

The fruit fly *Drosophila melanogaster* only grows to a size of two to three millimeters. Scientists use a brush to separate individual insects with differing characteristics.

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PHOTO: FRANK VINKEN

# THE METHUSELAH COCKTAIL

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# KNOWLEDGE FROM

— BIOLOGY & MEDICINE

Linda Partridge and her colleague Sebastian Grönke at the Max Planck Institute for Biology of Ageing in Cologne can't promise eternal life – but they are at least discovering ways to lead a healthier one. The researchers' findings in fruit flies and mice have revealed astonishing new insights into aging that will also benefit us humans.

The fruit flies of Sebastian Grönke that made it into the tubes marked “Rapa” have won the lottery. They reach an age of two months – an advanced age for flies – and are still fit and healthy. “Rapa” stands for “rapamycin”, a drug that’s one of the most promising anti-aging substances of our time.

Flies exposed to rapamycin – up to now prescribed as an immunosuppressive drug following kidney transplant – don’t just live considerably longer, they also stay healthier. The Max Planck researchers can measure such improvement by means of a specially devised fitness test for flies: “When rapamycin is added to their food, they still perform at the same level at 60 days old as they did when they were young,” Grönke explains.

In addition to a daily dose of rapamycin, their food contains two other drugs: lithium, known as a mood stabilizer to treat depression, and the drug trametinib, which is used to treat cancer patients. “The triple cocktail extends the usual lifespan of the fruit flies by almost 50 percent,” explains Linda Partridge, who heads the Department of Biological Mechanisms of Ageing at the Max Planck Institute in Cologne. The drugs have enabled some of the insects to live for as long as four months – the highest age ever attained through the administration of drugs. Typically, the flies die between two and three months of age.

On the strength of such results, Partridge is optimistic that such advances will also enable humans to remain generally healthy into very old age. “We won’t be able to do away with death,” she says, “but we can certainly reduce illness and infirmity in old age.” Her life as a researcher has mostly been devoted to unravel-

ing how cells, tissues, and organisms age. She now heads research teams at the Max Planck Institute in Cologne and at University College London. Numerous biological mechanisms are conserved across species, and that’s why scientists usually perform research on flies and mice. “Time isn’t on our side; life expectancy is continuing to rise in many western industrialized countries,” Partridge says. It has been calculated that in 2060, there will be more than ten million people over the age of 80 living in Germany. Every fifth baby born today could live to be 100.

When it comes to aging, genes only play a minor role. “Their contribution to life expectancy is less than 20 percent – perhaps even less than ten,” the biologist explains. Environmental factors, individual behavior, and a person’s social and economic status are far more important. Unhealthy habits such as overeating, sedentary lifestyle and smoking have been proven to reduce life expectancy.



Experiments on different animal species are continually revealing new ways in which the biological clock can evidently be turned back. Two years ago, for example, a discovery by Dario Valenzano, a colleague of Partridge at the Institute, caused a stir. He had found that fish fed micro-organisms from the guts of younger fish of the same species live longer. In another study, colleagues in the U.S. discovered that replacing the blood plasma of older mice with that of younger animals had an astonishing effect on the aging process.

In many animals, exercise and diet are the primary factors influencing the speed of aging. Mice that are allowed to consume only 60 percent of their normal diet live considerably longer and remain healthier than mice fed a standard ration. Rhesus monkeys have also been to live longer under dietary restriction when the diet contains sufficient quantities of vitamins and minerals. In recent years, however, Partridge and her team have come to suspect that the key to living a longer and healthier life into old age isn't calorie restriction; instead, it's the particular nutrients in an animal's diet. It is eating too many of these, they suggest, that will reduce life expectancy and increase the risk of age-related diseases.

