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# The prolongation of survival in mice by dietary antioxidants depends on their age by the start of feeding this diet

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**Abstract**

The effect of daily dietary supplements of an antioxidant mixture (AM) consisting of beta carotene, alpha tocopherol, ascorbic acid, rutin, selenium, and zinc on the survival of male C57BL/6 mice starting at 2, 9, 16, and 23 months of age was investigated. The survival of mice given AM starting at 2 and 9 months of age was found to increase significantly (from 86 to 108 days) compared to the control. The times, of 50, 90, and 100% mortality in mice given AM starting at 2 and 9 months of age increased by 16–9.5% compared to the control, whereas in mice given AM, starting at 16 and 23 months of age, no effect was observed. © 1996 Elsevier Science Ireland Ltd.

*Keywords:* Aging; Dietary antioxidants; Survival of mice

## 1. Introduction

The free oxygen radicals and other reactive oxidants caused by the aerobic metabolism may induce damage to DNA, proteins, and lipids, which is accumulated in human and animal tissue cells with age [1,2].

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The occurrence of oxidative damage in macromolecules makes us believe that the enzymatic and nonenzymatic antioxidant systems of the cells do not completely provide their efficient defense, so the organism, probably, needs a constant enhancement of the antiradical and antioxidant defense [1–4].

The accumulation of DNA lesions and prolonged residence of oxidized proteins and lipids in the cells of an aged organism also suggest a decrease in the efficiency of DNA repair and the elimination of altered protein and lipid molecules from the cellular structures with age [3–6].

There is abundant evidence that the lesions in DNA, proteins and lipids and their accumulation with age can be responsible for the development of cancer and various pathologies, which finally shortens the life span of an individuum [1,7,8]. Therefore, some authors tried to find a positive correlation between the level of the potential antioxidant defense in tissues and the longevity in various animal species. But these studies did not always give the expected results [2,4,9,10], although the dependence of the life span on the activity of the antioxidant system was demonstrated in some species. For instance, an increase in life duration has been shown in *Drosophila melanogaster* by overexpression of their two main enzymes of antioxidant defense: superoxide dismutase and catalase [11,12]. In earlier studies performed to attest the role of free radicals in aging, it has been shown that additional administration of free radical scavengers contributed to the life span [13–15]. These studies also indicate that an animal or human organism needs an enhancement of its antioxidant defense system, to reduce the age-dependent degenerative processes. The interest in using antioxidants as dietary supplements is also associated with the fact that such compounds may not only be potential geroprotectors but also antimutagens, radioprotectors, and means for chemoprevention of cancer [16,17].

Our recent studies demonstrated that dietary supplements of an antioxidant mixture (AM) containing beta-carotene, vitamins E and C, flavonoid rutin as well as zinc and selenium given to mice of various ages for 1.5 months exerted an antimutagenic effect, as judged from the level of *hprt*-mutations in spleen cells after gamma-irradiation of these mice [18]. In tissues of mice, given dietary supplements of AM, a modulation of expression of the hsp-70 gene [19] and activation of the genes for superoxide dismutase and catalase and the genes controlling apoptosis suppression was observed [20].

The aim of this study was to follow variations in the life span of mice kept on a AM-supplemented diet over prolonged periods. The experiments were performed on mice of several age groups. The results showed a significant increase in the life span of the mice that were introduced to the AM-enriched diet at early ages.

## 2. Materials and methods

Male C57BL/6 mice purchased from the Laboratory of Experimental Models of the Russian Academy of Medical Sciences were used.The maintenance and care of the mice were in strict compliance with the guidelines issued by the U.S. National

Institute of Health and the National Research Council [21]. The animals were housed in specific pathogen-free conditions in plastic cages with wood chip bedding in an animal room with a daily 12 h light/dark cycle at 20–22°C and $60 \pm 5\%$ humidity. Four parallel experiments on mice (of 2, 9, 16, and 23 months of starting age) were performed, two groups of animals, experiment and control, in each. The mice were fed a standard diet purchased from the enterprise 'Voronovo' (Moscow region, Russia). The composition of the diet was 20% proteins, 48.5% glucides, 3.5% fats, 11% cellulose, 6% ashes, 11% water, mineral and vitamin mix 35 g/kg diet. Mice in all groups irrespective of age received daily 15 g of this food (energy value 190 kJ) per animal and tap water *ad libitum*. The diet for the experimental groups was enriched by adding AM supplements [18] per 1 kg b.w.: beta carotene, 7.5 mg; alpha tocopherol acetate, 15 mg; ascorbic acid, 50 mg; sodium selenite, 25 $\mu$g; zinc gluconate, 38.4 mg (5 mg of elementary zinc) (all the reagents were from Twin Lab., Ronkonkoma, New York); rutin (quercetin 3$\beta$-rutinoside), 25 mg (Sigma, St. Louis). The content of the above AM components in the basic diet was 6.5–8 times as low. The freshly obtained food was used within at most 30 days. The mice were weighed every 1–2 months. Mortality was registered daily and calculated by the end of each month. The results for parallel groups were compared and the statistical significance of life span differences was determined by the Mann-Whitney $U$ test as described in [22]. A difference was considered statistically significant at the $P < 0.05$ level.

