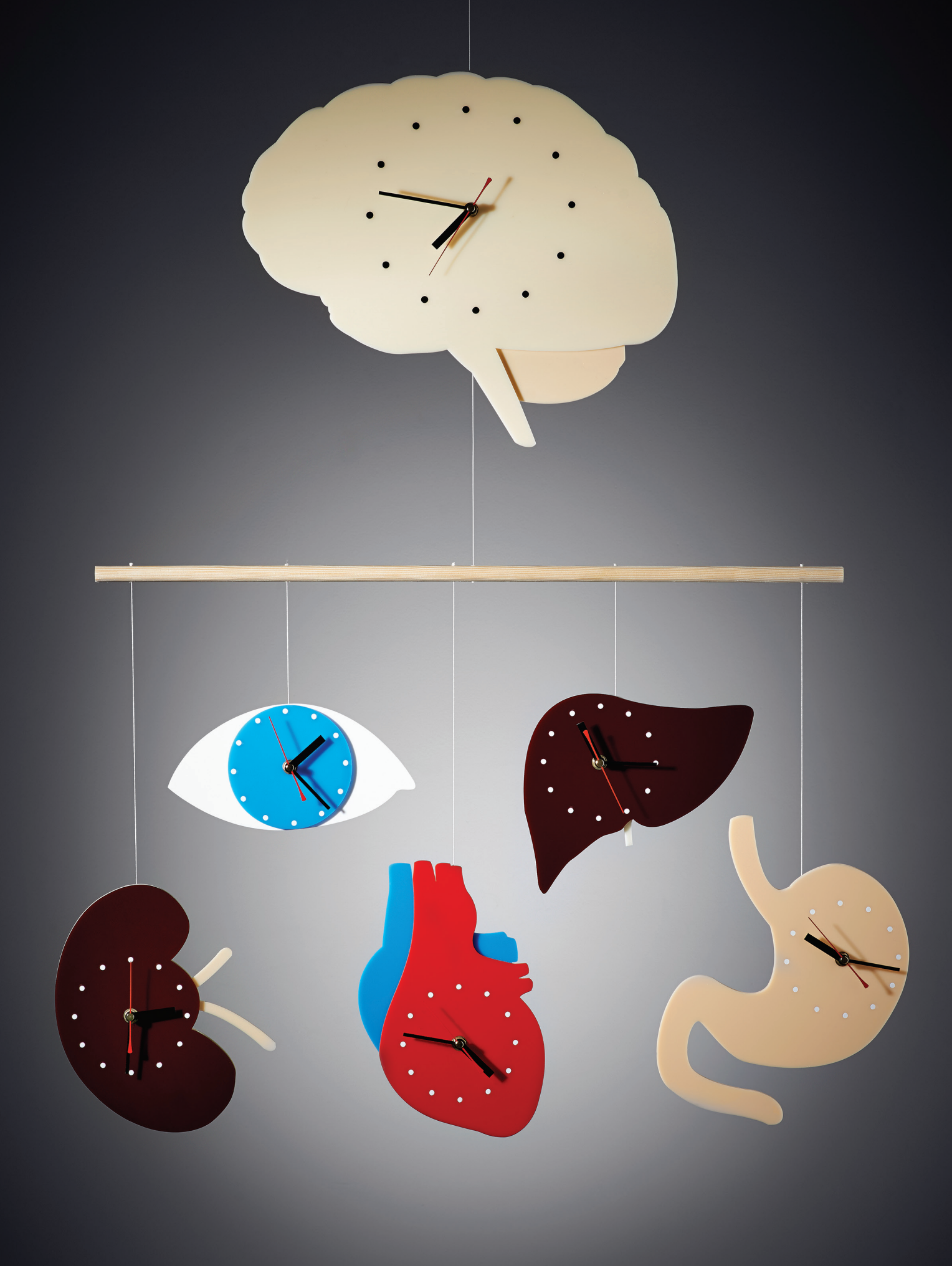

CHRONOBIOLOGY

THE CLOCKS WITHIN US

Genes in the liver, pancreas and other tissues (not just the brain) keep the various parts of the body in sync. Timing miscues may lead to diabetes, depression and other illnesses

By Keith C. Summa and Fred W. Turek

Illustration by Kyle Bean

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ANYONE WHO HAS EVER FLOWN EAST OR WEST AT 500 knots for more than a few hours has experienced firsthand what happens when the body's internal clock does not match the time zone in which it finds itself. Up to a week may be needed to get over the resulting jet lag—depending on whether the master clock, which is located deep inside the brain, needs to be advanced or slowed to synchronize when the body and brain want to sleep with when it is dark outside. Over the past several years, however, scientists have learned, much to our surprise, that, in addition to the master clock in the brain, the body depends on multiple regional clocks located in the liver, pancreas and other organs, as well as in the body's fatty tissue. If any one of these peripheral clocks runs out of sync with the master clock, the disarray can set the stage for obesity, diabetes, depression or other complex disorders.