In the laboratory, Sebastian Grönke points to an ordinary-looking shelf lined with dozens of cans. These contain chemicals for the flies' food. In a time-consuming process, Partridge's colleagues in London developed a fly food with a chemically defined formulation. This allows the scientists to modify the diet of the flies at will. This enabled Grönke to discover which nutrients prolong lifespan and improve health in old age. Fruit flies fed on a diet high in protein and amino acids die earlier. To compensate, the insects lay a large number of eggs. Conversely,

flies fed on a diet with less protein live longer. And this comes at the cost of reduced growth and lower fertility.

The scientists, however, wanted more detailed information. "Our results show that not all amino acids are equally important. Essential amino acids such as leucine, isoleucine, or valine are particularly important for longevity," Grönke explains. Essential amino acids are those that the body can't synthesize itself; they have to be ingested with food. They regulate a signaling pathway known as "TOR," which is involved in important aging processes in cells.

The researchers observed the same phenomenon in mice. One study has revealed that consuming lower quantities of certain essential amino acids can actually have a positive effect on health – including in humans. However, it's still unclear whether this also results in longer life. The scientists also used the genome information of the fruit flies to define their dietary amino acid requirements. "This allowed us to develop a specialized diet for fruit flies that contains neither too few nor too many amino acids," says Grönke. "Animals fed this food feel full earlier and therefore eat less. Nevertheless, they grow faster, become larger, and lay more eggs than other fruit flies fed with standard food. And, despite this, they live just as long," Grönke explains.

Reducing the quantity of essential amino acids has an impact on a network of cellular signaling pathways known as the "IIT network." This network is active in a wide range of organisms, from fruit flies to humans, and controls development, cell proliferation, growth, reproduction, and the stress response. The network includes "insulin/IGF-1" and "mTOR" signaling pathways, each with a large number of differ-



PHOTOS: FRANK VINKEN

The fruit flies in Linda Partridge's and Sebastian Grönke's laboratory live in finger-length tubes sealed with a gas-permeable stopper.





Fitness test for flies: only physically fit flies can climb up the tubes quickly. When the first ones have reached the top of the left-hand tube, the upper section is moved to the right, and the next round begins. Gently tapping the tube sends the flies back to the bottom and then they have to repeat their climb to the top. This procedure is repeated five times, until the fittest flies have reached the far right section.

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ent signaling molecules, the most important of which are IGF-1 and mTOR. Flies and mice that have the modified genes for these molecules age more slowly and become genuine Methuselahs. Like a precise sensor, the IIT network measures the nutrient status in the body and adjusts metabolic processes as required, based on the demand for and availability of food. Reduced food consumption evidently puts a brake on the activity of the network.

The drugs lithium, trametinib, and rapamycin also have an effect on various signaling molecules of the IIT network, reducing its activity. Each

individual substance extends the longevity of the flies by an average of eleven percent. A combination of two of them increases life expectancy by around 30 percent, while all three increase life expectancy by nearly 50 percent. At the same time, they complement each other when it comes to reducing side effects. Rapamycin on its own induces adiposity, while lithium appears to negate this.

Drugs that can slow down and promote healthy aging, it seems, are no longer a mere pipe dream. Metformin, used to treat diabetes, is another possibility. Its impact on life expectancy will soon be investigated in a large-scale

study in the U.S. involving several thousand participants. People who are unable to prolong their lives even with optimal nutrition could benefit from such drugs. Most of us, after all, find it difficult to stick to a dietary regimen for decades on end.

The researchers have also searched for other anti-aging candidate drugs. They trawled through a database of active agents with known effects and performed computer simulations to find out whether these bind signaling molecules in the IIT network.

They soon discovered what they were looking for. A drug known as tane-



spimycin had already proven its potential in the model organism most frequently studied by researchers on aging: the nematode *Caenorhabditis elegans*. It blocks the heat shock protein Hsp90 and reduces the number of “senescent cells.” These are mature cells that increase in number in old age and emit substances that facilitate chronic infection. As a result, they contribute to diseases associated with old age, such as heart attack and cancer. In young people, the immune system removes these cells. In the elderly, however, they are protected from destruction by precisely the Hsp90 protein.

