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A Healthy Internal Clock Keeps Weight Off

Staying up past bedtime, skipping meals, and snacking constantly all add up to weight gain, fatty livers, and high cholesterol levels for an unlucky group of mice whose internal biological clocks are genetically disrupted.

Researchers at Northwestern University and the Howard Hughes Medical Institute have identified wide-ranging molecular and behavioral changes in mice that have a faulty circadian system. In people, similar changes in body fat and metabolic activity are known as metabolic syndrome, which can lead to cardiovascular disease and type 2 diabetes.

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The research team, which included co-author Joseph S. Takahashi, a Howard Hughes Medical Institute investigator at Northwestern University, published its report on April 21, 2005, in *Science Express*, which provides rapid electronic publication of select articles from the journal *Science*. The study suggests a surprising new angle for understanding and eventually preventing and treating obesity and related disorders in people.

"Timing is critical to keep the metabolic symphony in tune," said corresponding author Joseph Bass, assistant professor of medicine and neurobiology at Northwestern University and head of the endocrinology and metabolism division at Evanston Northwestern Healthcare. Quoting Duke Ellington, Bass added, "It don't mean a thing if it ain't got that swing."

The mice have defective *Clock* genes that control daily rhythms in the brain and throughout the body, including sleeping and eating. The gene was discovered eight years ago by Takahashi's laboratory. Since then, Takahashi

and other researchers have shown that the *Clock* gene and a half-dozen other proteins run 24-hour oscillating clocks in most cells in the body and in a specific part of the brain that controls appetite and wakefulness. About 3-10 percent of the genes in any given tissue turn on and off in circadian rhythm.

The project started when circadian rhythm expert Fred Turek, lead author of the paper and professor of neurobiology and physiology at Northwestern, noticed that the *Clock* mutant mice gained more weight with age than other mice.

Experiments soon revealed the cause. Mice with the mutant *Clock* gene ate more than normal mice, so they gained more weight, especially on a high-fat diet, which was evident within six weeks of birth. The chubby *Clock* mutants put on about as much extra weight as did the normal mice that were switched to a high fat diet.

The *Clock* mutant mice lost both their alarm clocks and their internal dinner bells. Mice typically sleep during the day and then eat a meal at the beginning and at the end of their active nocturnal day, akin to breakfast and dinner. Instead, the *Clock* mutant mice skipped their meals, stayed awake far into the usual rest time, and snacked often.

The insomniac mice also were a little more sluggish, as measured by infrared sensors in their cages. The researchers removed the exercise wheels normally used to gauge mouse activity, because regular spins can help the mice reset their biological clocks, just as a daily walk might help a person sleep better at night.

In repeated round-the-clock measurements, the researchers found signs of further trouble emerging in the mice's early adult months. The circadian-challenged mice developed high cholesterol, high triglycerides, high blood sugar, low insulin, bloated fat cells, and lipid-engorged liver cells. Some of these changes appeared to be independent of the weight gain, Bass said.

Using sensitive techniques in Takahashi's lab, the researchers found changes in the key proteins in the hypothalamic region of the brain that manages feeding, energy balance, and sleep-wake regulation. Takahashi and his colleagues suspect the metabolic changes are caused more directly by misregulated genes in various tissues normally controlled by the *Clock* gene, rather than by the effects of the weight gain.

"It's like an orchestra," said Bass, a former HHMI postdoctoral fellow. "The tissues important in metabolism have to be conducted properly. But in the *Clock* mutant, each tissue plays to its own beat, which creates cacophony at the biological level that sets up the animal for obesity and metabolic disregulation."

"The *Clock* finding reinforces the idea of important interactions between circadian rhythms, sleep and metabolism," said Emmanuel Mignot, an HHMI investigator at Stanford University. He and his colleagues recently reported

that even partial sleep deprivation changes the blood levels of several appetite-regulatory hormones, including leptin and Ghrelin, an effect likely to increase food intake and obesity in the general population. "The study also shows the importance of working across multiple scientific disciplines—in this case, circadian rhythm and energy metabolism," Mignot noted. "Indeed, who would have considered *Clock* as a metabolic candidate gene?"