Mechanisms of Ageing and Development, 67 (1993) 227–237
Elsevier Scientific Publishers Ireland Ltd.

INHIBITION OF IRON ABSORPTION PROLONGS THE LIFE SPAN OF *DROSOPHILA*

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(Received April 1st, 1992)

SUMMARY

The life span of *Drosophila melanogaster* (Oregon R) males was found to be proportional to the logarithm of the iron content of the diet. Life span was also shown to be proportional to the rate of iron accumulation for *Drosophila*, mice and man. The total body iron content was found to correlate with the total calcium content of adult *Drosophila*. Iron content during the developmental stages, however, remained relatively constant and did not change with changes in the calcium concentrations. Dietary tea (*Camellia sinensis*) extracts were found to inhibit the ageing-related accumulation of iron and to prolong the life span of *Drosophila* by as much as 21.4%. It is concluded that iron accumulation is a significant factor contributing to senescence.

Key words: Iron; *Drosophila*; Ageing; Tea; Calcium; Life span

INTRODUCTION

Since there is no known mechanism for the excretion of iron, it is possible that iron overload is a risk for adult humans, especially men and postmenopausal women. Growing children and premenopausal women, in contrast, probably rarely experience iron overload because of growth and blood loss. This is indicated by the fact that serum ferritin, which is the best indicator of total body iron content, increases from 30 μg/l at age 15 to more than 90 μg/l at age 30 in males. After age 30 serum ferritin continues to increase gradually. In females the increase is delayed until

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after age 40 [1]. This observation has prompted Sullivan [2,3] to hypothesize that the greater incidence of heart disease in men and postmenopausal women compared with the incidence in premenopausal women results from the higher levels of stored iron in these two groups. In support of this idea are the observations that myocardial failure is a prominent feature of hemochromatosis, thalassemia major and dietary iron overload. Other disease states are also associated with iron. In patients with iron overload, functional impairment of monocytes and granulocytes has been reported [4]. Among iron miners in France the incidence of lung cancer is more than 3 times the number in the French male population in the same age group [5]. Elevated iron may be involved in rheumatoid disease [6,7]. In neuronal ceroid lipofuscinoses, higher concentrations of non-protein bound iron have been found in the cerebrospinal fluid of patients [8,9]. Many cases of severe spinal osteoporosis have been found in iron overloaded South African males [10]. It is also known that iron carbohydrate complexes are carcinogenic [11], that ferrous sulfate is mutagenic in bacteria [12] and that ferritin is clastogenic [13]. Weinberg [14] concludes in his review of the iron literature that 'procedures for preventing the accumulation of excess iron in a human or animal population would be expected to lower the incidence not only of infection and neoplasia but also of chronic cardiac failure'. We propose that these procedures might also reduce the rate of ageing.

The mechanism for tissue damage in iron overload conditions is not firmly established but it may involve free radical mechanisms where ferrous and ferric ions act as catalysts. This would explain why relatively small changes in iron concentrations might be responsible for extensive tissue damage. The Fenton reaction: $H_2O_2 + Fe^2 \rightarrow Fe^3 + .OH + OH-$, is widely believed to be a major source of damaging hydroxyl radicals but other reactions including iron-catalyzed lipid peroxidation have been proposed [15]. Tissue damage or cell death might occur if cellular membranes are made more permeable by iron catalyzed oxidation reactions with a resultant influx of calcium into the cell. Evidence that this may occur during the ageing process is suggested by the result of Persigehl et al. [16] where they found increased iron concentrations with ageing in human tissues with the greatest increase occurring in aorta. It has been established in other studies [17,18] that large increases in calcium in aortic and femoral arteries are found during human ageing. In diseased aortas, calcium and iron concentrations are considerably elevated not only in the areas containing plaque or aneurysmal tissue but also in the the areas free of plaque [19]. Other metal ions including manganese, zinc and copper were found to be unchanged. In other disease states, a close relationship between histologically observed calcification and iron deposition has been repeatedly observed in various forms of clinical calcinosis [20]. Rivenson et al. [21] have reported heavy mineralization with calcium and iron deposits in tracheal cartilages, kidneys, prostate gland and some arteries in ageing rats maintained on a high fat diet.