The two of us have dedicated ourselves to exploring the ins and outs of how these peripheral clocks work and to identifying the genes that regulate their activity. The first clock gene was isolated, or cloned, from fruit flies in 1984. One of us (Turek) was part of the team that in 1997 cloned and identified a different clock gene, the first discovered in mammals. As of the last count, researchers around the globe have identified dozens of genes that help the body keep time, including those going by such names as *Clock, Per* (for period) and *Tim* (for timeless).

Studies in our laboratory have focused on mice, but circadian clock genes have been identified in an amazing range of living organisms, from bacteria to fruit flies to humans. Many of these genes appear to be similar in a wide range of species—a sign that they have been central to survival throughout evolution.

The greatest progress so far has come in deciphering the role of clocks in disorders of metabolism, which is the set of processes by which the body converts food into energy and stores fuel for later use. (Among the more surprising finds: when you eat appears to be as important as what you eat in the regulation of weight gain.) Circadian rhythms do not explain every aspect of these complex conditions, of course, but we ignore our body's various clocks at our peril. Rapidly growing knowledge of these rhythms could radically change the ways diseases are diagnosed and treated in the future and improve people's ability to maintain their health.

MASTER CLOCK

FROM THE MOST COMPLEX organisms to the simplest ones, all of life on earth is governed by circadian rhythms that match the 24-hour day. Circadian rhythms are found even among the earliest lifeforms to emerge: cyanobacteria, single-celled blue-green algae now widespread throughout diverse habitats. These organisms derive energy from the sun through photosynthesis, using light to power the production of organic molecules and oxygen from carbon dioxide and water.

An internal clock enables each cyanobacterium to prime its photosynthetic machinery before sunrise, which enables it to start harvesting energy as soon as light starts to shine and gives

IN BRIEF

Embedded deep within the brain is a master clock that regulates the timing of many of the biological processes that occur in the human body.

Researchers have shown over the past few years that cellular (or regional) clocks can be found in the liver, pancreas and other parts of the body as well.

Routinely eating or sleeping at the wrong times may throw these peripheral clocks out of sync with the master clock in the brain.

A growing body of evidence suggests that these chronobiological disruptions predispose individuals to the development of obesity, diabetes, depression and other disorders. Resynchronizing the body's many clocks may, in the coming years, help to restore health and proper functioning.

PAGE 50: PHOTOGRAPH BY MITCH PAYNE

it a leg up on cellular organisms that merely respond to light. Similarly, the clock enables the cyanobacteria to turn off photosynthesis when the sun sets. In this manner, they can avoid wasting energy and other resources on systems that do not work at night. Instead resources can be diverted to reactions better suited for darkness, such as DNA replication and repair, which may be compromised by ionizing radiation from the sun's rays.

Bacterial strains carrying mutations in different clock genes may switch from the usual 24-hour cycles for turning genes on and off to periods, or "clock lengths," of 20, 22 or sometimes even 30 hours. In studies that grouped cells according to their altered cycles, Carl Johnson and his colleagues at Vanderbilt University showed in 1998 that cyanobacteria with a clock length that matched the environmental light cycle outcompeted those with a mismatch. For example, in a 24-hour light-and-dark cycle, normal cyanobacteria grow more quickly and divide more successfully than mutants with a 22-hour clock length. But when Johnson's team artificially set the light-and-dark cycle to 22 hours, those same mutants survived better than the normal bacteria. These experiments demonstrated clearly, for the first time, that the ability to properly coordinate internal metabolic rhythms to environmental cycles enhances fitness.

Although the human clock mechanism depends on different genes from those found in cyanobacteria, our circadian machinery shares many other similarities with that of these blue-green algae, suggesting that both processes arose separately during evolution to address the same biological needs and functions.

