Will it be possible to stop the clock and prevent the effects of ageing?

ANTI-AGEING

Live long and prosper

Researchers are learning about the molecular basis of ageing — and finding clues about how to treat diseases in the process.

BY KATHERINE BOURZAC

The Ames mice in Andrzej Bartke's lab look alike at birth. In every litter, however, some mice will have a genetic mutation that inhibits or prevents the production of growth hormone or insulin-like growth factor (IGF). These hormone-free mice will grow to a third of the size of their siblings without the mutation. But around middle age, their fortunes change: the tiny mice age differently.

As Bartke first observed in the early 1990s, when the normal mice started to appear hunched and grey, the dwarf mice stood apart. "They stay healthy and they look young," says Bartke, a gerontologist at the Southern Illinois School of Medicine in Springfield. To test whether they really did live longer, Bartke started a lifespan study. In 1996 he reported his results¹: normal Ames mice typically live about 720 days; male dwarf mice got an extra 350 days, and females lived another 470. Two of the dwarf females in the study lived for four years. This was the first solid evidence that a single genetic mutation could extend lifespan in a mammal.

By studying models such as the Ames mice, researchers like Bartke are learning that ageing is not an uncontrollable, entropic process. Using clues from studies of diets and other interventions, researchers are delving into underlying molecular mechanisms in the hope of developing drugs that thwart the process of ageing. Such therapies will protect against "potentially every age-related disease," says Felipe Sierra, a gerontologist at the US National Institute on Aging (NIA) in Bethesda, Maryland. "If we can get it to work in humans we will make a big impact on quality of life."

EAT LESS, LIVE LONGER

Much of the research into the mechanisms of ageing can be traced back to work in the 1930s by nutritionist and gerontologist Clive McCay at Columbia University in New York. McCay devised the caloric restriction diet, which involves reducing calories by about 30% without causing malnutrition. He pioneered the technique in mice and rats. Since then, caloric restriction has been found to extend lifespan in every species studied, including yeast, worms, flies and dogs.

In mice, caloric restriction extends life by 30–40%. It also broadly protects against age-related diseases, including cancer, diabetes and autoimmune disease. "Caloric restriction is the most powerful known intervention in ageing," says Luigi Fontana, a gerontologist at the Washington University School of Medicine in St Louis, Missouri. For this reason, researchers are using the diet to explore the mechanisms of ageing with a view to extending the work to humans.

Two major long-term studies are testing caloric restriction in non-human primates. Although both have demonstrated major

➔ **NATURE.COM**

For some of the latest research on anti-ageing:
go.nature.com/ixmhin

ASIASELECTS/GETTY IMAGES

JEFF MILLER

health benefits, they are far less conclusive about the effect on lifespan.

At the Wisconsin National Primate Research Center, the average lifespan of a male rhesus monkey is about 27 years. By this age, a normal monkey is stooped, with grey fur on its face and sagging skin on its torso. In contrast, a monkey the same age on a calorie-restricted diet has a lively look in its eyes, a full brown coat, and holds its tail up. These monkeys also have a threefold increase in resistance to age-associated diseases, says geriatrics researcher Richard Weindruch, who heads the Wisconsin study. Rates of cancer, diabetes, brain atrophy and cardiovascular disease are all much lower in these animals.

The second study, also in rhesus monkeys, is being conducted by the NIA at its Poolesville site in Maryland. In August 2012, the NIA group reported² that monkeys on a calorie-restricted diet not only had lower cancer rates, but also delayed onset of age-related diseases. Calorie restriction did not, however, seem to provide cardiovascular benefits or reduce the incidence of diabetes.

The biggest divergence in the two studies relates to lifespan extension. In 2009, the Wisconsin group published³ preliminary evidence that fewer calorie-restricted monkeys died of age-related diseases than the control group. Equivalent results from the NIA don't show this effect.

Researchers on both teams say this difference is probably the result of their study designs — the control diet, in particular. Julie Mattison, an experimental gerontologist and one of the leaders of the NIA study, says that their control monkeys have an especially healthy diet. “We didn't want to stack the deck with the diet,” she explains. In contrast, the control monkeys in the Wisconsin group are able to eat as much as they want. “This is more like what you see in the human population,” says Ricki Colman, a senior scientist at the Wisconsin Center. It makes sense that a diet providing minimal yet balanced nutrition will appear to be better in comparison with an unhealthy diet than with a healthy one.