## 3. Results

Our data on the survival of C57BL/6 mice in groups given and not given dietary supplements of AM are summarized and presented as semilogarithmic curves in Fig. 1. It is seen that the death of mice in groups A and B (starting at ages 2 and 9 months) begins when they are 13 or 14 months old and the mean life span (50% survival) in the control (without AM supplements) is 22.2 months. As for the control groups of aged mice (starting at ages 16 and 23 months, groups C and D), their 50% survival was registered by age 24.8 and 26.3 months, respectively. The survival was increased not in all groups of mice given dietary supplements of AM containing beta carotene, vitamins E and C, flavonoid rutin, selenium, and zinc. The statistically significant increase in survival (from 86 to 108 days) in comparison to the control, was registered in mice of 2 and 9 months of starting age, which were given daily AM dietary supplements ($P < 0.05$) (Figs. 1A and B), whereas in the groups of mice given AM beginning at 16 and 23 months there was no increase in survival in comparison with mice of the same age groups not given AM (Figs. 1C and D).

The times (days) of mortality of mice registered for all groups in four experiments are summarized in Table 1. It is seen that the 50, 90, and 100% mortality rates of the mice given AM starting at 2 and 9 months of age, occurred significantly later in comparison to the control. The time (days) after which the 50, 90, and 100% mortality was observed in mice given AM starting at 2 and 9 months of age was 16–9.5% greater in comparison to the control groups.

Table 1
Times for the 50, 90, and 100% mortality of mice given and not given dietary supplements of AM

| Experiment index and starting age of mice (month) | Number of mice in a group | AM delivery | Times of per cent mortality of mice (days) | | |
|---|---|---|---|---|---|
| | | | 50% | 90% | 100% |
| A | 118 | − | 670 | 863 | 948 |
| (2) | 88 | + | 780 | 997 | 1056 |
| | | | (16.4)[a] | (15.5) | (11.4) |
| B | 82 | − | 663 | 854 | 910 |
| (9) | 70 | + | 750 | 934 | 996 |
| | | | (13.1) | (9.4) | (9.5) |
| C | 72 | − | 744 | 885 | 921 |
| (16) | 56 | + | 775 | 904 | 946 |
| | | | (4.2) | (2.1) | (2.7) |
| D | 64 | − | 790 | 918 | 975 |
| (23) | 56 | + | 805 | 893 | 967 |
| | | | (1.9) | (0.0) | (0.0) |

( )[a] Percentage increment.

At the same time, for mice at starting age of 16 and 23 months, the 50, 90, and 100% mortality did not significantly differ from the control.

We weighed animals every 1–2 months in all age groups given and not given AM. The results of these measurements showed that both experimental and control mice equally varied in weight (data not given).

## 4. Discussion

The mean life span in the control groups of male C57BL/6 mice taken to the experiment at 2 and 9 months was 22.2 months, which is in line with the literature data [23,24]. But when we observed the mortality of mice at starting ages of 16 and 23 months; their mean life span appeared 24.8 and 26.3 months, respectively. We suggest this difference is due to that the 'short-livers' of the population (infection is excluded) are eliminated at earlier ages (beginning at 13 or 14 months).

On the basis of the data obtained, it can be suggested that any significant AM-related increase in the survival occurs only in those mice to which the AM-supplemented diet was given, beginning at early ages, whereas in aging mice introduced to the same diet, no marked increase in longevity takes place. Apparently, the greater the starting age of mice introduced to the experiment, the higher the proportion of 'long-livers' among them, and this is probably the reason why we failed to register any significant effect of dietary AM supplements on the life span of these 'selected' animals. Our observations are in agreement with the evidence

that a balanced level of antioxidants may reduce the oxidative damage to proteins and DNA thus decreasing the risk of degenerative processes causing premature aging and death [1,4]. The same data suggest that the antioxidant defense of an organism is limited and exogenous antioxidants are necessary to reinforce the endogenous defense systems, although there are earlier data indicating that some exogenous radical scavengers induce a compensatory depression of the endogenous antioxidant system [25] and a vitamin-E deficient diet results in enhancement of activity of some antioxidant enzymes [26]. Nevertheless, the data of the present study show that prolonged administration of AM to the diet of young mice (starting at age 2 and 9 months) leads to a significant increase in their survival, this

