

THE CURIOUS CASE OF CALORIC RESTRICTION

Reversing aging was once only fiction, but a special diet might **ADD YEARS** to the human life span

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ALTHOUGH Paul McGlothlin and Meredith Averill are in their early 60s, the married couple from New York State says that they feel at least 20 years younger. This is no idle claim: Their blood pressures, resting heart rates, and body fat percentages rival those of Olympic athletes. The slender duo is often mistaken for being much younger than their years. “Meredith doesn’t have any wrinkles,” McGlothlin points out. “She never wears makeup, but her face looks better than actresses who’ve had all kinds of plastic surgery.” So what’s their antiaging secret? For the past 16 years, McGlothlin and Averill have been eating a carefully controlled, calorie-restricted diet.

Scientists have known for decades that caloric restriction—reducing calorie intake without malnutrition—slows aging and extends life span in model organisms ranging from yeast to mice. Exactly why and how it confers these benefits in animals, and whether similar effects could be attained in humans, have been a mystery. Now, a flood of recent discoveries has brought scientists closer than ever before to the elusive Fountain of Youth.

Caloric restriction is about more than just being thin and fit. Something about eating a diet that is low in calories but nutritionally complete causes a dramatic reprogramming of cellular metabolism that can’t be replicated by exercise or by eating smaller amounts of high-calorie foods.

In laboratory animals such as fruit flies, roundworms, and mice, caloric restriction switches biochemical pathways on or off, resulting in higher insulin sensitivity, decreased inflammation, enhanced cardiovascular functioning, reduced muscle wasting with age, and improved resistance to cellular stress. Not only is

normal aging slowed, but calorie-restricted animals are also less likely to develop age-associated diseases such as diabetes and cancer. In mice fed a calorie-restricted diet, these effects translate to a greater than 30% increase in life span.

Nobody knows for certain why caloric restriction has such extreme health benefits, but scientists believe that from an evolutionary standpoint, it may help organisms cope with periods of famine. Pankaj

Kapahi, an assistant professor at the Buck Institute for Age Research, in Novato, Calif., says: “You can imagine that whenever food is limited in the wild, the animal goes into a sort of stasis. Then, when food is abundant, it will come out

RUNNING STRONG Although in their 60s, McGlothlin and Averill enjoy excellent health, which they attribute to a lifestyle of caloric restriction.



COURTESY OF PAUL MCGLOTHLIN & MEREDITH AVERILL

of that state and start eating so it can reproduce.” By shutting down growth and reproductive processes when food is scarce, the animal’s body can focus all of its metabolic resources on survival, which could improve the efficiency of energy production or the clearance of damaged cellular proteins. By eating less, modern humans might engage these ancient pathways to extend life span.

THE “WHY” of caloric restriction could forever be a mystery, but the past five years have witnessed impressive progress in elucidation of the “how.” According to Kapahi, a major goal of aging research has been to understand the mechanism of caloric restriction. As a postdoctoral fellow in Seymour Benzer’s lab at California Institute of Technology, Kapahi discovered that inhibiting the target of rapamycin (TOR) signaling pathway extends the life span of the fruit fly *Drosophila melanogaster* (*Curr. Biol.* 2004, 14, 885). Through the activity of the TOR protein kinase, the TOR pathway controls cell growth in response to nutrient availability.

The results led Kapahi to wonder whether the TOR pathway, which is evolutionarily conserved in organisms ranging from yeast to humans, might be the missing link between caloric restriction and long life span. “I found that flies with mutations

that reduce TOR signaling didn’t get further health benefits by caloric restriction,” he explains. These so-called epistasis experiments, in which the effects of one intervention (for example, caloric restriction) are masked by the effects of another (such as TOR mutation) in the same pathway, suggested that reduced TOR signaling is at least partly responsible for life span extension by caloric restriction.

Further studies in invertebrate model organisms (*Science* 2005, 310, 1193) provided compelling evidence for the vital role of TOR signaling in caloric restriction and aging, but data from higher animals were lacking. Last month, this situation changed when Richard A. Miller, a professor at the University of Michigan, and coworkers reported that rapamycin, a bacterial natural product that inhibits the TOR kinase, extends the life span of mice (*C&EN*, July 13, page 26; *Nature* 2009, 460, 392). In an accompanying commentary in *Nature*,

Matt R. Kaeberlein and Brian K. Kennedy, assistant professors at the University of Washington, Seattle, note that these findings “make TOR the first protein that has been shown to modulate life span in each of the four model organisms most commonly used to study ageing: yeast, worms, flies, and mice.”

Researchers think that when TOR signaling is blocked—whether from genetic mutation, rapamycin treatment, or caloric restriction—cells put the brakes on growth by decreasing protein synthesis, ribosome production, and amino acid transport. Simultaneously, the organism becomes more resistant to some forms of stress. Autophagy, or the recycling of damaged cellular components, increases.