Age pigments contain iron [22,23]. These particles may represent the site at which metal ions catalyze the production of free radicals and peroxidation reactions. This

subject has been reviewed by Halliwell and Gutteridge [15]. The observations of Perl et al. [24] of high accumulations of iron, calcium and aluminum in patients with amyotrophic lateral sclerosis and Parkinsonism-dementia suggest that iron may also be related to aluminum deposition.

The use of iron salts in the supplementation of baking flour is mandatory national policy in the USA and other countries and the use of iron dietary supplements is widespread among adults and the aged. Although it is known that some children and young women require such extra dietary supplements, the rest of the population may be exposed to an unnecessary hazard. Of all the metal ions that undergo univalent redox reactions, 'iron has been found to be the most active promoter of lipid peroxidation both in vitro and in vivo' [25]. In addition, iron is found in very high concentrations in most tissues. Since the role of iron ions and their complexes is widely acknowledged as a key factor in the promotion of non-enzymatic autoxidation of biological materials, it is not unreasonable to propose that iron excess is involved in senescence.

We have previously shown that iron accumulates during the adult and developmental stages of *Drosophila melanogaster* with an overall increase of 186% at 25°C [26]. Similar increases were found at 20 and 30°C. The rate of iron accumulation varied with environmental temperature with the logarithm of the rate proportional to temperature. The rate of iron accumulation with ageing was, thus, found to be proportional to the rate of ageing, suggesting that excess dietary iron may be an initiator of senescence. These results were in agreement with the previous observations of Sohal and Lamb for iron accumulation in *Musca domestica* [23]. Iron accumulation with ageing also occurs in other species. We have reported an increase with ageing in iron accumulation in the brain, kidney, heart, liver and bone of mice [27,28], with bone showing the largest increase (207%). Based on this result, we have also suggested that ageing-related osteopenia may be an iron overload disease. Previous work has also shown that changes in ambient temperature influence the rate of respiration of *Drosophila* [29,30]. The increased rate of iron accumulation at higher temperatures may, therefore, be linked to the increased activity of the mitochondrial respiratory chain at the higher temperatures.

Here we report that iron accumulation is related to calcium accumulation in *Drosophila* and that inhibition of iron absorption increases the life span of *Drosophila*.

MATERIALS AND METHODS

Oregon R *Drosophila* fruit flies were reared and maintained on yellow corn meal medium as previously described [31]. Flies were maintained in an environmentally controlled incubator at 25°C on a 12/12 h (light/dark) cycle at 70% relative humidity. Survival studies were done at 25°C with 100 male flies per group on Formula 4–24 Instant Medium (Carolina Biological Suppl C.). Data were analyzed according to

Student's *t*-test. A degree of certainty of greater than 95% ($P < 0.05$) was considered to be a significant difference in the median life span.

After drying overnight at 88°C flies were digested in Ultrex nitric acid (J.T. Baker Co.) for 5 days at room temperature. Iron and calcium were analyzed on a Varian 1250 atomic absorption spectrophotometer with carbon rod atomizer Model 90 at 248.3 and 422.7 nm, respectively. We used nitrogen as a sheath gas and atomized at 2200 and 2400°C for iron and calcium, respectively.

RESULTS

We have measured the dietary content of iron in *Drosophila* food and how it relates to life span. The normal food contains 47.4 μg/g of food. Adding ferrous gluconate or ferrous chloride to the food produced a decrease in life span which was proportional to the logarithm of the iron content of the diet (Fig. 1). We have, therefore, observed that increased dietary iron reduces longevity.

