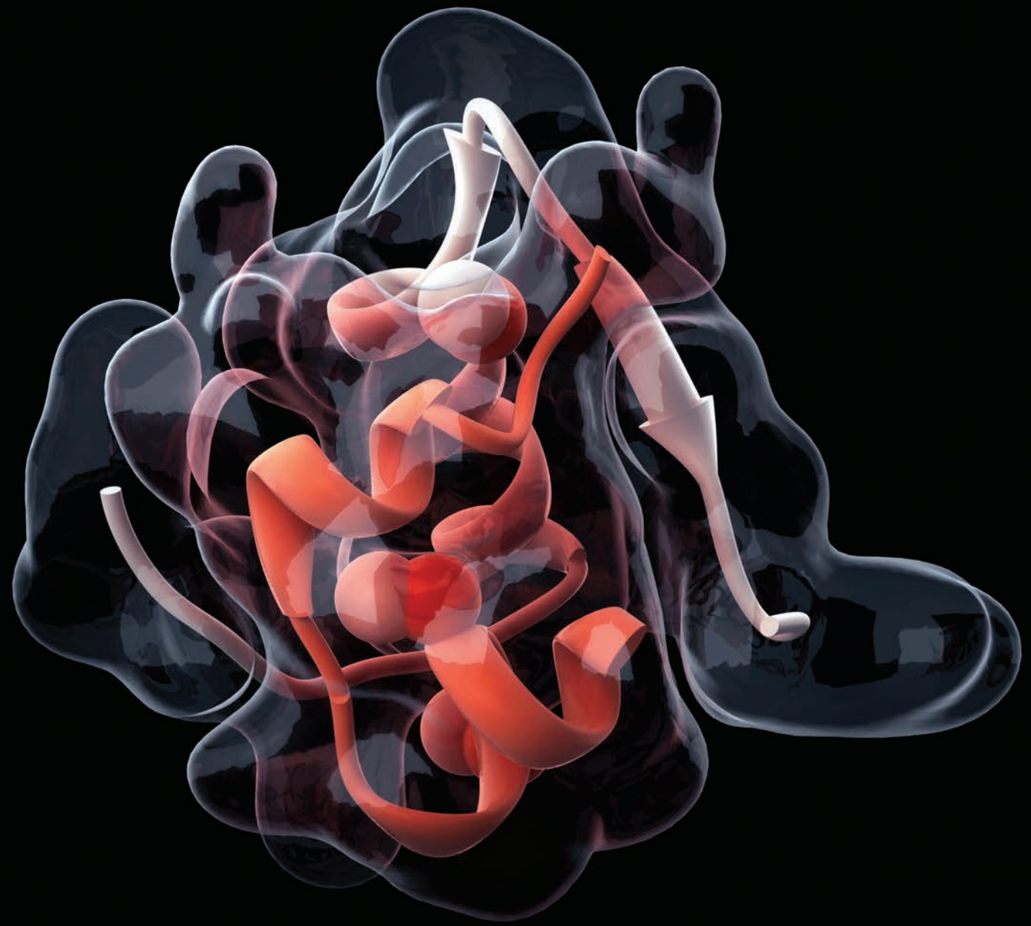

UNLOCKING THE SECRETS OF INSULIN

Discovering exactly how insulin interacts with cells could point researchers toward new, improved treatments for diabetes and other diseases.

BY ALEXANDER GELFAND

When Case Western Reserve's Michael Weiss, MD, PhD, first began investigating insulin as a resident at the Brigham and Women's Hospital in Boston in 1985, an adviser at Harvard Medical School tried to warn him off the topic. The adviser suggested that studying a protein whose mysteries already had been solved risked impairing the young scientist's career.

Fortunately, he didn't listen.

Earlier this year, Weiss and an international team of researchers produced the first 3-D image of the hormone-receptor complex to discover how insulin binds to the surface of cells. Their findings could lead to safer, more effective forms of insulin and possibly eliminate the need for insulin injections, carrying profound implications for diabetes patients.

Discovered in 1921, insulin has been used to treat diabetes since 1922 and is taken by a large proportion of the 25.8 million Americans with the disease. Researchers began mapping its molecular structure—the key to understanding how the hormone functions in the body—in the late 1960s.

When Weiss entered the field nearly three decades ago, most people thought insulin's structural questions had been answered. However, Weiss says, "Most of what we thought we knew about insulin in 1985 turned out to be incomplete—or even wrong."

A hormone and its receptor, a large and specialized molecule on the surface of many cells, fit together like a key and lock. When the key finds the lock, it turns

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something on; in the case of insulin, it tells our cells to absorb sugar (glucose) from our blood for use or storage as energy.

