Chapter

Hydrogen Water on Survival Rate after Fasting in Drosophila Model

Chung-Hsing Chao

Abstract

In this study, we use a Drosophila model to examine the effect of drinking hydrogen water on survival rate after fasting. The cells produce free radicals to help to absorb nutritious substances due to metabolism, which is a unique phenomenon for biological organisms. But if over the tension of free radicals can seriously affect the physiological functions, and even lead to death. Recently, scientists found that molecular hydrogen is a free radicals scavenger. However, no bio physiological mechanism and experiment have shown that by drinking hydrogen water, can eliminate the free radicals in animals and the evidence sufficient to influence the survival rate after fasting. Surprisingly, the results of the study support that hydrogen water may be helpful for the survival rate of the fasted fly. When the body loses oxygen free radicals due to food breakoff, hydrogen water may neutralize free radicals and reduce damage to cells. However, we also found that hydrogen water seems to be much help for relatively weak individuals, such as the mutant flies, and it is also favorable for individuals with stronger physique in wild *Drosophila melanogaster* females. In conclusion, the results show that flies can increase their survival rate by feeding hydrogen water under extreme oxidation stress.

Keywords: survival effect, hydrogen, Drosophila, fasting, free radicals, oxidative stress

1. Introduction

In recent years, there has been a great deal of attention toward the field of free radical chemistry. Our body generates free radicals of reactive oxygen species or reactive nitrogen species by various endogenous systems, exposure to different physiochemical conditions. To balance the free radicals and antioxidants is necessary for proper physiological function. If we can regulate free radicals to reduce the potential reactive oxygen species over tension on body's ability, it will not cause oxidative stress damage. Free radicals thus do not adversely alter lipids, proteins, DNA and trigger many diseases. As a result application of an external source of antioxidants likes oral hydrogen water can assist in coping this oxidative stress.

This metabolism phenomenon also occurs in many other animals or even insects that are less evolved. In 1946, the fruit flies were successfully carried out by National Aeronautics and Space Administration (NASA) for the biological experiments in space. About 75% of the genetic code of fruit flies has similarities to all human pathogenic genes. Insect life is much shorter than human. Drosophila flies also has found free radicals in their cells. The scientists take Drosophila to study aging effects much efficiently due to shortening the time in experiments. It is an important reason why studying *Drosophila melanogaster* in fruit flies is very popular among biological researchers. They also found many mutant species of fruit flies, which are related to the regulatory function of oxidative stress attacked by free radicals in cells. Therefore, we study the survival effects of drinking hydrogen-water on fruit flies after fasting.

According to past research reports, fasting or severely hungry animals will rapidly accumulate a large number of free radicals in cells because of lack of well physiological functions. Excessive free radicals, however, can damage the cell's genetic material and cell membranes, eventually killing the fruit fly. And the flies of the mutant species are more likely to accumulate free radicals in vivo because the chromosome of the Drosophila cell, an enzyme gene that removes peroxidase free radicals by hydrazine, is removed so that the number of free radicals is out of control. Although the fruit fly of this mutant species can still develop to adults, its lifespan is shorter than that of wild fruit flies. I used this kind of fruit fly to do experiments because they have accumulated many free radicals in the body, and alkaline hydrogen water (negatively charged) can neutralize positively charged free radicals, so feeding hydrogen-water on the mutant flies are more evident than wild species. In the course of the experiment, I found that the mutant flies fasting (stop feeding water), causing the flies to die. It proved that the fruit fly did have poor anti oxidative stress, so I did not fast-feed the fruit flies in advance during the experiment. Only for the wild flies, fasting beforehand.

In the study, the experimental result shows that the mutant fruit flies of *Drosophila melanogaster* have a much stronger response to hydrogen water than wild ones. It appears to support a possibility that hydrogen-water seems to be a definite help for individuals who are more likely to accumulate free radicals. Therefore, it is the best way to use the mutant flies directly for anti-free radicals experiments. There will be more significant results. It is also possible to test older flies that are more sensitive to alkaline electrolyzed water because the larger the flies, the more free radicals will accumulate in the body. In our experiments, we found that the older the flies, the more they can't afford the pressure of fasting. On the other hand, the open trial can also directly use strong alkaline hydrogen water to feed mutant flies. In addition, the moisture of the feeding environment in the test tube during the experiment is also vital; otherwise, the fruit flies will die a lot and affect the experimental result. In the same operation, the problem of escaping of the flies is also to avoid. Otherwise, if the trial flies are too few, they are not representative.