Many of the proteins controlled by TOR signaling still need to be identified, but some known targets of the TOR kinase play key roles in protein synthesis and have also been implicated in life span extension. These include S6 kinase, a protein that activates components of the protein translation machinery, and the eukaryotic initiation factor 4E binding protein, a translational repressor. In addition, Kapahi and colleagues showed recently that HIF-1, a protein whose expression is activated by TOR signaling, causes increased stress of the endoplasmic reticulum, a cellular site of protein translation and folding, and reduces the life span of *Caenorhabditis elegans* roundworms (*PLoS Genet.* 2009, 5, e1000486).

ALTHOUGH the TOR pathway has captured much of the limelight in recent years, other biochemical pathways such as the insulin/insulin-like growth factor 1 (IGF-1), AMP kinase, and Sir2 pathways likely play supporting parts, at least under some conditions. “The conclusion that people are coming to is that all of these signaling pathways that respond to growth factors and nutrients are talking to each other,” Kaeberlein says. “I think a very appealing hypothesis is that TOR coordinates several of the responses that go along with caloric restriction, including turning down protein synthesis and modulating the response to glucose and the insulin pathway.”

Nevertheless, some researchers have been skeptical that caloric restriction could extend human longevity. And the enormous cost and logistical difficulties of studying caloric restriction in humans over the course of an approximately 80-year life span makes most such studies unfeasible. Last month, however, a research team led by Richard Weindruch, a professor at the University of Wisconsin, Madison, reported groundbreaking findings from a 20-year study of caloric restriction in a close human cousin, the rhesus monkey (*Science* 2009, 325, 201). The monkeys, which were placed on calorie-restricted diets at 7–14 years old, are now in old age.

The effects of caloric restriction were dramatic: Calorie-restricted monkeys not only appeared younger, but they also showed significantly reduced incidences of diabetes, cancer, and cardiovascular disease, as well as age-associated brain atrophy and muscle wasting. Compared with calorie-restricted monkeys, control animals had a threefold higher rate of death from an age-related cause.

At the time of the report, the calorie-restricted monkeys had an 80% survival rate, whereas only 50% of the control animals were still alive. Although Weindruch and coworkers won't know for another 15 years whether caloric restriction extends the maximum life span of rhesus monkeys (normally about 40 years), these results clearly demonstrate that caloric restriction lengthens the average life span of a primate species. The finding bodes well for the prospects of extending human longevity through caloric restriction.

Studies so far show definite health benefits for practitioners. Luigi Fontana, a research associate professor of medicine at Washington University in St. Louis and director of the Division of Nutrition & Aging at the Italian National Institute of Health, in Rome, has been studying members of the Calorie Restriction Society, a group of people who voluntarily restrict calorie

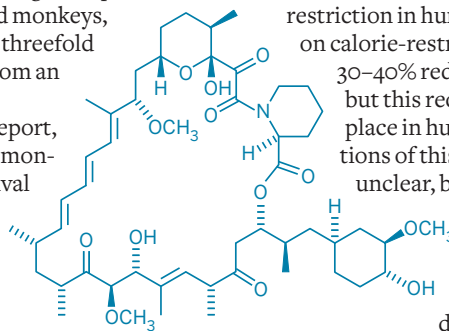
intake to improve health and slow aging. Members of the society, which include McGlothlin and Averill, sometimes refer to themselves as “CRONies” (Caloric Restriction with Optimal Nutrition).

Results are encouraging (*Proc. Natl. Acad. Sci. USA* 2004, 101, 6659). “The data suggest that most of the metabolic adaptations to caloric restriction in mice and monkeys are also occurring in humans,” Fontana says. “On the basis of their metabolic profiles, CRONies have practically zero risk of developing cardiovascular disease or stroke. We've also measured the elasticity and efficiency of the left ventricle and found that these people have hearts that are 15 years younger in terms of biology,” he says. In addition, “preliminary data suggest that many hormones and growth factors implicated in cancer are also reduced,” he says.

Fontana notes one important difference, however, in the effect of caloric restriction in humans and in mice: Mice on calorie-restricted diets show a 30–40% reduction in IGF-1 levels, but this reduction does not take place in humans. The implications of this finding are currently unclear, but low IGF-1 levels have been linked with increased life span and reduced cancer risk. Fontana discovered that strict vegetarians (vegans) on a low-calorie, low-protein diet have reduced

IGF-1 levels (*Rejuvenation Res.* 2007, 10, 225). “I asked six of the CRONie people to go for three weeks on a diet that contains the same amount of calories they normally consume, but lower protein, and, in fact, IGF-1 levels dropped,” Fontana says (*Ageing Cell* 2008, 7, 681). These results raise the possibility that in addition to caloric restriction, protein restriction might be important for slowing aging in humans.