Understanding exactly how a hormone attaches to its receptor makes it possible to develop improved versions of the hormone that can be used to treat patients more effectively. Weiss has spent much of the past 25-odd years helping understand the structural basis of how insulin really works. In a paper published in January in the journal *Nature*, he and his colleagues unveiled the 3-D image, getting them one step closer to understanding it all.

A PATH LESS TRAVELED

When Weiss began his research, diabetes was hardly a hot topic. Type 2 diabetes, which is linked to obesity, had yet to emerge as a global epidemic that disproportionately affects underprivileged and minority communities in the United States, and researchers believed they had already determined the structure of insulin at the atomic level. If not for a supportive mentor, Weiss, who is now Cowan-Blum Professor of Cancer Research, chair of the Department of Biochemistry and a professor of biochemistry, biomedical engineering and medicine at Case Western Reserve, might never have bothered with insulin at all. But the late physicist Leo Neuringer, PhD, with whom Weiss studied while working on his doctorate at MIT, had a son with Type 1 diabetes. Neuringer encouraged anyone who would listen to study the only medication used to manage it. For years, Weiss says, he was teased by his peers at the MIT Magnet Laboratory for being the only person foolish enough to answer Neuringer's call.

It was a wise move—and a lucky one. A fellow resident, Steve Shoelson, had done insulin research at the University of Chicago while working on his own MD and PhD. He introduced Weiss to a network of senior scientists, including Chicago biochemist Donald Steiner, MD, who were trying to get a better handle on the protein's workings. By 1991, Weiss and Shoelson began to make progress of their own. Key encouragement to their efforts was provided by Eugene Braunwald and Marshall Wolf at the Brigham & Women's Hospital and by Martin Karplus, who was Weiss' PhD mentor in the Department of Chemistry at Harvard University.

A NEW APPROACH

Weiss and Shoelson, now head of the Section on Pathophysiology and Molecular Pharmacology at the Joslin Diabetes Center in Boston, used a molecular imaging technique called nuclear magnetic resonance (NMR) spectroscopy to discover that a mutant insulin was active even though a portion of the hormone was disordered and detached—findings that didn't mesh with the established crystal structure of insulin. Weiss and Shoelson's work—published in *Nature* in 1991 with coworkers Q.X. Hua, PhD, and M. Kochoyan, PhD—provided evidence that scientists had unknowingly been studying an inactive shape of insulin. A complementary study also in 1991 by the late English structural biologist Guy Dodson, DPhil, at the University of York, showed that an inactive analog could retain the classical structure. Although the classical crystal structures provide insight into how the hormone is stored in the pancreas, it was a misunderstanding that may have stymied progress in insulin therapy for many years.

That realization sparked a multi-year international project. Key progress was made in the biochemical and structural understanding of the insulin receptor by a team in Melbourne, Australia, led by Colin Ward, PhD, and Michael Lawrence, PhD. The team's goal was to unlock the mysteries of the active shape the molecule takes when it is released into the bloodstream so it can bind to its receptor—a project that involved the coordinated efforts of Weiss, Jonathan Whittaker, MD, associate professor in the Department of Biochemistry at Case Western Reserve School of Medicine, Steiner, Dodson—who died just a few weeks before the recent publication—and others as far afield as the Czech Republic.

The new structure reveals an intricate dance between insulin, a small protein made up of two delicately twisting strands of amino acids, and its much larger receptor, a "transmembrane" protein that sits partly outside of a cell and partly inside it.

The group combined a refined form of X-ray crystallography with cutting-edge computational methods (led by Lawrence) and painstaking biochemical experiments by Weiss and Whittaker. Their efforts not only confirmed some prior hypotheses, but also revealed surprising results. Among other findings, Weiss, Lawrence and their colleagues demonstrated that both the insulin molecule and the extracellular