The possible general involvement of iron in senescence is further indicated by the relative rates of iron accumulation in different organisms (Fig. 2). The rate for mice is for iron accumulation in the heart of C57BL/6J male mice in our laboratory. The human value is for the rate of increase of serum ferritin in human males. As discussed above, serum ferritin is directly proportional to body iron stores in male humans. The *Drosophila* values are for whole body iron accumulation rates found in our laboratory at four different environmental temperatures (11, 20, 25 and 30°C). The rate

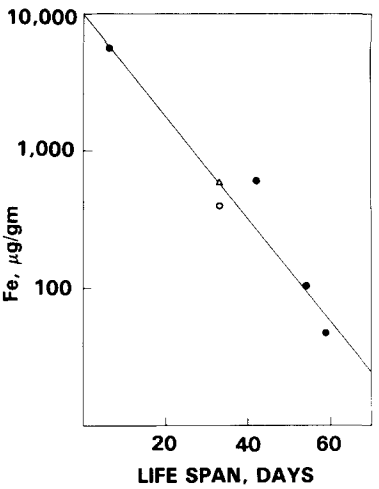

Fig. 1. Semilogarithmic plot of iron content of *Drosophila* food and median life span. ●, ferrous gluconate added; ○, △, ferrous chloride added. Both ferrous compounds were added to Instant *Drosophila* medium to achieve the different iron concentrations.

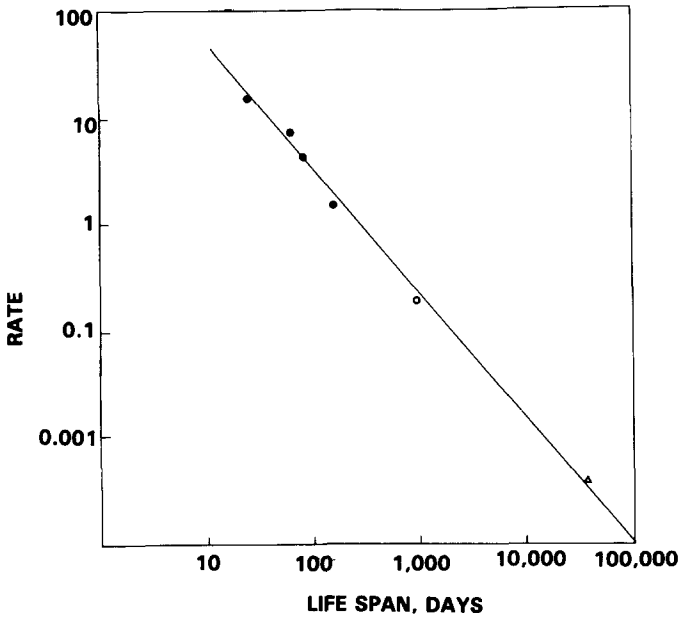

Fig. 2. Rate of iron accumulation versus age for whole *Drosophila* at different temperatures, ●; mouse heart, O; and human serum ferritin, Δ. Rate is ng iron/mg dry wt./day accumulated. Life span is the median life span in days.

