Following Herodotus, the ancient Greek father of history, searching for the mythical "Water of Life" to gain everlasting youth
Part 2: Replace "mythical water" with fasting and fiddling around with genes

The quest for longevity enhanced the understanding of the role of metabolic pathways on lifetime, but no pragmatic antiaging dietary scheme can be recommended yet.

Fantasizing, like the ancient Greeks, to attain everlasting youth by just drinking or bathing in "mythical water" appears absurd nowadays. But some scientists today are not that far from similar dreams. They want to learn from Hydra vulgaris, a tiny freshwater polyp, how humankind can be treated almost to immortality. This tiny laboratory animal model owns stem cells able to exchange themselves when aged. It was claimed that by keeping adult Hydra in laboratories and influencing their genes and hormones, at least 5% of them might live up to 1400 years. Those who die will die because of accidents and diseases but not old age (1). More realistic attempts to extend our life and remain healthy focus on nutrition and toying around with our genetic setting.

Antiaging diets as defined in science

Examples of nutritional topics concerned with health and well-being are the problem of obesity, the controversial promotion of a carbohydrate reduced- in favor of a high protein diet, as well as the pros and cons of being a vegetarian. Dietary regimes to reduce weight and avoid "carb" favoring protein or vegetarian food are supposed to lower the biological age.

The term "antiaging" is widely exploited when promoting cosmetics or unverified nutritional supplements. To discuss antiaging diets in science, medicine, and public health should follow a definition of antiaging involving "molecular mechanisms of aging" being the "hallmarks or pillars of aging" (2-4). Yet, fully understanding the definition of "antiaging" based on "molecular mechanism" is reserved for those scientists working in the field of molecular biology or at least for experts interested in the most recent results of relevant laboratory investigations.

Fasting – the oldest wisdom of humankind for health

Without knowing what molecular mechanisms are at work in a living cell, Hippocrates already suggested that nutrition determines health and disease. In adding up reasons for falling sick and what should be done to support your health, he mentioned …"the mode in which the inhabitants live, and what are their pursuits, whether they are fond of drinking and eating to excess, and given to indolence, or are fond of exercise and labor, and not given to excess in eating and drinking." The advice given in the 5th century BCE is still very appropriate today.

What Hippocrates suggested here might go back to prehistoric times and is one of the oldest pieces of wisdom of humankind and implies "fasting". Fasting is "abstinence from food and drink or both for health, ritualistic, religious, or ethical purposes". Fasting has found its way into religions and philosophies such as Buddhism, Christianity, Islam, Judaism, and others. To

approach fasting within the framework of modern science, you must be clear about what you mean by fasting while exploring its physiological effects.

Types of fasting and religion

Numerous studies investigate the health effects of fasting as a commitment to religious beliefs. There are various ways of fasting, differing among religious dedications, which makes it difficult to compare study results and come to an overall conclusion. All in all, religious fasting as a "non-pharmacological intervention" benefits health and longevity. Greek Orthodox Christians observe 180 to 200 days of fasting over the year. Fasting resembles vegetarianism, influences the body mass index (BMI), and has a favorable effect on the lipid pattern (5). Of the three primary forms of fasting, the "Orthodox" fasting is an example of "dietary restriction (DR)", depending on bread, fruits, legumes, nuts, seafood, and vegetables (6). Roman Catholics, Lutherans other religious groups in Christianity are on a similar type of fasting, limiting dietary intake ad libitum to fruits, vegetables, and whole grains, and don't consume meat which influences risk factors for metabolic and cardiovascular diseases (7).

The second important fasting type is "alternate-day fasting (ADF)," with feast and fast periods over the day. A good example is Ramadan. Millions of Muslims don't eat or drink from sunrise to sunset during Ramadan. How these religious customs are followed varies according to different groups. There are various studies about the health effect of Ramadan fasting, but no overall conclusion could be accomplished (5). In addition, testing the impact of fasting on humans is problematic because health and longevity depend to a great extent also on socioeconomic status, as well as environmental factors such as behavior like smoking and alcohol consumption.

Caloric restriction without causing malnutrition – major study type for fasting

Still, nutrition plays an essential part in how healthy we are and how long we are alive, and what we eat, as well as how much we eat is under the control of the individual. One major study type for fasting, "caloric restriction (CR)," developed into a very active topic of research conducted mainly with laboratory animals. CR decreases caloric intake without causing malnutrition. The decrease in caloric intake varies but often comprises 20 to 40% kilocalories of the ad libitum food intake (8). It appears that CR has the potential to influence the risks and progression of diseases and is beneficial for health and long life (9-11).