2. Materials and methods

The untreated tap water is almost neutral at about pH 7.0 with a positive oxidative potential of +150 mV. The tap water after filtered and electrolysis treated, the cathode hydrogen dissolving water belongs to be alkaline at a pH over 8.0 with a relatively high negative reductive potential of −150 to −300 mV. Recently, a lot of literatures [1–3] found that drinking hydrogen water increases the activity of a critical detoxifying enzyme of superoxide dismutase in the body. It protects against free radicals toxic damage on protein, enzyme, DNA, lipid, and membrane in cells (see **Figure 1**). Since the alkaline hydrogen water molecules with an extra amount of free electrons, which can neutralize the highly reactive free radicals before free radicals take away free electrons from intracellular molecules.

Water, which constitutes over 70% of the body, is involved in virtually every function of life. It forms the bodily fluids, such as blood, lymph, cerebrospinal fluid, saliva, and digestive fluids to regulate the metabolism of joint lubrication,

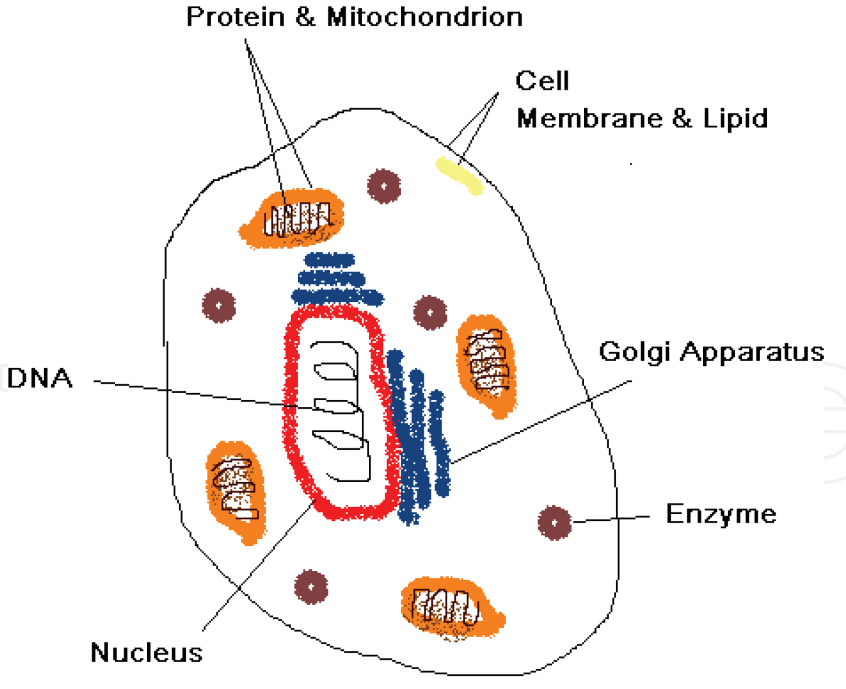

Figure 1.
Free radicals toxic damage on protein, enzyme, DNA, lipid, and membrane.

detoxification, and maintaining the blood pressure. While the water molecules around the cell membranes, they show a long-range ordering feature and like an epitaxial liquid crystal with distinct properties from the bulk state [1]. Shirahata et al. [2] showed that drinking hydrogen water increases the activity of a critical detoxifying enzyme, superoxide dismutase (SOD), which exists in humans, animals, plants, and micro-organisms, as an essential antioxidant to protect cell under oxidative stress damage in vitro. Superoxide is one of the primary reactive oxygen species in the cell. So SODs serves a key antioxidant role. This antioxidant function exerted by molecular hydrogen has been proved [3]. In the rat model, drinking hydrogen water showed signs in the decrease of the peroxided lipid level in their urine. When comparing to tap water to hydrogen-water, the rat drinks hydrogen-water can extended to live 20–50% longer [4]. Finally, we studied the survival rate of drinking hydrogen-water on Drosophila flies after fasting, which related to the regulatory function of oxidative stress attacked by free radicals in cells.

Drosophila lacking SOD1 has a dramatically shortened lifespan, whereas fly lacking SOD2 will die before birth. Lacking SOD3 does not show any visible defects and exhibit an average lifespan, though more sensitive to hyper oxidative injury. *Drosophila melanogaster* is a species of fruit flies. Drosophila fly is typically used in research because it can readily rear in the laboratory. Since Charles W. Woodworth used *Drosophila melanogaster* as modal organism, eight Nobel prizes have awarded to research using Drosophila. It continues to be widely applied to biological research in genetics, physiology, microbial pathogenesis, and lifespan [5]. Wild-type fruit flies are yellow-brown, with brick-red eyes and transverse black rings across the abdomen. Females are about 2.5 mm long, and males are slightly smaller with darker backs (see **Figure 2**).