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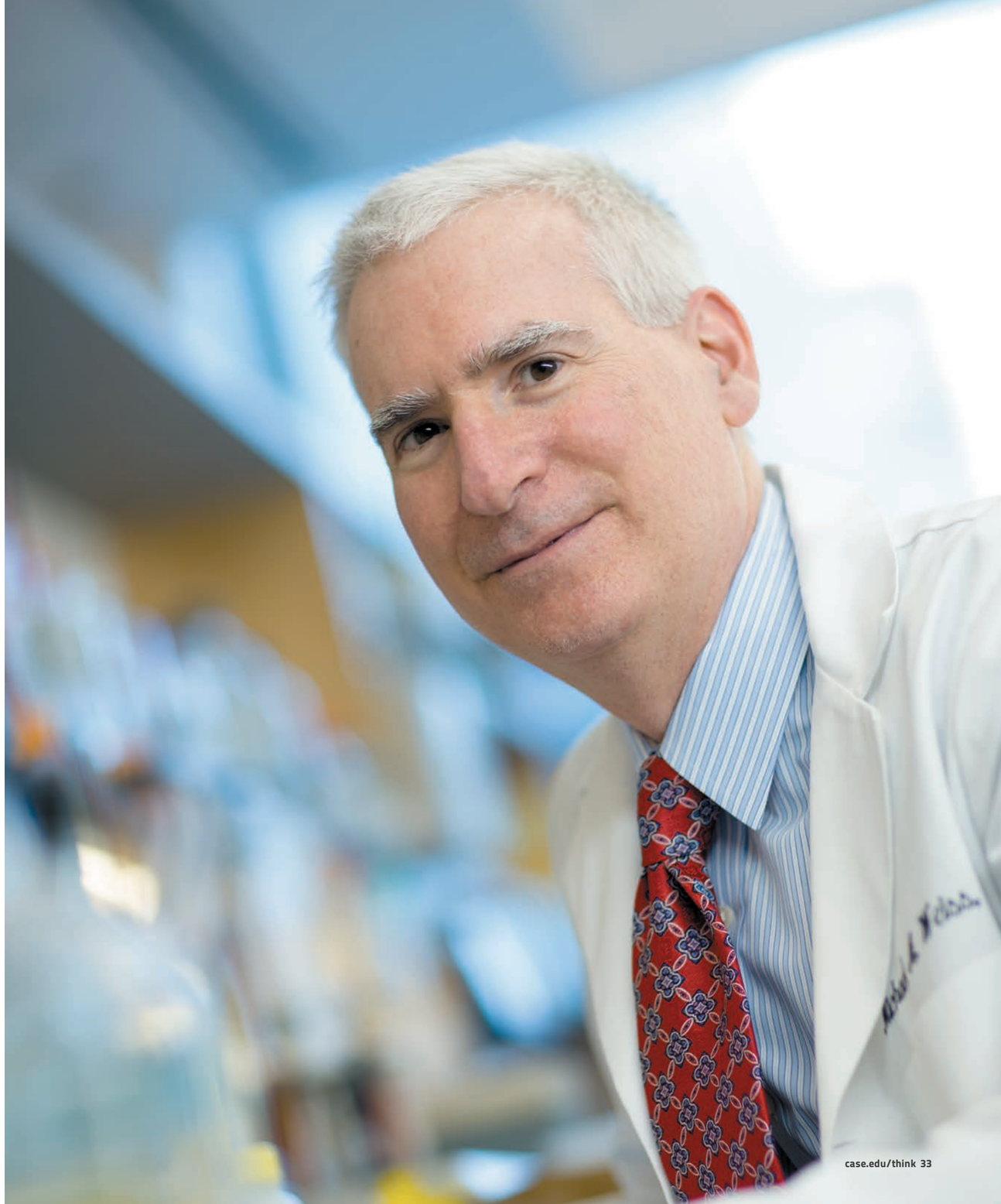

Insulin Therapy is
Tricky Business

Under normal circumstances, our bodies produce only as much insulin as we need, pumping out more of the hormone as our blood sugar rises during a meal and throttling back production as it falls. But people with diabetes who rely on insulin injections cannot match nature's finesse. No matter how carefully they monitor their blood glucose and adjust their dosages, their glucose levels can either rise too high, leading to hyperglycemia (which can lead to serious complications), or fall too low, leading to hypoglycemia (which can result in coma).

Scientists began tinkering with additives to formulations of animal insulins in the 1930s and 1940s to render its effects more predictable. Their ability to create modified forms of the human hormone rapidly accelerated with the advent of genetic engineering in the 1980s. Today, fast-acting insulin analogs can be administered around mealtimes to handle temporary spikes in blood sugar, as well as long-lasting ones to supply a steady background level of the protein. Some people with diabetes also use insulin pumps—cellphone-sized devices that can be worn on a belt or against the skin—to deliver insulin through a catheter, avoiding the need for multiple daily injections by syringe.

Nonetheless, navigating the shoals of hyper- and hypoglycemia remains difficult, even as clinical trials led in part by endocrinologists at Case Western Reserve School of Medicine have proven that maintaining tight control over blood glucose is critical to avoiding long-term complications.

One solution lies in next-generation "smart pumps," designated as the artificial pancreas, that continuously monitor a patient's blood glucose and use software algorithms to deliver just the right amount of insulin on demand. For maximum performance, however, these pumps will require much faster-acting insulin analogs than those currently on the market. Analogs that are less temperature-sensitive than ordinary insulin also would be helpful. The original form must be refrigerated and spoils when exposed to extreme heat or cold. Ultra-concentrated forms of both long-lasting and short-acting insulin also would help in treating those who require very large doses of the hormone, such as obese Type 2 patients with extremely high insulin resistance. Case Western Reserve School of Medicine's Michael Weiss, MD, PhD, has been developing analogs like these for years. Now, with a better understanding of how insulin binds to its receptor, he and his colleagues hope to make further progress even more quickly.

portion of its receptor rearrange themselves upon binding. During that process, the molecule and the receptor change their shapes in subtle ways as they embrace one another at the atomic level. For Weiss, the weeks leading up to the final results were nerve-racking, partly because he'd relied on relatively untested experimental procedures.