The Wisconsin and NIA teams plan to pool their data to come up with more definitive results, say Mattison and Colman. The two groups have been collecting microarray data about genes that are up- or downregulated in monkeys on the diet. This should help them to understand the mechanisms underlying ageing, and the beneficial health effects of the diet. However, conclusive results will not be available until after all the monkeys have died, which won't be for another decade at least. “As a guy who's done lots of mouse lifespan studies, I can say this requires patience,” says Weindruch.

HUMAN GUINEA-PIGS

While researchers wait for statistical proof of the diet's effects in primates, some people have elected to go on the diet anyway. CRONies — the label adopted by those on a diet of

A hunger for life: an elderly rhesus monkey fed a calorie-restricted diet (left) appears younger and has better health than normal monkeys of a similar age (right).

Caloric Restriction with Optimal Nutrition — voluntarily eat 30% fewer calories than recommended by the US Department of Agriculture. That can be as low as 1,400 calories a day for men, and 1,120 for women.

Fontana, who studies the CRONies, says most of the health benefits seen in animals on the caloric restriction diet also appear in humans. He says that people who started caloric restriction in middle age and stayed with the regimen for eight years have a “fantastic” cardio-metabolic profile. He adds that he has seen subjects in their late 70s with the blood pressure of teenagers.

Fontana's group has published data showing that caloric restriction protects against atherosclerosis⁴ and leads to greater heart elasticity and heart-rate variability⁵ — a marker of cardiac health. “These studies are proving, in humans, that it's possible to completely prevent obesity, diabetes and cardiovascular diseases,” Fontana says. “This is the most powerful thing I've seen in my life as a physician.”

Fontana is compiling some of the first molecular data from humans on the diet. Using biopsies from fat and muscle tissue, he will examine patterns of gene expression and hormone levels to see if they correlate with those found in animals on the diet.

It's unlikely, however, that large numbers of people will ever sign up to such a curtailed diet. After all, it's hard enough getting people to limit themselves to only the recommended amount of calories. Fontana says in his experience, US men who are not dieting tend to eat more than

the recommended upper limit of 3,000 calories a day. Even researchers who study caloric restriction rarely practise it — none of those interviewed for this report do.

Instead, the value of caloric restriction is that it demonstrates that in principle it is possible to prevent many age-related diseases with one intervention. “We've spent the last 80 years trying to treat one disease at a time,” says Rafael de Cabo, an experimental gerontologist at the NIA and the principle investigator of its monkey calorie-restriction study. Learning about the processes underlying ageing, he says, may make it possible to tackle multiple age-related diseases at once.

Caloric restriction also provides a useful tool to study the genetics of the ageing process in animal models. Previous studies into the genetics of longevity have focused on breeding short-lived invertebrates such as worms or flies that lack or can't express certain genes. Comparing the life-spans of these model animals with normal ones reveals whether the missing genes are important in ageing. To figure out which genes are important in caloric restriction researchers put established animal models on the diet; if the diet doesn't extend lifespan, the missing gene is probably vital to the process.

There is one fundamental unknown factor about caloric restriction: how does eating less lead to such drastic longevity and health benefits? Most researchers believe that two pathways are central to the process. One is IGF — the same pathway Bartke found was key to the long life of the Ames dwarf mice. The second is TOR (target of rapamycin), which is involved in both protein translation and intracellular clean-up. A third possible pathway involves the sirtuins — a group of seven

RISKY RAPAMYCIN

Rapamycin has been shown to increase lifespan in mice, perhaps by slowing the progression of cancer. But potential immune-related side effects have driven the search for safer alternatives with a similar mechanism of action.

A slice of uterus from a mouse given rapamycin appears perfectly healthy.

A similar slice from a control mouse shows signs of cystic endometrial hyperplasia, a risk factor for cancer.

related proteins that caused a lot of excitement after their discovery in 1999. In mice that can't make the proteins SIRT1 or SIRT3, the effects of caloric restriction are blocked. Some researchers, including Harvard University's David Sinclair, say this means that these proteins are critical to the diet's effects, and play an important role in ageing.

But the proposed connection between sirtuins and ageing is contentious. The disagreement came to a head in 2010, when a review of the field in *Science* by Fontana, Valter Longo at the University of Southern California, and Linda Partridge at University College London excluded sirtuins from the molecular mechanisms behind caloric restriction. The review prompted a flood of correspondence to the journal. Researchers including Sinclair and biologist Leonard Guarente at the Massachusetts Institute of Technology in Cambridge were adamant that sirtuins are connected with lifespan extension in mice on caloric restriction — only to have the review's authors reply that they were not convinced by the data for mammals. Sirtuins are likely to be connected to health and disease, but not ageing, they wrote.