PERIPHERAL CLOCKS

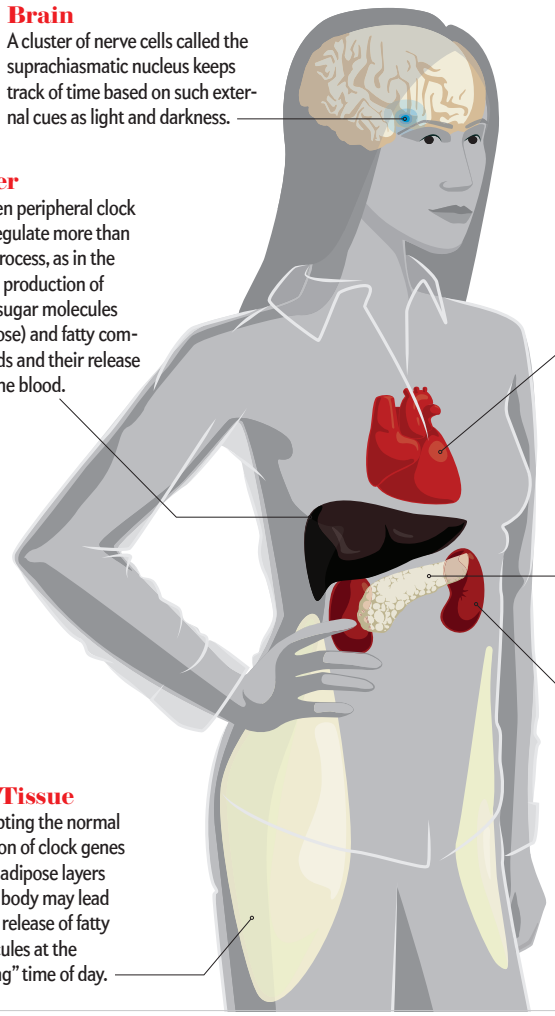
RESEARCHERS ORIGINALLY assumed that there was but a single clock that acted like a metronome and regulated myriads of biological processes throughout the body. In the 1970s they traced this putative clock to the suprachiasmatic nucleus of the brain, just above where the optic nerves cross. But about 15 years ago signs began emerging that subordinate timing mechanisms existed in other organs, tissues and individual cells as well. Investigators started finding evidence that the same clock genes that were active in the brain were periodically turning on and off in the individual cells of the liver, kidneys, pancreas, heart and other tissues. These cellular clocks, we now know, regulate the activity of 3 to 10 percent—and in some cases perhaps as much as 50 percent—of genes in various tissues.

At about the same time, a number of scientists began wondering whether circadian rhythms played any role in the process of aging. Turek asked Amy Easton, then a graduate student at Northwestern University, to conduct a few experiments on mice that had mutations in the *Clock* gene. While examining daily running behavior in older mice, she realized that they tended to be fat and to have difficulty climbing into the running wheels in their cages. This observation inspired us to focus some of our research efforts on metabolism and circadian rhythms. In a series of tests, published in *Science* in 2005, we demonstrated a relation between alterations in the *Clock* gene and the development of obesity and metabolic syndrome, which is a cluster of physiological abnormalities that puts individuals at higher risk of heart disease and diabetes. To receive a diagnosis of metabolic syndrome, a person must experience at least three of the following conditions: excess fat in the abdominal area, as opposed to on the hips; high amounts of triglyceride fats in the

HOW IT WORKS

The Body's Many Cellular Clocks

Life on earth moves to the rhythms of a 24-hour day. In humans, a master clock in the brain synchronizes subordinate clocks in various cells of the body. Specifically, certain genes direct the production of proteins at different times of day, which ramp up or inhibit biological processes. Health problems may occur if these clocks fall out of sync.



Brain
A cluster of nerve cells called the suprachiasmatic nucleus keeps track of time based on such external cues as light and darkness.

Liver
A given peripheral clock can regulate more than one process, as in the liver's production of both sugar molecules (glucose) and fatty compounds and their release into the blood.

Heart
Clock genes signal the heart before dawn to prepare it for the rigors of being awake. This daily nudge may help explain why so many heart attacks occur early in the morning.

Pancreas
Clocks in different tissues can balance one another, allowing, say, insulin from the pancreas to modulate the glucose produced by the liver and ingested from food.

Kidney
The retention and release of such substances as sodium, potassium and chloride (which help to regulate blood pressure) are controlled by clock genes in the kidney.

Fat Tissue
Disrupting the normal function of clock genes in the adipose layers of the body may lead to the release of fatty molecules at the "wrong" time of day.

Illustration by Josy Conklin

blood; low levels of HDL, the so-called good cholesterol, in the blood; high blood pressure; and high levels of glucose in the blood (indicating a difficulty in processing sugar).

This work triggered an explosion of interest in the effects of circadian rhythms on metabolism. Previous studies of shift workers—who experience chronic misalignment between their internal clocks and the solar day—had shown that they have a greater risk of developing metabolic, cardiovascular and gastrointestinal diseases, among others. But shift workers commonly exhibit other unhealthy behaviors, such as insufficient sleep, poor diet and lack of exercise. Thus, researchers had trouble distinguishing between cause and effect. By providing genetic evidence linking the internal clock and metabolic health, the *Clock* mutant mice helped to propel the study of circadian rhythms into a more precise, molecular era that allows more definitive conclusions.