The wild-type and mutant-type flies obtained from the *Drosophila melanogaster* stock center at the National Health Research Institutes in Taiwan. Peroxide reductase-1 is one of the mutant-type fruit flies. *Drosophila melanogaster* flies have every six tubes of females and males of 2 day-old-adult flies, 30 flies per tube. Four ones (20/tube) of females and four ones (30 tubes/tube) of males collect within 2 day-olds of adult flies. These flies gently placed to the bottom of the vial (9.5 cm tall × 2.4 cm diameter) and top with cotton swabs and balls with pure neutral water and

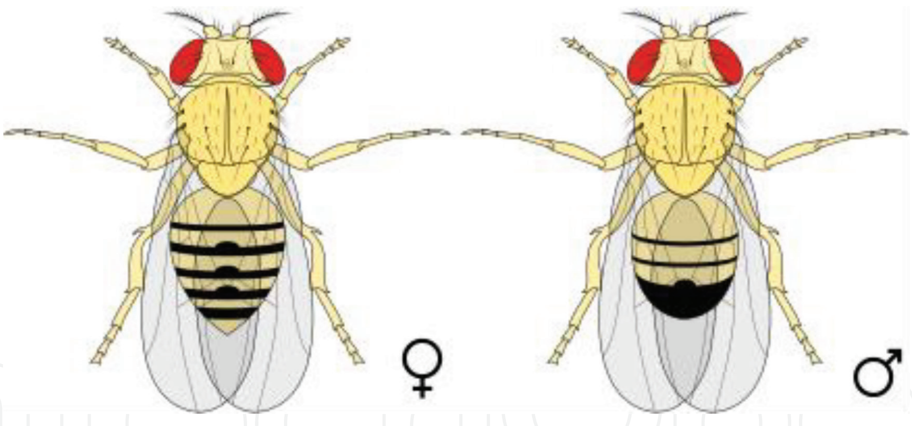

Figure 2.
Female (left) and male (right) of Drosophila melanogaster [6].

alkaline hydrogen water (pH 8.5 and 9.5) in the standard medium supplement at room temperature of 50–70% relative humidity. Before experiments, each group carefully collected 2 day-old males and females of adult flies, respectively. The experimental procedures are as follows: 1. Wild-type flies have fasted for 12 hours and then feed water, while the munt flies do not. 2. Using wetted cotton swabs inserted into the test tube for two feeds a day. 3. Observe the death of the flies every 12 hours until all the flies have died. 4. All experiments treatments kept at the standard medium. 5. If the feeding tube wetted with water droplets and change it periodically.

3. Experimental results

For the control group, every two tubes are wild and mutant species of fruit flies feed with pure water of pH 7. One is female fly, and another is male fly. For the treatment group, every four tubes are wild and mutant species with the female and the male ones fed with alkaline hydrogen water of pH 8.5 and 9.5, respectively. Generally, regardless of whether it is a wild fruit fly or a mutant fruit fly, in the fasting state, feeding of hydrogen water has a significant increase in the survival rate (see **Figures 3**–**6**).

3.1 The mutant and wild species of Drosophila

If we compare with the results of the mutant and wild species, the mutant fruit fly of males to feed weak alkaline hydrogen water of pH 8.5 is the most significant change in survival among them (see **Figure 3** vs. **Figure 5**; **Figure 4** vs. **Figure 6**). In the case of feeding mutant flies with pure water of pH 7, for example, when fasting for more than 4 days, half number of deaths occur. If weak alkaline hydrogen water of pH 8.5 fed, it will delay to more than 9 days to happen it (see **Figure 3**). That is about a twice of increase in the lifespan. Therefore, a weak alkaline hydrogen water of pH 8.5 seems to have a positive effect on the survival rate of flies in the state of starvation.

3.2 The females and males of Drosophila

Past research results have shown that the average lifespan of females is longer than that of males. Our experimental results showed that the effect in response to the feeding of hydrogen water, the male-fly was significantly more than the female-fly for all the wild and mutant species. While the case of feeding wild flies with pure water, for example, fasting for more than four to 6 days, to dead a half (see **Figures 5** and **6**).

