*Experimental Gerontology*, Vol. 19, pp. 393–399, 1984
Printed in the USA. 

0531-5565/84 $3.00 + .00


# THE EFFECT OF DIETARY METHIONINE ON THE COPPER CONTENT OF TISSUES AND SURVIVAL OF YOUNG AND OLD MICE

HAROLD R. MASSIE and VALERIE R. AIELLO

Masonic Medical Research Laboratory, Utica, NY 13504

(*Received* 25 *June* 1984)

**Abstract**—We tested the possibility that methionine might improve the lifespan of male C57BL/6J mice, based upon the ability of methionine to chelate copper. Old mice given 0.05M methionine in their drinking water for 42 days had lower brain copper concentrations ($p < 0.05$). The decrease in liver, kidney, and heart copper was not significant when compared to unsupplemented controls. The lifespan of old mice was unchanged by feeding 0.05M methionine. Young mice, however, experienced a 16.9% decrease in their average lifespan and a decreased maximum lifespan when given supplemental methionine. We conclude that dietary supplements of methionine may be useful for removing copper from the brain but they also can increase the rate of senescence in mice.

## INTRODUCTION

METHIONINE IS an essential amino acid needed for normal metabolic function. Lippman (1980, 1981) has suggested that methionine may also function as an inhibitor of senescence based upon its ability to inhibit superoxide radical production from metabolically-active human mitochondria.

Other observations suggest a protective role for methionine in senescence. Amyloid formation is inhibited by methionine (Kornejewa, 1970). A combination of vitamin E and methionine induces glutathione peroxidase activity (Kruhlykova and Shtutman, 1976). Methionine decreases lipid peroxidation induced by carbon tetrachloride (Hafeman and Hoekstra, 1977). The eye lenses of old humans show oxidation of methionine in membrane fractions with extensive oxidation occurring in cataracts (Garner and Spector, 1980). Methionine protects rats from carcinogenesis induced by aflatoxin $B_1$, N-2-fluorenylacetamide, 1,2-dimethylhydrazine (Rogers, *et al.*, 1980) and ethionine (Farber and Ichinose, 1958). Nucleolar volume and polymerase activity in the liver may depend upon dietary methionine (Bailey, *et al.*, 1976).

A diet low in methionine leads to elevated cholesterol levels and atherosclerosis in rats, mice and monkeys (Mann, *et al.*, 1953; Fillios and Mann, 1954; Mann, 1961; Clandinin and Yamashiro, 1980) but has no effect on pigs (Hill, *et al.*, 1971).

In bacteria, certain methionine auxotrophs of *Escherichia coli* lose their colony-forming ability when deprived of methionine, resulting in "methionineless death" (Breit-

man, *et al.*, 1971). A decay of messenger RNA "with a survival curve similar to that of an aging process," also occurs during methionine starvation (Silengo, 1973).

One possible explanation for the favorable effects of methionine may be its ability to chelate copper ions with an equilibrium constant of 14.75 (Chaberek and Martell, 1959). It is known that dietary methionine counteracts the elevated levels of copper in blood plasma and livers of chickens fed diets containing high concentrations of copper (Jensen and Maurice, 1979). This suggested to us that dietary methionine might influence the increase in brain copper known to occur in mice and humans with aging (Schroeder, *et al.*, 1966; Massie, *et al.*, 1979a). Older rats absorb methionine at a slower rate (Pénzes, *et al.*, 1968), and it disappears more rapidly from the tissues of old rats than from young (Barrows and Roeder, 1961). Thus, the increase with aging in both brain and serum copper in mice and humans (Harman, 1965; Herring, *et al.*, 1960; Yunice, *et al.*, 1974; Massie, *et al.*, 1979b) could be due to insufficient dietary methionine. Harman (1965) has proposed that elevated copper levels may accelerate aging by acting as a catalyst for the production of free radicals. Higher dietary levels of methionine should, therefore, improve survival if they are able to lower tissue copper concentrations.

Here we report that high dietary methionine lowers brain copper concentrations but does not increase lifespan.

## MATERIALS AND METHODS

### *Biological sample and diet*

Male C57BL/6J mice obtained from Jackson Labs., Bar Harbor, Maine, were used for all experiments. Mice were purchased at one month of age and introduced into our colony. Purina laboratory chow (which contained 18 ppm copper in the ash) and tap water were given *ad libitum* to the aging colony. According to the label the Purina laboratory chow contained 23.4% protein, 4.5% fat and 0.43% methionine. Animals were kept at 22°C and lights were on 12 hours and off 12 hours.