CLOCKS AND METABOLISM

SOON AFTER investigators realized that circadian rhythms help to regulate metabolism, they began studying the peripheral clock found in the liver, which plays a pivotal role in metabolism. In 2008 Katja Lamia, Kai-Florian Storch and Charles Weitz, all then at Harvard Medical School, conducted experiments using mice in which a critical circadian clock gene had been deleted only in liver cells. (Unlike people, mice are active primarily at night and sleep during the day, but the sleep-wake cycle is otherwise similarly regulated.) In essence, these mice had no clock in the liver and normal clocks elsewhere in the body. During their daytime rest period (when mice do not eat as much), they experienced extended bouts of low blood sugar levels, or hypoglycemia. This drop is dangerous because the brain can begin to shut down within minutes if it does not receive enough glucose to meet its energy demands.

Further experiments showed that the low glucose levels occurred because the rhythms that usually control when the liver produces and secretes the sugar molecule into the blood had disappeared. Thus, the liver clock contributes to the maintenance of normal blood glucose levels over the course of the day, ensuring a constant and adequate source of energy to support the ongoing functions of the brain and the rest of the body.

Not surprisingly, an opposing counterregulatory system is required to limit excessive blood glucose in response to feeding. The primary hormone responsible is insulin, which is produced by so-called beta cells, found in the pancreas. After a person eats a meal, glucose enters the bloodstream, triggering the secretion of insulin. This hormone, in turn, acts like a brake on rising sugar levels by promoting the removal of glucose and its storage in the muscles, liver and other tissues.

As a follow-up, Billie Marcheva and Joseph T. Bass (an original member, along with Turek, of the circadian metabolism research team at Northwestern) carried out a series of studies to determine the role of the biological clock in the pancreas. They found that the pancreatic clock is critical to maintaining normal blood sugar levels and that disruption of the clock severely compromises pancreatic function, resulting in diabetes. Diabetes is a metabolic disorder in which the body produces too little insulin or is insensitive to it. Too much sugar ends up locked out of cells and floating in the bloodstream.

Marcheva and Bass began by examining isolated pancreatic tissue from mice that had mutations in circadian clock genes. They saw that the amount of insulin secreted in response to glucose stimulation was reduced drastically. Next, they generated mice in which the clock was deleted only in the pancreas. The animals developed diabetes early in life and exhibited a profound reduction in insulin secretion.

Rapidly growing knowledge of circadian rhythms could radically change the way diseases are diagnosed and treated in the future.

These examples illustrate a key point about the function of clocks in different tissues: they may have drastically different roles. In cases such as the liver and pancreas, they even regulate opposing physiological processes. Yet when they are integrated into a functioning system, these tissue clocks precisely synchronize their timing to maintain the body's homeostasis; that is, they provide for relatively stable levels of key molecules in the face of varying conditions in the external environment. Taken a step further, the master circadian clock can be conceptualized as a conductor of an orchestra that keeps multiple peripheral tissues—the instruments—properly timed relative to one another and to the environment, thus optimizing the function of the system.

MULTIPLE ROLES

ANOTHER OVERARCHING discovery is that the clock in a given tissue can affect more than one process in that tissue. Indeed, each clock can regulate multiple processes. For example, the liver clock regulates entire networks of genes necessary for the production and metabolism of glucose. In addition, in 2011 Mitch Lazar of the University of Pennsylvania and his colleagues demonstrated that the liver clock also determines how much fat accumulates in liver cells.

In this instance, Lazar and his co-workers determined that a clock gene called *Rev-erbα* acts like a timer for an enzyme that controls access to the genetic instructions found within the DNA molecule. The target enzyme in question—histone deacetylase 3 (HDAC3)—affects the process by which certain strands of DNA are wound into coils so tight that the hereditary information inside cannot be used by the cell to drive its biological processes.

Using a genetic trick, Lazar and his team showed that blocking the *Rev-erbα* clock gene, which in turn prevented HDAC3's activity, resulted in the development of a condition known as hepatic steatosis, or fatty liver. It turns out that one of HDAC3's functions is to turn off the genes that control the production of fatty

molecules during the night (when mice are active and need to use their fat stores for energy). The loss of the clock gene causes the amount of HDAC3 to decline, which in turn leaves the genes responsible for the synthesis of fats in the liver stuck in the on position. This hyperactivity, in turn, causes abnormal accumulation and deposition of fat (adipose) in liver cells, a process that disrupts liver function and commonly accompanies obesity and diabetes.