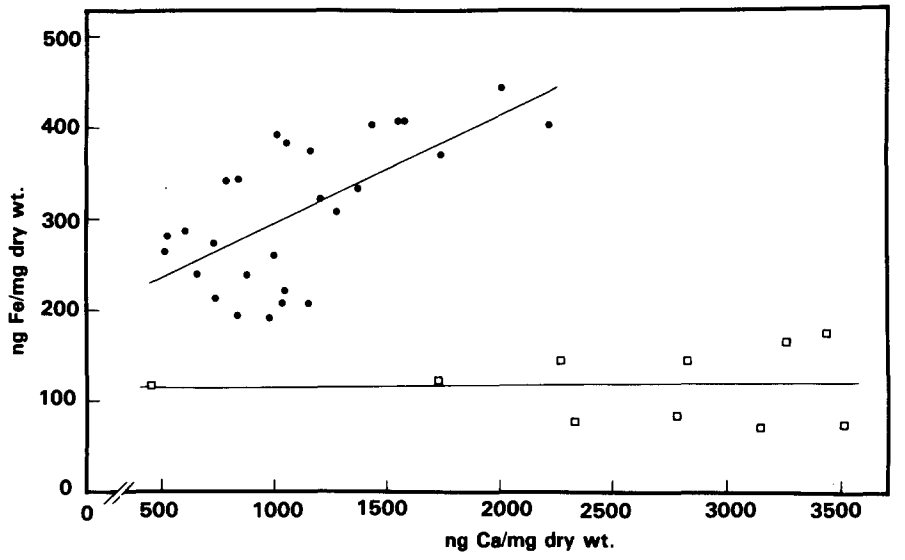

Fig. 3. Correlation between iron and calcium in adult, ● and developmental stages, □, of Oregon R male *D. melanogaster* at 25°C.

of iron accumulation in a wide range of different organisms is, therefore, inversely proportional to life span.

We have previously reported that total body calcium increases with ageing in *Drosophila* [32]. During development, from the egg to the late pupal stage, the large changes in total calcium are not related to changes in iron (Fig. 3). For the adult stage, however, there was a close correlation between total body iron and calcium, when iron and calcium were measured on identical fly samples ranging in age from 0 to 64 days. The result suggests but does not establish iron accumulation as a cause of ageing-related calcification.

Based on this observation, it seemed to us that inhibition of excess iron accumulation might be expected to alter the ageing process. We found that dietary tea (*Camellia sinensis*) prevented the ageing-related accumulation of iron in *Drosophila*. When the water used to make up Instant *Drosophila* medium was replaced, beginning at one day of adult age, with an aqueous extract of tea, the amount of iron in old flies remained as low as that found in young flies (Fig. 4). In addition, when adult flies were maintained on the medium containing tea for the entire adult stage there was an increase in life span with the entire survival being shifted to higher values (Fig. 5). Dilutions of the tea extract also improved life span but not all of the extensions were significant. Green tea improved the median life span to a lesser extent (8.3%). A brand of decaffeinated tea, however, failed to significantly improve survival (Table I).

In an effort to find the factor in tea responsible for improved survival, we examined the influence of some known components of tea on life span. None of the compounds tested, including caffeine, catechin, epicatechin, tannic acid, theobromine and theophylline, had any significant effect on life span (Table II). We are thus unable to explain the life extension achieved with tea except to suggest that the solvent

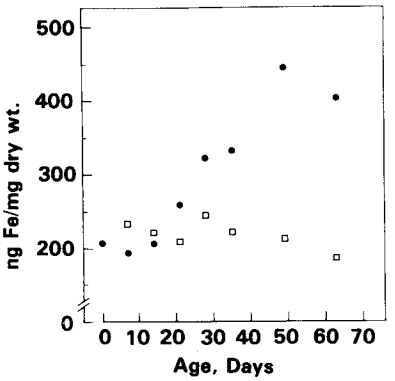

Fig. 4. Iron content of whole *Drosophila* (male Oregon R) versus age for control diet, ● and diet where water was replaced with brewed (one tea bag in 100 ml water at 100°C for 5 min) tea, □.