“mitochondria”, the power generators of the cell, and, this was also sufficient to extend the lifespan of the animals. The IIT network therefore controls various life-extending processes in different tissue types.

and measured the impact on life expectancy. In the young animals, the results were as expected. The mice on a restricted diet lived to a very old age and were still comparatively healthy even at an advanced age. In contrast, animals fed a restricted diet only later in life experienced little or no benefit.

## SUMMARY

The IIT network has a major impact on how organisms age. Pharmacological and genetic inhibition of this cellular signaling network results in increased life expectancy.

Dietary restriction and reduced consumption of essential amino acids decrease the activity of the IIT network. Fruit flies and mice therefore live longer in laboratory tests when they are given a restricted diet.

A combination of rapamycin, lithium, and trametinib significantly increases the life expectancy of fruit flies. The drugs also reduce activity of the IIT network.

The adipose tissue possesses a type of memory for environmental influences and lifestyle. For this reason, dietary modification in mice only extends life expectancy if it is implemented at young age.

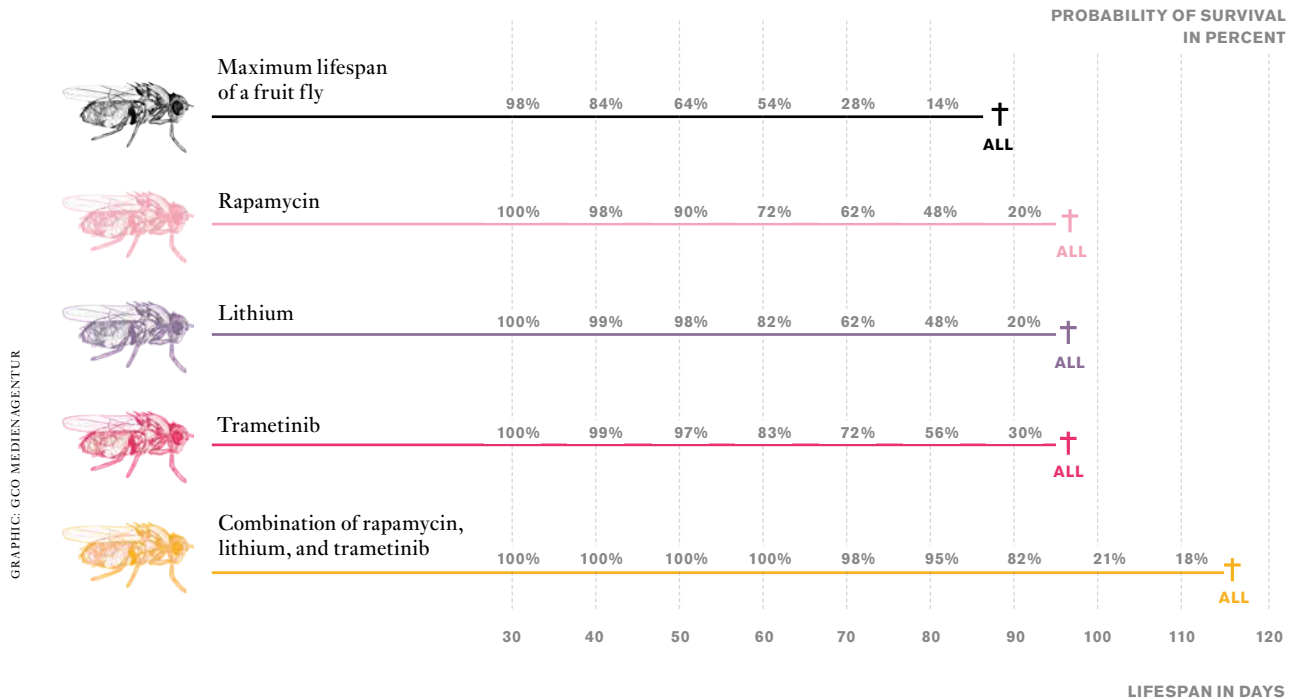
External influences also have varying effects on different tissue types, depending on age. For example, the Max Planck researchers put young and older mice on a restricted diet