### *Survival studies*

Animals were removed from the aging colony at 42 and 581 days of age. Mice were placed 7–8 per cage in plastic cages with stainless steel tops. Corncob bedding and distilled water bottles were changed weekly. L-methionine (Sigma or Nutritional Biochemical Co.) was added to the drinking water for the experimental groups. A 0.05M methionine solution was prepared every 3 weeks and stored at 4°C. Mice were given fresh solution bottles weekly. Cages were monitored daily for deaths. Mice were weighed every two weeks until 150 days of age and thereafter monthly. Fighters or injured animals were removed from the group and placed in separate cages. Whenever possible fighters were removed from the experiment during the first few weeks. All animals were allowed to eat Purina laboratory chow pellets without restriction.

### *Data analysis*

Student's *t*-test was used to establish significant differences between groups for both metal content and average survival times.

### *Metal determinations*

Mice were sacrificed between 9 a.m. and 11 a.m. (Eastern Standard Time) in order to avoid possible diurnal changes. Organs were isolated and perfused with 0.1M HEPES buffer (pH 7.8). Single organs were then placed on acid-washed microscope slides and dried overnight in an oven at 88°C. We found that longer drying times did not decrease organ weights. Whole organs were digested in ULTREX $HNO_3$ (J.T. Baker Chemical Co.). Acid digestion was allowed to proceed for 7 days at room temperature. The thin fat layer formed on top of the liver samples was removed by aspiration. Some liver copper may have been lost by this procedure, but it was probably less than 1% of the total. Samples were analyzed on a Varian 1250 atomic absorption spectrophotometer with carbon-rod atomizer Model 90. Both young and old organ samples were checked by the method of standard additions for possible age-related interference with copper detection. None was found under our conditions.

TABLE 1. COPPER CONTENT OF ORGANS FROM C57BL/6J MALE MICE FED 0.05M METHIONINE IN THEIR DRINKING WATER FOR 42 DAYS BEGINNING AT 538 DAYS OF AGE.

| *Organ* | *Copper Content ng/mg dry wt.*[1] | *Number* | *Significance* |
|---|---|---|---|
| liver, control | 12.04 ± 1.26 | 7 | n.s.[2] |
| liver, fed | 11.53 ± 1.37 | 6 | |
| kidney, control | 17.18 ± 1.00 | 7 | n.s. |
| kidney, fed | 16.72 ± 0.95 | 6 | |
| brain, control | 16.29 ± 0.77 | 7 | $p < 0.05$ |
| brain, fed | 14.42 ± 1.72 | 6 | |
| heart, control | 21.15 ± 2.36 | 7 | n.s. |
| heart, fed | 18.81 ± 3.74 | 6 | |

[1]Data are averages ± standard deviation.
[2]n.s. indicates not significant ($p > 0.05$).

## RESULTS

In order to find effective chelating compounds we routinely fed various compounds to old mice for 42 days. Feeding 538 day old mice 0.05M methionine for 42 days failed to significantly change the copper content of liver, kidney or heart (Table 1). The copper content of brain, however, was reduced from 16.3ng to 14.4ng/mg dry wt., with a degree of certainty of greater than 95%.

The lowering by methionine of brain copper concentrations to the levels found in young mice (Massie, *et al.*, 1979a) suggested that feeding excess dietary methionine to old mice might increase longevity. Feeding 0.05M methionine in the drinking water beginning at 581 days of age did result in greater stabilization of total body weight (Figure 1) but it failed to prolong the average lifespan (Figure 2). The average lifespan for the methionine group was 757 days and for the control 755 days (Table 2). It should be noted that the control group for this experiment was unusual. The weight versus age curve declined at a faster than normal rate (Figure 1) and the 755 day value for the average survival time was less than the 900 day value which we normally find. The control group also showed two abrupt drops in survival just prior to and after 800 days of age (Figure 2). The methionine-supplemented group, in contrast, showed an even decline with age. It is probable that the control group contained one or more fighters which we failed to recognize. Such an individual could cause early weight loss and premature death for his cage companions. The two abrupt declines in survival before and after 800 days of age in the control group (Figure 2) suggest that this is a likely possibility. Therefore, the survival curve for the control group should be regarded with suspicion. In view of this, methionine may in fact be toxic to old mice when compared to the expected survival curves.

When young (42 day old) mice were given 0.05M methionine for life, the body weight versus age curve showed essentially no difference between the control and the methionine group (Figure 3). Surprisingly, the average lifespan of the methionine group decreased by 16.9% ($p < 0.05$) (Table 2). Even the maximum lifespan decreased in this experiment (Figure 4). Since the weight versus age curves were essentially identical it is unlikely that differential food intake was responsible for the increased aging of the methionine group.