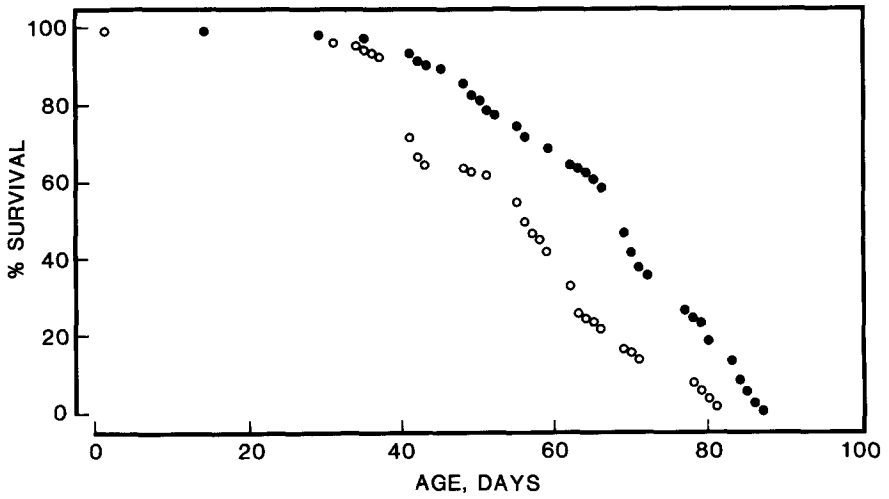

Fig. 5. Survival versus age for Oregon R *D. melanogaster* males at 25°C; water control, O; brewed 'Tetley' tea, ●.

TABLE I

SURVIVAL TIMES OF *D. MELANOGASTER* (OREGON-R MALES) MAINTAINED AS ADULTS, BEGINNING AT 1 DAY OF AGE, ON MEDIUM CONTAINING TEA

Substances	*80%* survival	*Median survival (days)*	*20% survival*	*% Change from control median*	*Mean survival ± S.D.*
Tea (Tetley brand)	50	59	69	+18.0	60.3 ± 12.1**
Tea 1:10 dilution	39	53	59	+6.0	52.3 ± 14.2
Tea 1:100 dilution	41	51	62	+2.0	52.1 ± 14.8
Control	40	50	63	—	52.1 ± 14.2
Tea (Tetley)	50	68	80	+21.4	66.1 ± 15.2**
Contol	40	56	66	—	55.0 ± 14.7
Tea (Salada brand)	43	56	63	+16.7	54.4 ± 9.9**
Tea 1:10 dilution	43	52	58	+8.3	51.9 ± 9.8*
Tea 1:100 dilution	37	48	58	0	48.5 ± 11.7
Control	38	48	58	—	48.9 ± 11.2
Green tea	54	65	76	+8.3	64.5 ± 13.2**
Green tea 1:10 dilution	47	60	71	0	59.8 ± 13.2
Green tea 1:100 dilution	48	57	68	−5.0	58.1 ± 12.4
Control	49	60	68	—	59.4 ± 11.4
Decaffeinated tea	47	59	71	+3.5	59.8 ± 12.3
1:10 dilution	41	54	68	−5.3	55.8 ± 13.0
1:100 dilution	39	54	67	−5.3	56.0 ± 11.5
Control	47	57	68	—	57.5 ± 11.7

*Indicates significant at $P < 0.05$.
**Indicates significant at $P < 0.01$.

TABLE II

SURVIVAL TIMES OF *D. MELANOGASTER* (OREGON-R MALES) MAINTAINED AS ADULTS, BEGINNING AT 1 DAY OF AGE, ON MEDIUM CONTAINING CONSTITUENTS OF TEA

Compound	*Concentration*	*80% survival*	*Median survival (days)*	*20% survival*	*% Change from control median*	*Mean survival ± S.D.*
Caffeine	1.0 mg/ml	47	58	66	−3.3	57.9 ± 9.8
Caffeine	0.1 mg/ml	48	61	69	+1.7	60.4 ± 11.3
Caffeine	0.01 mg/ml	47	54	69	−10.1	57.8 ± 12.3
Control	0	47	60	70	—	59.9 ± 11.2
Catechin	1.0 mg/ml	42	56	62	−5.1	55.1 ± 10.5
Catechin	0.1 mg/ml	42	54	64	−8.5	54.5 ± 12.8
Catechin	0.01 mg/ml	47	56	70	−5.1	57.2 ± 12.1
Control	0	45	59	68	—	57.5 ± 12.5
Epicatechin	1.0 mg/ml	45	58	62	+7.4	55.5 ± 11.6
Epicatechin	0.1 mg/ml	45	56	63	+3.7	55.8 ± 10.7
Epicatechin	0.01 mg/ml	46	55	63	+1.8	56.1 ± 10.2
Control	0	45	54	66	—	55.9 ± 11.9
Tannic acid	1.0 mg/ml	57	67	72	−2.9	65.6 ± 10.6
Tannic acid	1.0 mg/ml	57	69	77	0	67.8 ± 11.9
Tannic add	0.1 mg/ml	49	69	75	0	64.7 ± 13.0
Contol	0	52	69	75	—	64.6 ± 14.4
Theobromine	0.50 mg/ml	45	54	69	0	56.8 ± 14.4
Theobromine	0.05 mg/ml	46	54	69	0	57.7 ± 11.5
Theobromlne	0.005 mg/ml	48	55	66	+1.8	57.0 ± 11.2
Control	0	48	54	65	—	56.8 ± 10.9
Theophylline	1×10^{-3} M	37	46	53	0	44.4 ± 12.0
Theophylline	1×10^{-4} M	40	48	57	+4.4	48.6 ± 9.8
Theophyilline	1×10^{-5} M	40	48	55	+4.4	48.0 ± 8.1
Control	0	40	46	53	—	47.6 ± 7.8