Clock genes also function in adipose tissue and affect multiple metabolic processes from there. In addition to serving as an energy storage depot, fat functions as an endocrine organ through its production of the hormone leptin; that is, it secretes hormones into the blood that alter the activities of other organs in the body. Georgios Paschos and Garret FitzGerald, both then at the University of Pennsylvania, and their colleagues recently engineered mice lacking an intact clock in fat cells (adipocytes) and found that the animals developed obesity and shifted their normal patterns of food intake to the daytime. As a result, fatty molecules were coursing through their body at the "wrong" time, disrupting their brain's ability to regulate the timing and intake of food. This change in feeding behavior appears to be specific for animals lacking an adipocyte clock because mice with deleted pancreatic or liver clocks retain normal feeding rhythms.

The observation that these animals shifted their feeding patterns and gained excess weight without clocks in adipocytes agrees with prior studies demonstrating that the timing of food intake can have a significant effect on how efficiently the body stores and utilizes the fuel it consumes. Indeed, in 2009 Deanna Arble, then a graduate student working with us at Northwestern, reported that mice given access to a high-fat diet only during the "wrong" time of day gained significantly more weight than animals fed the same diet only during the dark phase. These weight differences persisted despite similar overall caloric intake and physical activity in each group.

More recently, Satchidananda Panda and his group at the Salk Institute for Biological Studies in La Jolla, Calif., have extended these findings, showing that restricting intake of a high-fat diet in mice to an eight-hour window during their normal time for eating (the dark phase) prevented obesity and metabolic dysfunction without any reduction in caloric intake. In fact, these animals had metabolic health profiles similar to mice that ate just a low-fat diet. The benefit appears to stem from improved coordination of the metabolic cycles in the liver and other tissues.

Interestingly, these experiments in mice may be relevant for individuals with night eating syndrome, a disorder in which people consume an overabundance of calories at night and develop obesity or metabolic syndrome, or both. Perhaps this condition arises in part from a defect in regulating the circadian timing of hunger, an asynchrony that could predispose patients to weight gain and the misregulation of their metabolic processes.

Recently a study of dieters led by Marta Garaulet of the University of Murcia in Spain and Frank Scheer of Harvard found an association between the timing of lunch and success with weight loss. Individuals who had an earlier lunch tended to lose more weight while dieting than those who ate later. More clinical research must be done on whether eating times influence the development of obesity, diabetes and related conditions, but such findings raise the possibility that circadian feeding strategies might one day serve as entirely new, nonpharmacological complements to standard treatment regimens.

CIRCADIAN MEDICINE

OTHER WORK with humans suggests that detailed investigation of people's circadian rhythms may one day produce greater insight into their metabolic disorders, leading to more appropriate treatments. For example, Till Roenneberg and his colleagues at the Ludwig Maximilian University of Munich have studied the sleeping patterns of thousands of people around the world and described a common form of chronic circadian disruption they refer to as "social jet lag." Representing the time difference between habitual sleep cycles during the work (or school) week and free time on the weekends, this measurement provides a quantification of the weekly disruption of the internal clock, which may be equivalent to traveling across three to four time zones twice a week for someone who wakes at 6 A.M. on weekdays and sleeps until 9 or 10 A.M. on weekends. The researchers have discovered a positive association between the magnitude of social jet lag and body mass index, suggesting that the disruption of circadian cycles contributes to weight gain.

In addition to delving further into understanding the mechanisms underlying the connection between clock genes and metabolic disorders, researchers have recently produced provocative results linking circadian rhythms with many other conditions. Ties have been found between circadian disruption and ailments of the heart and stomach, as well as various cancers, neurological and neurodegenerative diseases, and psychiatric illnesses—among others. Indeed, a handful of small studies suggest that, in some cases, disrupted sleep cycles may be a contributing cause—and not just an effect—of severe depression in people who are already prone to the illness. Similarly, experiments in mice and hamsters over the past five years have shown that conditions resembling chronic jet lag impair learning and memory and disrupt neuronal structures in certain regions of the brain.

Deeper understanding of the role that our internal clocks play in our body has the potential to revolutionize medicine. Taking into account knowledge of optimal clock function—such as when glucose production is best turned on and off in the course of 24 hours—could lead to the development of what we refer to as "circadian medicine." Physicians who are able to incorporate information about circadian rhythms and sleep-wake cycles most effectively into their diagnosis and treatment of disease will be better positioned, we believe, to improve health, prevent disease and maximize the benefits of the therapies that their patients require. SA

MORE TO EXPLORE

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