Our mice consumed about 4ml of liquid per day. The daily intake of methionine from

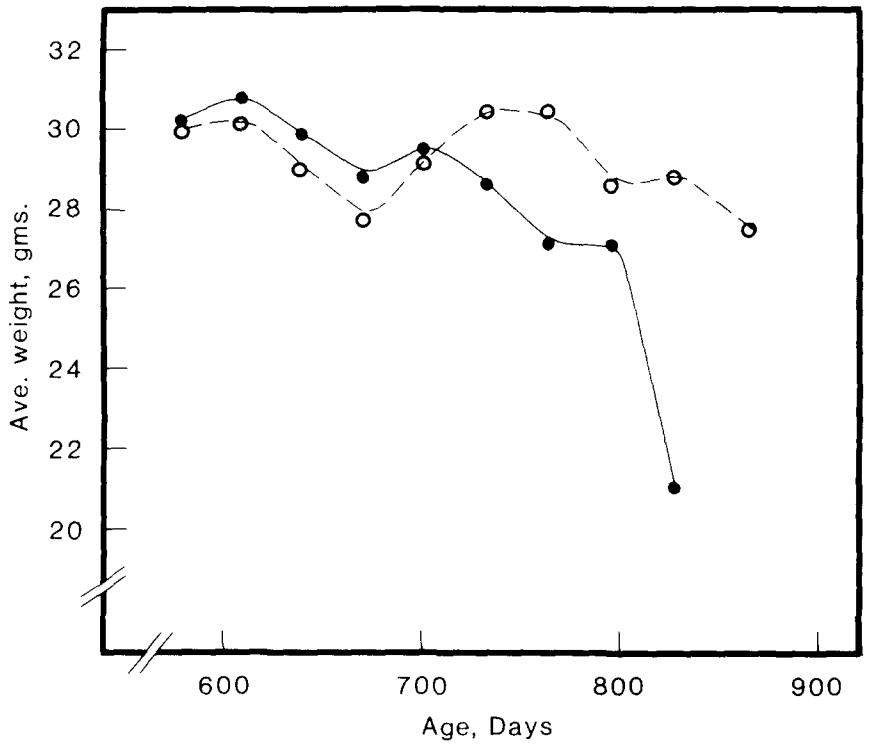


FIG. 1. Average weight in grams versus age for control (——) mice and mice fed 0.05M methionine (----) in their drinking water for the remainder of their life, beginning at 581 days of age.

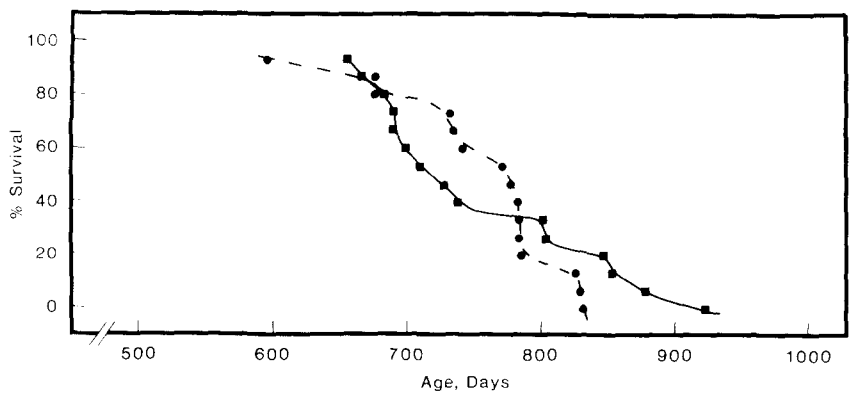


FIG. 2. Survival curves for control (●) and mice receiving 0.05M methionine (■) in their drinking water for the remainder of their life, beginning at 581 days of age.

TABLE 2. CHANGES IN LIFESPAN FROM CONTINUOUS FEEDING OF METHIONINE.

| *Age began, Days* | *Methionine, M* | *Average life span, Days*[1] | *Number of Animals* | *% Change* | *P Value* |
|---|---|---|---|---|---|
| 42 | control | 921 ± 109 | 8 | – | |
| 42 | 0.05 | 766 ± 113 | 8 | −16.9 | $0.01 < p < 0.05$ |
| 581 | control | 755 ± 65.5 | 15 | – | |
| 581 | 0.05 | 757 ± 86.4 | 15 | 0 | $p > 0.05$ |

[1]Data are averages ± standard deviation.