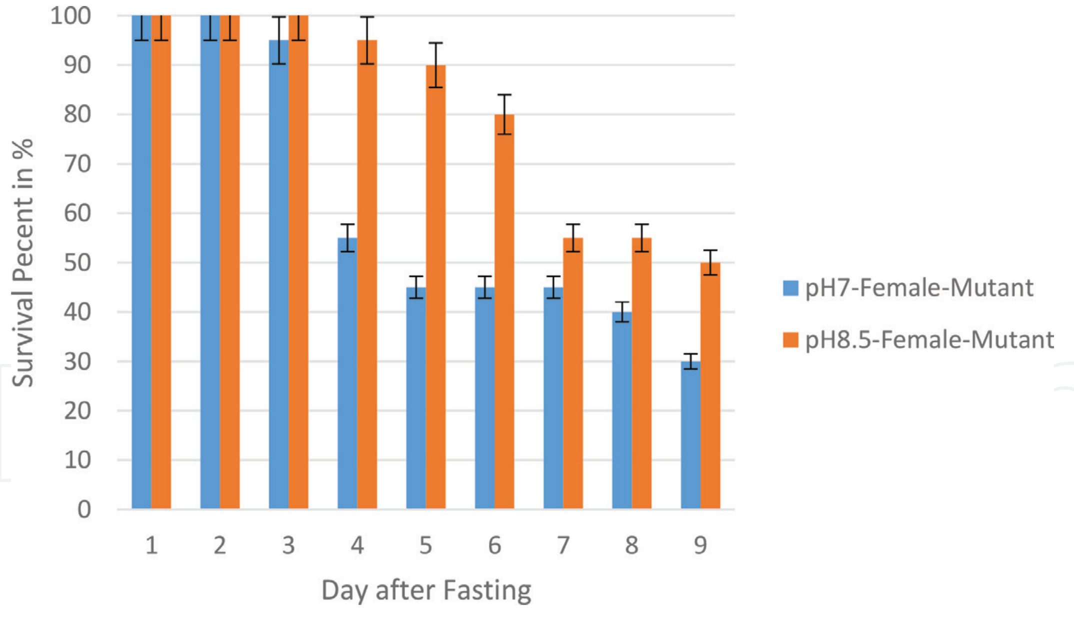

Figure 3.
Survival percent in % of female-mutant fruit flies vs. day after fasting. Note: pH 7: pure water; pH 8.5: weak-alkaline-H2-water.

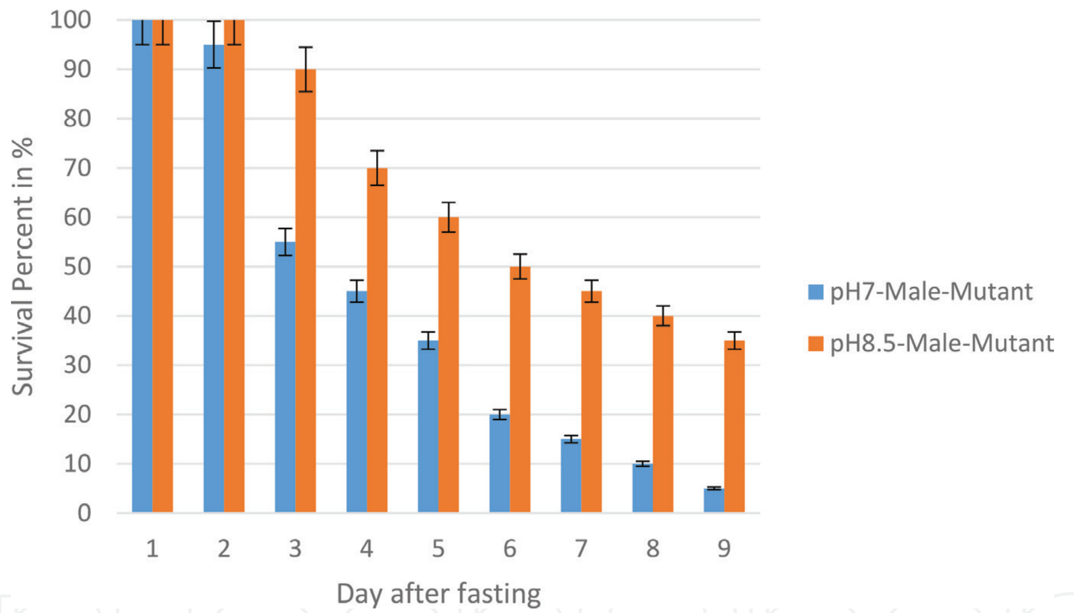

Figure 4.
Survival percent in % of male-mutant fruit flies vs. day after fasting. Note: pH 7: pure water; pH 8.5: weak-alkaline-H2-water.