TABLE III

SURVIVAL TIMES OF *D. MELANOGASTER* (OREGON-R MALES) MAINTAINED AS ADULTS, BEGINNING AT 1 DAY OF AGE, ON MEDIUM CONTAINING TEA AND IRON (FERROUS GLUCONATE)

Substance	*80% survival*	*Median survival (days)*	*20% survival*	*% Change from control median*	*Mean survival ± S.D.*
Tea	50	68	80	+21.4	66.1 ± 15.2**
0.01 M ferrous gluconate	36	41	56	−26.8	46.4 ± 12.4**
Tea + 0.01 M ferrous gluconate	47	65	70	+16.1	60.0 ± 12.9*
Control	40	56	66	—	55.0 ± 14.7

*Indicates significance at $P < 0.05$.
**Indicates significance at $P < 0.01$.

used to produce decaffeinated tea probably also removes the factor or factors responsible for the increased survival of *Drosophila*.

Dietary tea also prevented the reduction in life span induced by excess dietary iron. A final concentration of 0.01 M ferrous gluconate in the *Drosophila* food medium, for example, reduced the median life span by 26.8% compared to the water control. Replacing the water with tea extract, however, increased the median life span by 16.1% even in the continued presence of 0.01 M ferrous gluconate (Table III). This observation adds additional credence to the idea that some factor in dietary tea prolongs survival by inhibiting iron absorption.

DISCUSSION

The principal result of this investigation is that dietary extracts of tea can prolong the life span of *Drosophila*. The mechanism for this effect appears to be inhibition of iron absorption. This is consistent with the report of Alarcon et al. [33] where tea was used to inhibit iron absorption by 41 to 95% in thalassemia patients where the major cause of death was iron overload. The ability of dietary tea to inhibit iron absorption has prompted others to warn that tea drinking may even contribute to the pathogenesis of iron deficiency [34]. We have found that tea is an effective tool for changing the ageing-related accumulation of iron in *Drosophila*. The reduction in total body iron achieved with dietary tea is also accompanied by an increase in life span. We have tested four different commercially available brands of tea and have found increases in the median life span ranging from 8.3 to 21.4%, with black tea giving the largest increase in life span. Unfortunately, tea is not a well characterized substance and we are unable to say at this time what factor is responsible for both the reduction in iron absorption and the increase in life span. We found that several known components of tea were not responsible for the effect, including caffeine, catechin, epicatechin, tannic acid, theobromine and theophylline.

We have also found that the rate of iron accumulation is inversely proportional to life span and that excess dietary iron increases the rate of ageing for *Drosophila*. The apparent correlation between iron and calcium accumulation in adult flies is consistent with the previous reports of others demonstrating the association of iron deposition with histologically observed calcification [16,19–21].

Overall, it appears that numerous lines of evidence point to the involvement of iron overload in degenerative diseases and in the ageing process itself. The current practice of adding iron supplements to numerous food products for human consumption may not, therefore, be without risk. Whether or not dietary tea could prove to be of benefit in controlling the ageing-related increase in iron accumulation found during human senescence remains to be seen.

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