Investigation with rats in the first half of the 20th century found that CR reduced the growth of the animals but increased their life span (12). From the 70th onwards, mice were included as laboratory animals, and CR became the predominant method to look into antiaging effects (13). It emerged that CR not only enlarged life span but also diminished the risk of cancer, cardiovascular diseases, type 2 diabetes, autoimmune diseases, and neurological degeneration due to old age (11).

Meal size and frequency of feeding

Not only CR as such, but reduced meal size and changing feeding frequency deletes the onset of diseases and aging. Sophisticated feeding schemes are used, such as time-restricted feeding (TRF) and intermittent- or periodic feeding (IF or PF). TRF for 4 to 12 hours reduces the risk of type 2 diabetes mellitus and high blood cholesterol. IF means to change fasting and ad libitum eating from one day to the other, which protects from obesity, several non-communicable diseases, and oxidative stress and increases the median life span of mice. PF differs from IF because fasting and ad libitum periods extend over days. Similar effects as mentioned above were achieved by fasting-mimicking diets (FMD), which means fasting for five days within a month or every 3 to 4 months. Fasting time and energy restriction involve metabolite-controlled longevity pathways and significantly affect human organ functions (14). At least in laboratory male mice, CR alone could extend the life span by 10%. A daily intermitted fasting and feeding alteration between sleep-wake times increase mice's life span by 35% (15, 16).

The genome and metabolic pathways

Results obtained under controlled conditions in laboratory mice and additional laboratory animal models may hint at similar effects in humans. Humans might benefit in the same way as mice from CR. Improved technology now allows us to follow up on complicated metabolic processes on the cellular level and the genome. Even DNA and protein structures are visualized directly using Cryo-electron microscopy (17, 18).

Research interest shifted to the molecular level and started using invertebrate models, such as nematode worms and fruit flies (19). Essential metabolic functions affected by aging and obesity could be investigated by following up on “complex inter-relationships between metabolic, immune, and inflammatory pathways” (20-22). For the experts, “mechanistic target of rapamycin (mTOR), adenosine 5`-monophosphate (AMP)-activated protein kinase (AMPK), insulin and insulin-like growth factor 1 (IGF-1) like receptors, FOXO-family transcription factors, and nicotinamide adenine dinucleotide (NAD)-dependent sirtuin deacetylases are familiar molecular mechanisms. CR and DR influence metabolic pathways involving the innate-immune system, antigen production, and inflammation.

An “immunometabolic regulator” was detected recently. Responding to CR, the factor was identified to be PLA2G7. PLA2G7 is an enzyme playing a role in the transport of triacyl glycerides and cholesterol in the blood. It degrades platelet aggregation and activates inflammation. It was found for mice and humans that CR inhibited the ”expression of the gene Pla2g7 encoding the platelet activating factor acetyl hydrolase PLA2G7”. CR of 14% for two years in healthy humans positively affected the immune function of the thymus, adipose tissues, and macrophages (23, 24). Clinically increased amounts were associated with “metabolic, immune and inflammatory dysfunction” and several serious diseases, including stroke, COPD, autoimmune- and cardiovascular diseases, and cancer.

Influence of genes on lifespan

Not only do nutrition and immunological reactions influence life span, but also our genes. Studying the life span of humans is difficult. Research depends on animals, from rodents and dogs down to worms. In a large yearlong multicenter intervention project, five genes in mice

were identified with effects on life span as tested in the worm Caenorhabditis elegans (Bou Sleiman et al.) (25). The investigators compared their results with several datasets available to identify phenotypes related to longevity (26). Loci influencing a long life vary according to sex, age, and litter size. A single locus for longevity was detected on chromosome 3. A locus for males was only detected after excluding early death. The increase in body weight increases premature death and lifespan related to variation in genotypes.

The genes identified in mice were silenced in the worm model. Of the five genes, only one increased life span, suggesting that the others are pathological and not related directly to aging. The enzyme pyruvate dehydrogenase kinase 1 (Pdk1) linked the gene involved in longevity to its role in cell metabolism. A locus near the Fgd6 influenced "health span, parental life span, and longevity in humans" (27). For the study of Bou Sleiman et al., early nutrition influenced longevity and growth. However, this finding needs further exploration since other investigations were not conclusive yet (26, 28, 29).