If hydrogen water supplied, fasting time of female fly and male fly extended for more than 7 days. However, hydrogen water seems to have a less help on the survival rate of wild female flies in the state of starvation (see **Figure 5**). This result implies that hydrogen water appears to be of much more help to individuals with weaker constitutions than to individuals who have stronger ones.

3.3 The alkaline hydrogen water for pH 8.5 and 9.5

Tested for the relationship between the alkaline pH (pH 8.5 and 9.5) and the survival response. Our previous results have shown that drinking hydrogen water seems to be helpful for the survival rate of Drosophila in the case of starvation. Therefore, it is worthwhile to analyze hydrogen water in alkalinity further the impact on the survival rate of Drosophila. When the pH of the hydrogen water

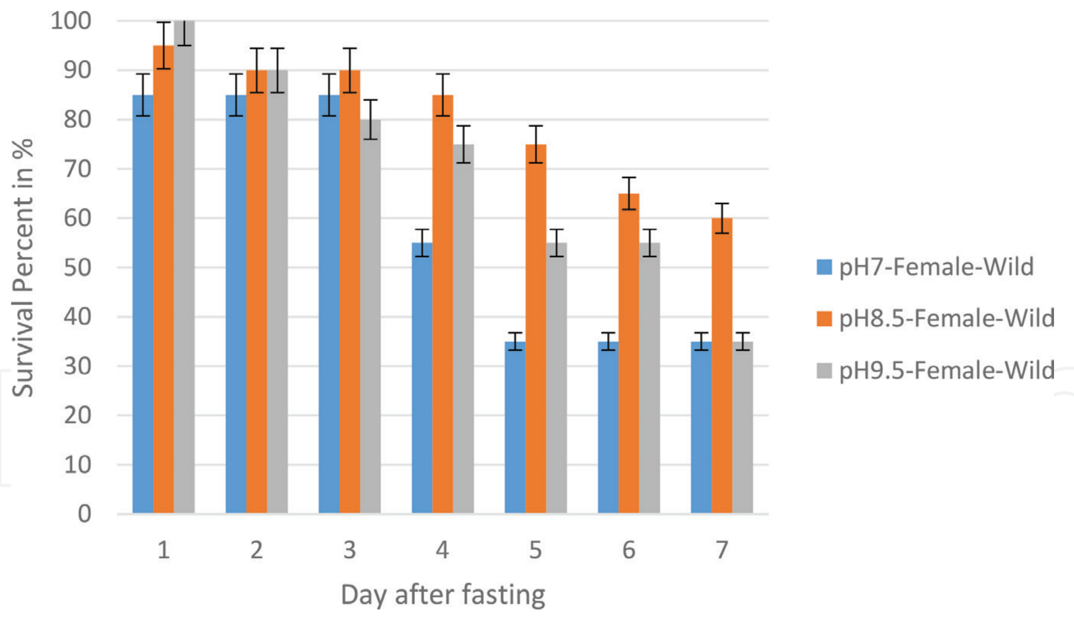

Figure 5.
Survival percent in % of female-wild flies vs. day after fasting. Note: pH 7: pure water; pH 8.5: weak-alkaline-H2-water; pH 9.5: medium-alkaline-H2-water.