Could the effects of caloric restriction lengthen the human life span beyond roughly 120 years? “I'm positive that caloric restriction will work in humans to extend median life span,” Fontana says. “In terms of maximum life span, I don't know.”



Rapamycin

“Healthy aging people will not only have a positive effect on the economy, but they’re also wiser and will actually bring benefits to society that can’t even be measured.”

Weindruch remarks, “I think it’s more important and useful, and this is the direction aging research is going, to move away from considering life span extension as the gold standard of aging retardation. There are other important aspects, such as how long animals stay healthy, the so-called health span.”

Even knowing that caloric restriction improves human health and could extend life span, most people probably won’t be rushing to adopt the CRONie diet. “Caloric restriction is not about only eating half

a hamburger or half a pack of french fries but is really a major change in the quality of your diet,” Fontana says. “You cannot be on a typical American diet and just eat half or a third less food than you used to eat.”

McGlothlin and Averill, who describe the CRONie lifestyle in their 2008 book “The CR Way,” meticulously plan their diet with a dedication that many might find difficult to maintain. Each day, they weigh their food and use a detailed software program to ensure that caloric and nutritional requirements are met. “My diet on the average day is 1,900 calories,” says McGlothlin, who is 5’11” and weighs 136 pounds. “There are no throw-away calories, but rather all our calories have some purpose behind them in terms of the nutrients supplied.” According to McGlothlin, a typical meal might include a barley dish, a mixture of vegetables tossed with olive oil dressing and walnuts, and salmon.

BECAUSE many people have difficulty adhering to a low-calorie diet for a week, much less a lifetime, some pharmaceutical companies are attempting to develop caloric restriction mimetics—drugs that would offer all the benefits of caloric restriction without requiring drastic changes in eating habits. Initial studies in yeast suggested that activation of the Sir2 pathway is important for life span extension by caloric restriction (*Genes Dev.* 1999, 13, 2570). Therefore, many efforts to identify caloric restriction mimetics and antiaging drugs in general have focused on the class of histone deacetylases known as sirtuins



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(SIRT)s, of which Sir2 is a member.

In particular, resveratrol, a natural compound found in red grapes that lengthens the lives of yeast and worms, has attracted intense scientific scrutiny and media coverage as a SIRT-activating compound that might mimic caloric restriction. However, recent studies have questioned the importance of the Sir2 pathway in caloric restriction, and trials of resveratrol in mice have been disappointing. “So far, feeding resveratrol to mice doesn’t increase life span, and overexpressing SIRT1 doesn’t increase life span,” Kaeberlein says. “Resveratrol may mimic some aspects of caloric restriction, but thus far it hasn’t given the phenotype that is probably most important from an aging perspective, and that is the actual slowing of aging.”

Miller and colleagues’ finding that rapamycin, an inhibitor of the TOR pathway, extends life span in mice suggests that TOR inhibitors are a promising new class of caloric restriction mimetic. Rapamycin analogs are already used clinically to prevent rejection of transplanted organs and to treat some forms of cancer. Although the immunosuppressive side effects of the current forms of rapamycin could prevent their use as antiaging drugs, Miller says, “someone might design a drug that has the beneficial effects of rapamycin but is not immunosuppressive.”

On the other hand, some scientists

WHICH MONKEY LOOKS OLDER? Owen (left panels) and Eeyore (right panels) are both 27 years old, but Eeyore, who has been fed a calorie-restricted diet for the past 20 years, looks more youthful in terms of facial features, coat, and posture.

doubt that the beneficial effects of caloric restriction could ever be completely mimicked by simply popping a pill. “I don’t believe in caloric restriction mimetics myself because I don’t think that a single compound or even two or three compounds can replicate the effects of the many factors that are working together in caloric restriction,” Fontana says. “What I also firmly disbelieve is that someone can smoke and drink, be sedentary, and eat a high-fat diet, and then with a combination of caloric restriction mimetics, he will live longer and healthier. That doesn’t make sense.” However, Fontana acknowledges that caloric restriction mimetics could prove beneficial for people

who have healthy lifestyles but don’t practice extreme caloric restriction.

Whether or not caloric restriction mimetics come to fruition, Kapahi believes that educating people and governments about the health benefits of caloric restriction could have a major impact not only on human life span but also on health care costs and worker productivity. “The general perception of the public is that it’s wrong to work on aging,” he says. “But I think the way to think about it is, healthy aging people will not only have a positive effect on the economy in general, but they’re also wiser people, and they will actually bring benefits to society that can’t even be measured.”

“Oh yes,” McGlothlin says when asked whether he wants to live to the ripe old age of 130. “I hope for a longer life, but I’m most concerned about the now. I like being able to read the newspaper without glasses. If I decide I want to go out and play basketball with some kids, I can do it, and I can keep up with them. There are a lot of things that people write off as gone in their 60s, and Meredith and I haven’t written them off at all—we’re just enjoying life like it’s always been for us from young adulthood.” ■