"The genetics of a long life" is an exciting research field but is hampered by significant limitations. In animal models, besides mice, also flies, yeast, and worms, more than 2000 genes interconnected to longevity were discovered (29). However, dietary and pharmacological intervention may have species-dependent genetic effects. Thus, genetically dependent longevity in mice still is not yet well understood and needs more exploration (26). Mainly, non-Mendelian associations in the frequency of phenotypes are difficult to detect, and this might be true for genetic variants related to longevity (30). Whether the genes identified by Bou Sleiman et al. influence longevity in humans still need to be proved. That mainly refers to the FGD6 locus mentioned above. To manipulate our genes to achieve a long life probably is not advisable. Similarly, pharmaceutical intervention with medicaments like rapamycin and metformin should not be considered because of side effects. So, when influencing genes should not be advisable, what about antiaging diets instead?

Conclusions - from a "pragmatic perspective," dietary intervention for longevity seems unrealistic

Outlined above, the four main diets with antiaging potential are: Calorie restriction, which means torturing yourself but feeling good; Time-restricting meal intake for being a good Muslim; Vegetarian diets, forced upon us, so to save the world from overheating. That is because cattle emit methane causing global warming. Ketogenic diets are marketed with the battle cry against the devilish "carb."

Research with laboratory models of various kinds increased the understanding of metabolic pathways. Clinical application and intervention, for instance, obesity, benefitted from the research results, which may be true even more in the future. However, it is questionable whether healthy people can prolong their life by following one or the other diet regime. What seems to work under strictly controlled circumstances for rodents might not work for humans or are even contra-productive. The antiaging diets could be compared with some sort of drug. A medicine that works also has side effects. The difference between both outcomes usually depends on the doses. A too-high dose is harmful or even deadly. Side effects of calory restriction include a low

tolerance against a cold environment, loss of interest and inability of sexual activities, being moody, constantly tired, not sleeping well, and being prone to infections (31).

Of all the antiaging diets, calorie restriction probably is the least to work in the "real world". In the 80th of the last century, a well know scientist created a "CR Society" under the promise "The 120 Year Diet: How to double your vital years" (32). He could mobilize only a few followers, and he died at the age of 79 years. Constantly fighting the temptation to eat more but should eat less is too demanding.

Vegetarian diets stand the "real world" challenge and are commonly followed. Whether they really prolong the life of those under the scheme is doubtful, but there is a positive influence on health. Vegetarians, compared to "nonvegetarians", usually have lower blood lipid patterns, lower blood pressure, and a lower risk of developing ischemic heart disease, hypertension, stroke, type-2-diabetes, and certain types of cancer. A low intake of saturated fat and high dietary fiber, together with high consumption of fruits and vegetables, is favorable. However, a vegetarian needs to have a good knowledge of nutritional issues to avoid deficiencies, including vitamins B and D, omega-3 fatty acids, and the minerals calcium, iron, and zinc (33).

The financial interests behind the propagation and the marketing of low-carbohydrate diets prevented the "mainstream" from knowing about adverse health consequences. Short-term "carb" restriction may lower the weight, but being under ketogenic diets for longer increases the risk of cardiac functions, osteoporosis, kidney damage, lipid abnormalities, cancer, and sudden death (34).

A recent review of antiaging diets concluded for the benefit of "otherwise healthy people" that from a "pragmatic perspective," dietary intervention for longevity seems unrealistic. That doesn't mean to stop working against malnutrition for under- or over nourished populations. But it is difficult to think about a dietary regime as such, which is promising to prolong your life. At best healthy nutritional intake decreases the risk of diseases causing death. Public health should assist in geroscience and adopt biomarkers to identify population groups at risk for early death, compared with groups living under more favorable conditions enjoying a longer high-quality life span (3).

Literature:

1. Cohen J. Death-defying experiments. Science. 2015;350(6265):1186-7.
2. Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, et al. Geroscience: linking aging to chronic disease. Cell. 2014;159(4):709-13.
3. Lee MB, Hill CM, Bitto A, Kaeberlein M. Antiaging diets: Separating fact from fiction. Science. 2021;374(6570):eabe7365.
4. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194-217.
5. Trepanowski JF, Bloomer RJ. The impact of religious fasting on human health. Nutr J. 2010;9:57.