Analyzing gene activation in various tissues showed that the liver adapted quickly to the new diet. By contrast, in adipose tissue, the researchers observed a memory effect of the previous diet. Activation of fat metabolism genes in the precursor cells of the actual fat-storing, adipocyte cells can be influenced by lifestyle in youth, but not later on. As a result, the mice that only began to fast later in life did indeed lose weight, but the genes in the adipose tissue remained active at a similar level to that of animals with a normal diet. In addition, the changes in the composition of lipids in old mice were less marked. The various lipid molecules have different functions: triglycerides, for example, are used by the body to store fat, phospholipids are components of cell membranes, and cholesterol act as signaling substances.

Adipose tissue therefore has an important part to play in the body’s aging process. There, dietary habits chiefly affect the mitochondria. If the animals consume less food, these cellular power generators are produced in greater numbers – but only if the animals eat less early on in life. In the case of mice, “early on” means after just a few months, as a further study by the researchers in Cologne has shown. This memory effect may be based on “epigenetic” alterations of genes, in which DNA is tagged by small molecular groups, switching genes on or off. In this way, environmental factors can have a relatively rapid impact on genes, and their effects can even be passed on to the next generation.

Tanespimycin is associated with severe side effects, however, and needs to be administered with caution. “Even so, it might be feasible to combat old age-related diseases with short-term localized administration, such as in cases of macular degeneration of the eye, the commonest cause of age-related blindness,” Partridge explains. This prompted the researchers to investigate whether drugs targeting the IIT network might delay aging throughout the body, even though they are activated only in certain tissues. They analyzed whether the brain, muscle, gut, and adipose tissues of a fruit fly produce the same proteins when the IIT network is less active. “That clearly wasn’t the case, as we discovered. The various cell types respond differently,” Partridge explains. Of the 6,000 proteins studied, 2,400 are synthesized at differing levels in the brain, muscle, gut, and fat. The gut, for example, produces more enzymes that control the quality of protein synthesis when the IIT network is less active.

“That local factor alone can prolong an organism’s overall lifespan,” Partridge explains. On the other hand, if the IIT network is suppressed in a fly’s fat tissue, entirely different proteins are synthesized. These improve the quality of the



Studies in the U.S. have shown that some epigenetic changes are age-related. On the basis of around 350 such DNA modifications, scientists can determine the biological age of a person with astonishing accuracy. The epigenetic clock ticks at the same pace in all the cell types studied – whether in cells that are continuously being regenerated or in those that originally developed in the embryo. The precise impact of these DNA modifications is still unknown.

Dietary restriction clearly has an effect on age-related epigenetic changes. It also stimulates lipid metabolism reprogramming. This protects the body from the harmful effects of fat deposition in the liver, as well as against insulin resistance – a typical sign of age-related, type-2 diabetes.

If the results of the studies on mice are transferred to humans, it is clear

that anyone who wants to live a long time needs to start early. “Our research shows that such dietary habits need to be adopted at an early age. The foundations for a healthy old age are already laid in early adulthood,” Linda Partridge explains.

A further discovery made in the laboratory by the researchers in Cologne might offer an incentive to start thinking about your own aging process early on.

Together with colleagues from Leiden University Medical Center in the Netherlands, they took blood samples from tens of thousands of people and searched for molecules that indicate the remaining lifespan a person is likely to have. Following extensive analysis, the scientists identified 14 biomarkers, including various amino acids, the ratio of “good” to “bad” cholesterol, fatty acids, and signaling molecules involved in infections. Initially, the scientists aim

to employ these biomarkers in age research on animals and in clinical studies on humans. However, blood biomarkers may eventually help young people find out, with a high degree of certainty, whether they will suffer from certain illnesses in old age. Then everyone would be able to decide for themselves whether they should exercise more and eat a healthier diet, or whether they should even take preventive medication to improve their health in old age.