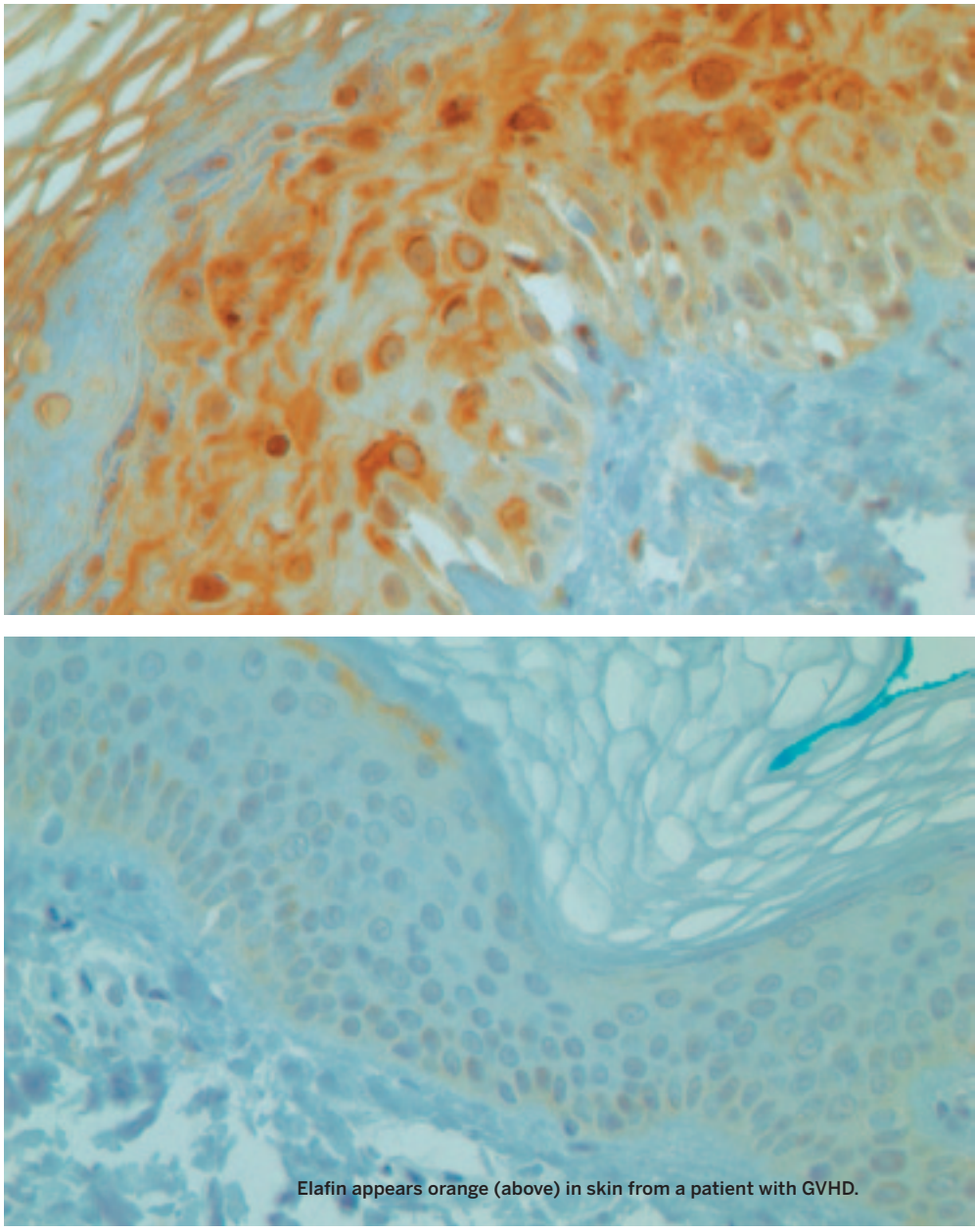

Elafin appears orange (above) in skin from a patient with GVHD.

BRYAN COFFING, M.D.

Jon Sekiya

SCOTT SODERBERG, U-M PHOTO SERVICES

New Joints from Old Bone

DO YOU SUFFER FROM A PAINFUL DISLOCATED SHOULDER? HAVE PREVIOUS operations failed to fix the problem? You could be a candidate for a new reconstruction procedure that uses cadaver bone and cartilage to essentially sculpt a new shoulder.

Jon K. Sekiya, M.D. (Residency 2001), an associate professor of orthopaedic surgery, and his research team analyzed defects in the humeral head, or ball part, of injured shoulder joints to determine how defects affect instability following standard surgical repairs. They found that even small defects and divots in the humeral head have biomechanical consequences that can affect joint stability. Standard surgical procedures stabilize the joint by tightening ligaments and repairing damaged tissue, but do nothing for bone defects.

"In situations where there's missing bone, the soft tissues experience forces much higher than they can withstand, and they fail," says Sekiya.

Using the new reconstruction technique, U-M surgeons match cartilage and bone from cadaver shoulder joints to the shape and consistency of the patient's joint and then attach the donor material to areas where the patient's own bone and tissue are missing. Sekiya says the procedure can stabilize shoulder joints with recurrent dislocations and after previous operations have failed. —SP

MORE ON THE WEB

Health Briefs

Scientists have known for years that people with low amounts of vitamin D in their bodies are more susceptible to heart failure — but why? A new study led by Robert Simpson, Ph.D., professor of pharmacology, has found a connection between heart disease and a genetic variant that alters an enzyme required to produce vitamin D hormone in the body. People who had high blood pressure and this genetic variant were twice as likely to develop congestive heart failure.

MORE ON THE WEB

U-M physicians say costly stress tests and beta blockers prior to surgery aren't necessary for most patients. New guidelines from the American College of Cardiology and the American Heart Association confirm that these medications and tests should be reserved for high-risk patients undergoing complicated surgeries.

MORE ON THE WEB

Despite advances in the diagnosis and treatment of heart disease, chances of surviving cardiac arrest outside a hospital haven't changed since the 1950s. A new U-M study found that only 7.6 percent of 142,740 people whose heart suddenly stopped beating outside a hospital survived. Providing CPR, cardiac defibrillation, or restoring a pulse on scene prior to transport were the most important predictors of survival. —SP

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