6. Sarri KO, Tzanakis NE, Linardakis MK, Mamalakis GD, Kafatos AG. Effects of Greek Orthodox Christian Church fasting on serum lipids and obesity. BMC Public Health. 2003;3:16.
7. Bloomer RJ, Kabir, M.M., Canale, R.E., Trepanowski, J.F., Marschall, K.E., Farney, T.M., Hammond, K.G. Effect of a 21 day Daniel Fast on metabolic and cardiovascular disease risk factors in mmen and women. Lipids in Health and Disease. 2010;9:94.
8. Spindler SR. Caloric restriction: from soup to nuts. Aging Res Rev. 2009;9(3):30.
9. Mattson MP, Allison DB, Fontana L, Harvie M, Longo VD, Malaisse WJ, et al. Meal frequency and timing in health and disease. Proc Natl Acad Sci U S A. 2014;111(47):16647-53.
10. Panda S. Circadian physiology of metabolism. Science. 2016;354(6315):1008-15.
11. Speakman JR, Mitchell SE. Caloric restriction. Mol Aspects Med. 2011;32(3):159-221.
12. McCay CM, Crowell, M.F., Maynard, L.A. The effect of retarded growth upon the length of life span and upon the ultimate body size. The Journal of Nutrition. 1935;10(1):16.
13. Weindruch R, Walford RL, Fligiel S, Guthrie D. The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake. J Nutr. 1986;116(4):641-54.
14. Di Francesco A, Di Germanio C, Bernier M, de Cabo R. A time to fast. Science. 2018;362(6416):770-5.
15. Acosta-Rodriguez V, Rijo-Ferreira F, Izumo M, Xu P, Wight-Carter M, Green CB, et al. Circadian alignment of early onset caloric restriction promotes longevity in male C57BL/6J mice. Science. 2022;376(6598):1192-202.
16. Deota S, Panda S. Aligning mealtimes to live longer. Science. 2022;376(6598):1159-60.
17. Cohen J. The dream vaccine. Science. 2021;372(6539):227-31.
18. Milne JL, Borgnia MJ, Bartesaghi A, Tran EE, Earl LA, Schauder DM, et al. Cryo-electron microscopy--a primer for the non-microscopist. FEBS J. 2013;280(1):28-45.
19. Kapahi P, Kaeberlein M, Hansen M. Dietary restriction and lifespan: Lessons from invertebrate models. Ageing Res Rev. 2017;39:3-14.
20. Fontana L, Partridge L, Longo VD. Extending healthy life span--from yeast to humans. Science. 2010;328(5976):321-6.
21. Kahn CR, Wang G, Lee KY. Altered adipose tissue and adipocyte function in the pathogenesis of metabolic syndrome. J Clin Invest. 2019;129(10):3990-4000.
22. Komatsu T, Park S, Hayashi H, Mori R, Yamaza H, Shimokawa I. Mechanisms of Calorie Restriction: A Review of Genes Required for the Life-Extending and Tumor-Inhibiting Effects of Calorie Restriction. Nutrients. 2019;11(12).
23. Rhoads TW, Anderson RM. Caloric restriction has a new player. Science. 2022;375(6581):620-1.
24. Spadaro O, Youm Y, Shchukina I, Ryu S, Sidorov S, Ravussin A, et al. Caloric restriction in humans reveals immunometabolic regulators of health span. Science. 2022;375(6581):671-7.
25. Bou Sleiman M, Roy S, Gao AW, Sadler MC, von Alvensleben GVG, Li H, et al. Sex- and age-dependent genetics of longevity in a heterogeneous mouse population. Science. 2022;377(6614):eabo3191.
26. de Magalhaes JP. The genetics of a long life. Science. 2022;377(6614):1489-90.
27. Timmers P, Wilson JF, Joshi PK, Deelen J. Multivariate genomic scan implicates novel loci and haem metabolism in human ageing. Nat Commun. 2020;11(1):3570.
28. English S, Uller T. Does early-life diet affect longevity? A meta-analysis across experimental studies. Biol Lett. 2016;12(9).

29. Tacutu R, Thornton D, Johnson E, Budovsky A, Barardo D, Craig T, et al. Human Ageing Genomic Resources: new and updated databases. Nucleic Acids Res. 2018;46(D1):D1083-D90.
30. de Magalhaes JP, Wang J. The fog of genetics: what is known, unknown and unknowable in the genetics of complex traits and diseases. EMBO Rep. 2019;20(11):e48054.
31. Most J, Tosti V, Redman LM, Fontana L. Calorie restriction in humans: An update. Ageing Res Rev. 2017;39:36-45.
32. Walford RL. The 120-year diet. Am J Clin Nutr. 1988;47(1):162.
33. Craig WJ. Nutrition concerns and health effects of vegetarian diets. Nutr Clin Pract. 2010;25(6):613-20.
34. Bilsborough SA, Crowe TC. Low-carbohydrate diets: what are the potential short- and long-term health implications? Asia Pac J Clin Nutr. 2003;12(4):396-404.

Frank P. Schelp is responsible for the content of the manuscript, and points of view expressed might not reflect the stance and policy of the Faculty of Public Health, Khon Kaen University, Thailand

For comments and questions, please contact <awuso11@gmail.com>