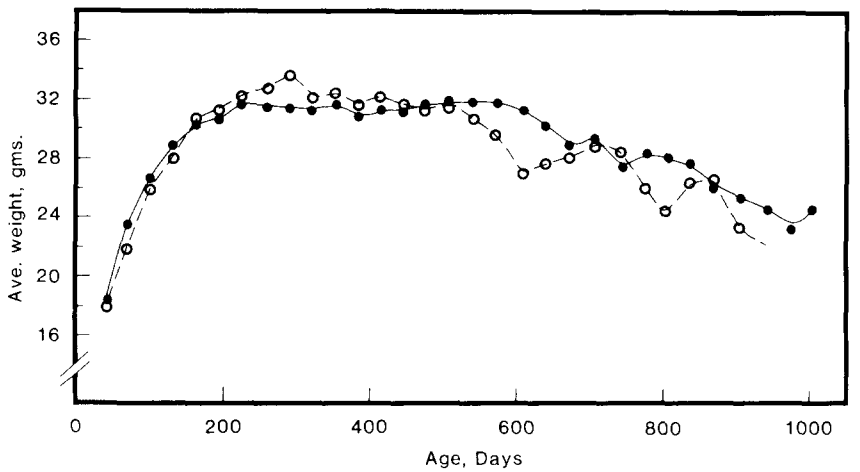


FIG. 3. Average weight in grams versus age for control (——) mice and mice fed 0.05M methionine (---) in their drinking water for life, beginning at 42 days of age.

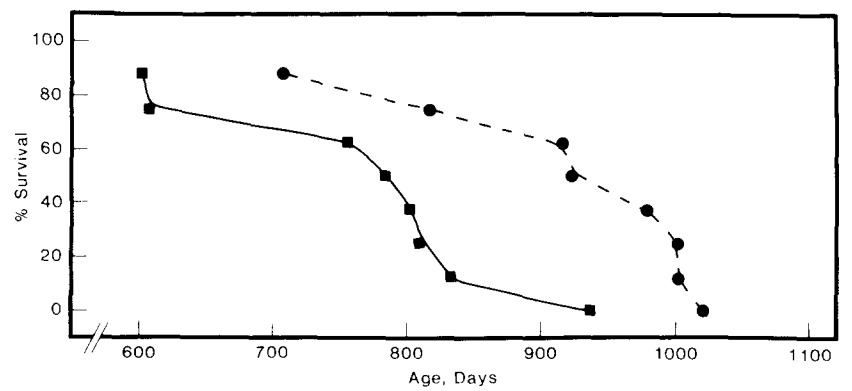


FIG. 4. Survival curves for control (●) and mice receiving 0.05M methionine (■) in their drinking water for life, beginning at 42 days of age.

the drinking water was thus 29.8mg/day. The total solid food consumed was about 5000mg/day and contained 0.43% methionine. Thus, methionine represented approximately 1.03% of the total solid intake. Assuming an average weight of 30gm for our mice, the total intake of methionine for supplemented mice was 1.67gm methionine/kg body weight/day or 11.2 millimoles methionine/kg/day.

In conclusion, dietary methionine was found to lower the copper levels in the brains of old mice but the average lifespan was unchanged. In contrast, feeding methionine to young mice shortened their lifespan. These results indicate that dietary methionine can shorten instead of lengthen the lifespan.

## DISCUSSION

Harter and Baker (1978) observed a decreased rate of weight gain, lower blood hemoglobin and increased spleen iron levels for chicks fed 1% or more of dietary methionine. At 0.50% methionine there was little change from the control chicks. Our body weight versus age curves (Figures 1 and 3) indicate an absence of weight loss for the methionine-fed mice. It, therefore, seems unlikely that we used a methionine concentration where overt toxicity was a problem. It is clear, however, that methionine shortened lifespan in our experiments with young mice. Identification of the mode of action of methionine-induced reduction of longevity might lead to a better understanding of senescence. One possible mechanism could be the methionine-induced nuclear and nucleolar lesions reported by Shinozuka *et al.* (1971).

The amount of methionine given in this experiment was not large when compared to the methionine content of many food proteins. Milk protein from cows, for example, contains 2.6% methionine and egg albumen 4.6% methionine. Bird (1978) has described methionine as the most toxic of the nutritionally important amino acids. Thus, while rats can consume 1.5% methionine with no apparent decrease in the rate of growth (Benevenga and Harper, 1967), guinea pigs, rabbits and man are less tolerant of methionine (Hardwick, *et al.*, 1970). Our mice consumed a total of 11.2 millimoles methionine/kg body weight/day. A dose of 10 millimoles/kg/day results in inanition and death within 65 hrs for guinea pigs. A single intravenous dose of 2.8 millimoles/kg of methionine to humans produces nausea, vomiting, hypotension, tachycardia, fever, disorientation and liver disfunction (Floyd, *et al.*, 1966). It isn't clear why species differences are so great. Several proposals have been made, but none fully explains why methionine should be so toxic (Bird, 1978). Our results suggest that methionine may be changing copper metabolism, especially in the brain. The use of methionine as a means for slowing senescence is not supported by our data. In fact, diets high in methionine may actually accelerate senescence especially in those species showing greater sensitivity to methionine such as man.

Our results showing a slight but significant removal of brain copper with methionine suggest that methionine might be effective in the treatment of certain disorders of copper metabolism such as Wilson's disease where copper is known to accumulate in the brain.

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