Sirtuins are still controversial. "The field is polarized, and that's OK," says Brian Kennedy, president of the Buck Institute for Research on Aging in Novato, California. While at MIT, working under Guarente, Kennedy helped to establish the role of sirtuins in ageing. Now, however, he focuses mostly on TOR.

AN ANTI-AGEING PILL?

To mimic the beneficial effects of caloric restriction in a drug, it's not necessary to know exactly how it works. Large-scale studies can test promising compounds in mammals, looking for any evidence of lifespan extension. Several are already underway, including the NIA's rigorous Interventions Testing Program (ITP).

Started in 2004, the ITP encompasses three study sites where five compounds are screened each year in genetically heterogeneous mice eating food from the same supplier and sleeping on the same bedding. These studies are designed to be able to detect a 10% change in average lifespan with high confidence, even if data from one of the sites proves to be unusable.

The greatest recent success is rapamycin, which targets TOR. (The 'target of rapamycin' pathway is named after the drug that shuts it down.) A 2009 study⁶ showed that adding rapamycin to a mouse's food starting at 600 days old — roughly equivalent to a human age of 60 years — increases lifespan by 14% in females and 9% in males.

These numbers have convinced many researchers who study ageing. Arlan Richardson, a biologist at the University of Texas Health Sciences Center in San Antonio, has been doing ageing research for 40 years. He has seen many supposed anti-ageing drugs come and go, including vitamins E and C and melatonin. "I never thought we'd have the potential for an anti-ageing pill," he says. "Rapamycin is a breakthrough." His group is testing rapamycin in marmosets.

It isn't clear whether rapamycin extends lifespan by slowing cancer growth, by intervening in the mechanisms of ageing, or both, says Richard Miller, a pathologist at the University of Michigan in Ann Arbor who heads the ITP. He says his Michigan group has been looking into this question, and has unpublished data showing that the drug slows down ageing in multiple normal cell types, suggesting a broad anti-ageing effect.

However, rapamycin has several negative side effects that are likely to keep it out of the

running as a potential anti-ageing drug. It is known to cause cataracts in mice, and in humans it is used as an immunosuppressant to prevent organ-transplant rejection. This suggests that it might have serious immune-related consequences. "I don't view the studies we're doing as a precursor to clinical trials," says Miller.

The next step is to figure out which mechanisms in the TOR pathway are responsible for the positive effects of rapamycin, and then develop a more targeted drug. Understanding mechanisms "helps in terms of scoping out potential side effects", says David Glass, a specialist in muscle diseases at the Novartis Institutes for Biomedical Research in Cambridge, Massachusetts.

Miller's group is trying to discern these effects by studying rapamycin in various different tissue types in the mice. Other researchers are testing derivatives that may have more targeted effects. Kennedy's team, for example, is currently working with Biotica Technology, a drug development company in Cambridge, UK, that makes rapamycin derivatives, in the hope of finding a version that works without the side effects.

Not all compounds screened by the ITP show as much promise as rapamycin. Resveratrol, a compound found in red wine that targets sirtuins, had been shown to extend lifespan in obese mice fed a high-fat diet — but it failed to produce results in the ITP studies at any dose, says Miller. Many researchers still hope that resveratrol has positive effects. Sirtis Pharmaceuticals, a Cambridge, Massachusetts-based biotechnology company owned by GlaxoSmithKline, is testing resveratrol derivatives in ulcerative colitis and psoriasis. Meanwhile, the ITP will soon publish data on two new life-extending compounds, says Miller.

Ageing isn't a disease, and lifespan extension will be almost impossible to prove in humans. Instead, Glass, Sierra and others hope that research on ageing interventions will change the way we think about disease and drug development, and lead to treatments that tackle multiple age-related diseases at once. Major causes of death worldwide, including cancer and cardiovascular disease, share a common risk factor: age. Tackling one disease at a time isn't working, says the NIA's de Cabo. "Ageing is the leading risk factor for all chronic diseases," he says. "Postpone ageing, and you postpone these diseases." ■

Katherine Bourzac is a freelance journalist based in San Francisco.

1. Brown-Borg, H. M. et al. *Nature* **384**, 33 (1996).
2. Mattison, J. A. et al. *Nature* **489**, 318–321 (2012).
3. Colman, R. J. et al. *Science* **325**, 201–204 (2009).
4. Fontana, L. et al. *Proc. Natl Acad. Sci. USA* **101**, 6659–6663 (2004).
5. Stein, P. K. et al. *Ageing Cell* **11**, 644–650 (2012).
6. Harrison, D. E. et al. *Nature* **460**, 392–395 (2009).