"If something's been done a thousand times," he says, "then you're confident the technique works. But if it's a new approach, you get nervous."

In the mid-'90s, Stevan Hubbard, PhD, professor of biochemistry and molecular pharmacology at New York University, had described the structure of the receptor portion that resides inside the cell. He relates in a *Nature* article earlier this year that a structure of the outside portion of the receptor had been reported in 2006 by Weiss' Australian collaborators, but no one had managed to capture an image of the entire receptor—or even a piece of it—with an insulin molecule docked to it. To achieve the unprecedented resolution needed for this picture, the international team had dissected the primary hormone-binding portion of the receptor and relied on some recent advances in crystallographic techniques.

The picture of the interaction remains incomplete. Despite their breakthrough, Weiss and his colleagues still had to settle for a partial view. Yet Hubbard and other leading researchers call the latest results a major leap forward. In fact, Hubbard says the work opens new possibilities for engineering enhanced forms of insulin.

TARGETING BETTER TREATMENTS

For years, Weiss has been using computers to design altered forms of insulin in hopes of improving diabetes treatment. In 2009, he founded a company, Thermalin Diabetes LLC, to commercialize several of these insulin analogs. The Thermalin pipeline includes ultra-concentrated forms of insulin for patients who require especially large doses of the hormone; an ultra-rapid-acting form for use in the insulin pumps employed by some patients; and a heat-stable version of the protein for use both in pumps and in developing parts of the world where the electrical grid is incomplete and refrigerators are rare.

With a new model of insulin and its receptor in hand, Weiss is now working on a paper to explain exactly how his ultra-stable, fast-acting analog works, and he already has begun revisiting its design. In time, he hopes to use the model to develop more and better analogs.

But this latest breakthrough in insulin research has implications that go beyond diabetes. For example, a special form of the insulin receptor is found in the brain. Tony Hollenberg, MD, chief of the Division of Endocrinology, Diabetes and Metabolism at the Harvard-affiliated Beth Israel Deaconess Medical Center in Boston, notes that evidence exists that insulin resistance, the underlying cause of Type 2 diabetes, also contributes to Alzheimer's and other neurodegenerative disorders. Weiss, who watched his grandmother succumb to dementia, would like to create an insulin analog that would bind better to the receptor in the brain and stave off the illness. In addition, a substance called insulin-like growth factor (IGF) has been linked to certain kinds of cancer. Insulin and IGF are very similar, and their receptors are closely related—so much so that they can swap partners, insulin binding to the IGF receptor, and vice versa. As a result, a better understanding of how insulin binds to its own receptor could offer researchers clues about how to go about finding new cancer treatments.

Weiss considers it a privilege to work with an international team on a project that benefits society, even as it advances science. Now that a lifetime's worth of research is finally starting to bear fruit, his mounting optimism is almost palpable. "The next five years," he says, "are going to be even more exciting than the last five." ⌑

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What is
Diabetes?

Diabetes is a group of disorders characterized by elevated levels of blood glucose that arise when the body either can't produce enough insulin or no longer responds to it properly.

Type 1 diabetes accounts for roughly 5 percent of all diagnosed cases of diabetes in adults; Type 2 diabetes accounts for 90 to 95 percent of the remainder. Symptoms of either type of diabetes, when untreated, include fatigue, blurred vision and increased thirst and urination. Serious long-term complications range from increased risk of cardiovascular disease to blindness and kidney failure.

In Type 1 diabetes, the body's own immune system destroys the pancreatic cells that produce insulin. Once known as "the wasting disease," prior to the introduction of insulin therapy in the 1920s, the disease was "100 percent fatal," says George Grunberger, MD, a former researcher in the Diabetes Branch of the National Institutes of Health and founder of the Comprehensive Diabetes Program at Wayne State University.

Type 2 diabetes, which is closely linked to obesity and lack of exercise, results from insulin resistance; although the body continues to produce insulin, its cells no longer respond to the hormone as they should. In the United States, the burden of Type 2 diabetes falls disproportionately within underprivileged communities, including minorities and women. The Department of Health and Human Services reports that African Americans are twice as likely as non-Hispanic whites to be diagnosed with diabetes, and Latinas are 17 times more likely to die from the disease than non-Hispanic white women.

Adverse changes in diet and lifestyle also are spurring an epidemic of Type 2 diabetes in the developing world. The World Health Organization estimates that more than 80 percent of diabetes deaths occur in low- to middle-income countries, and it expects total deaths to rise by more than 50 percent over the next 10 years. While early-stage Type 2 diabetes can be controlled by diet and exercise, about one-third of patients require supplemental insulin.