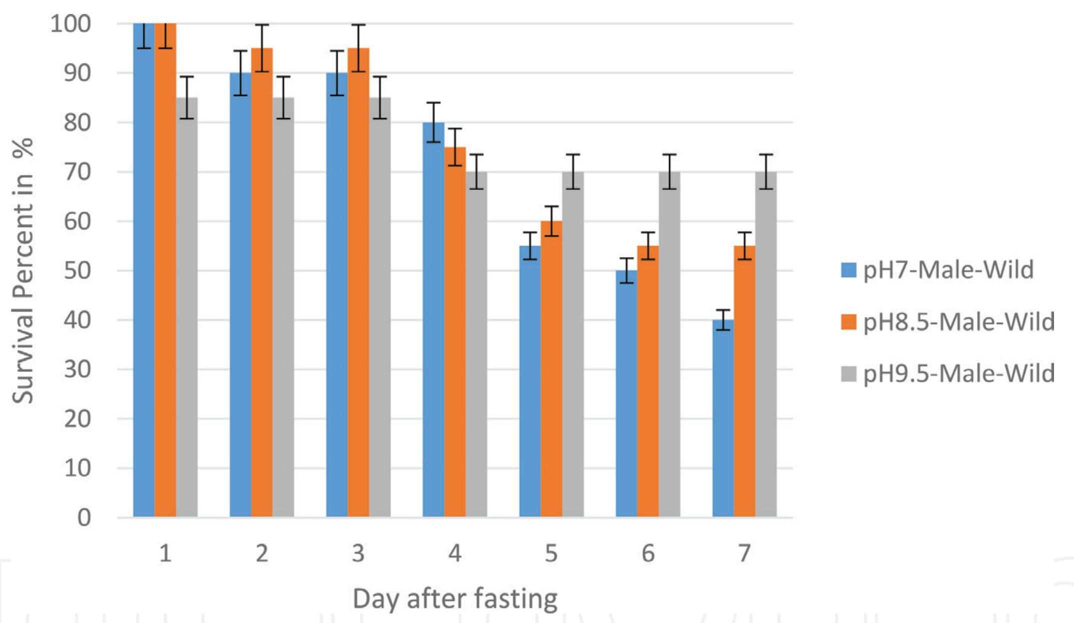

Figure 6.
Survival percent in % of male-wild flies vs. day after fasting. Note: pH 7: pure water; pH 8.5: weak-alkaline-H2-water; pH 9.5: medium-alkaline-H2-water.

increased from 8.5 to 9.5, after fasting time more than 4 days the significant increase in the survival rate of the male fly observed in **Figure 6**. However, for the female fly, the result is not the same. Interestingly, although the wild species of female fruit fly, hydrogen water increased from pH 8.5 to 9.5, the survival response was slightly decreased. That is to say, further addition in alkalinity, however, has the opposite effect on the female flies, and the survival rate does not rise any longer.

4. Results and discussions

In the past, many works of literature have reported that human aging may be related to the function of free radicals to destroy cells. However, why do free radicals come? For example, the role of cells depends on the oxidation and decomposes the nutrients in the body. It is like gasoline, which burns oxygen to release heat energy and promote steam. However, in the process of redox, cells produce a byproduct, oxygen free radicals. The oxygen molecule (O_2) itself has 16 electrons, but it loses one electron in the process of the redox reaction, and the oxygen that becomes a single electron becomes very unstable, and it will destroy the function of the cell and even cause disease. Interestingly, if the neutral water is decomposed using a water electrolysis generator, the water molecules will change to negatively charged alkaline hydrogen water. If the electrolytic liquids are separated, acidic water will act like oxygen free radicals to destroy the cells. However, hydrogen water is the opposite, with an extra free electron, which can combine with free radicals to neutralize the ability to equilibrate free radicals before free radicals take away an electron from intracellular molecules. Therefore, hydrogen water may have antioxidant function and protect cells from free radical damage.

5. Conclusions

This experiment supports that drink hydrogen water may help the fasting fly survival rate. When oxygen free radicals in the intestine are out of balance, hydrogen water may neutralize free radicals and reduce damage to cells. Drosophila has an innate immune system similar to humans, and the structure and physiology of the fruit fly's gastro-intestine, cardiac, and neurological diseases resemble that of humans. Even if the genes and mechanisms that they are involved whether conserved or not, the biological process in a similar genes species often provides a valuable framework to study anti-oxidative stress effect and allow development of potential clinical applications.

When Drosophila intestinal oxygen free radicals increase due to fasting, the increased reactive oxygen species in the intestine pass through nitric oxide, erythrocytes, and another non-nitric oxide signal to activate transcription of NF-κB protein in the fat of Drosophila. The factor, Drosophila liver, promptly initiated in the response of cells to many stimuli, including oxidative stress, cytokines, free radicals, ultraviolet radiation, and immune response of the antibacterial peptide, causing a systemic immune response in the Drosophila. The biological model of Drosophila used to simulate that the immune response of human organs is closely related to intestinal health [7].

However, this does not mean that drinking hydrogen water is beneficial to all healthy individual because, in the body, some enzymes that regulate free radicals, which can balance the problems caused by the accumulation of free radicals. Therefore, drinking a significant amount of hydrogen water in a healthy state may be interference to the body's natural regulation mechanism. In other words, as healthy people do not need and should not take medicine, only those who are sick need to follow the doctor's instructions. All in all, the function of hydrogen water on the human body needs further experiments to be further studied. This experiment can only support the flies in the extreme unfavorable environmental pressure; feeding hydrogen water may increase its survival rate.

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