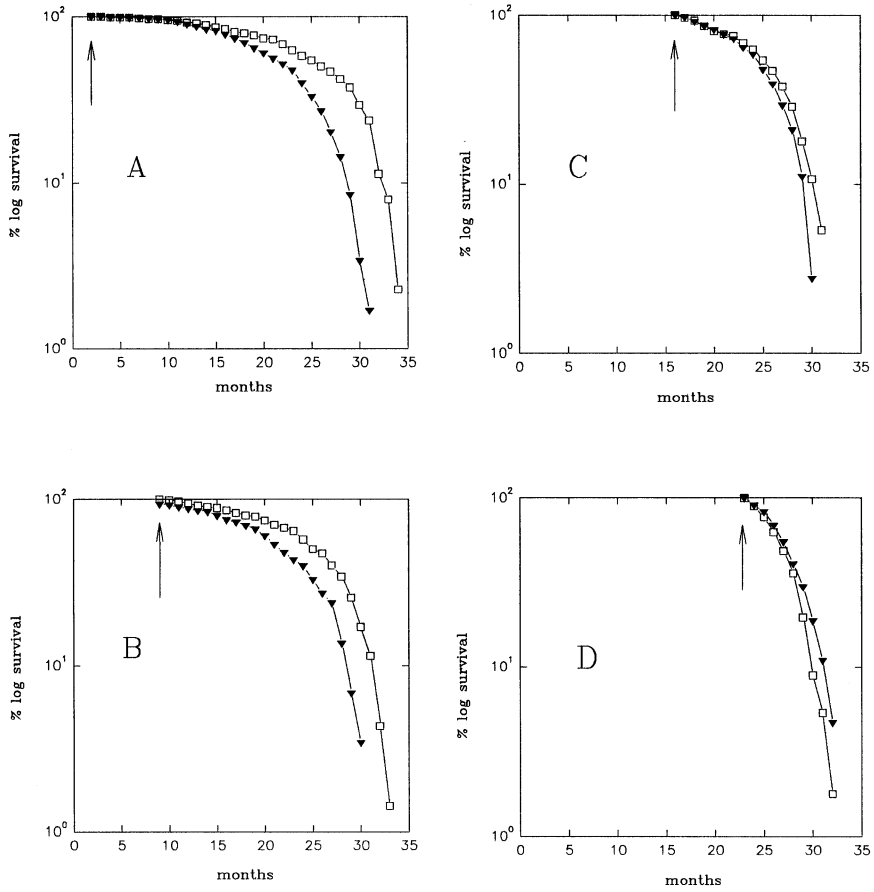


Fig. 1. Survival curves for male C57BL/6 mice not given (▼) and given (□) the AM as dietary supplements at various starting age. The mice were taken to the experiment starting at 2 (A), 9 (B), 16 (C), and 23 (D) months of age. The abscissa: observation time (months). The ordinate: % log survival. The arrows show the starting age of mice groups (taken to the experiment).

not being observed for aged mice (starting at ages 16 and 23 months). Our previous experiments showed that, in mice given dietary supplements of the same AM, a decrease in the frequency of radiation-induced *hprt* mutations took place but we found that in aged mice mutations that cannot be prevented by AM were mostly induced [18]. It was also shown that prolonged administration of AM to the diet of mice produced an increase in the expression of genes encoding heat shock proteins, superoxide dismutase, catalase, and the genes to control the suppression of apoptosis in splenocytes [19,20]. These data suggest that the AM components, when used at moderate doses as supplements to the diet of mice, activate the defense systems of the organism and prolong the life span of these animals. Daily consumption of a vitamin-antioxidant mixture by young and aged donors for 4 months enhanced the antiradical activity and decreased the frequency of spontaneous and in vitro $\gamma$-ray-induced micronuclei in peripheral blood lymphocytes [27]. The components of our antioxidant mixture enter the organism of animals and humans with normal food, they not only can be direct scavengers of radicals but also may be involved in various pathways of homeostasis regulation and be partners in various processes at the molecular and cellular levels [28–32] to reduce finally the consequences of action of harmful endogenous and exogenous factors. The components of AM are also agents capable of preventing cancer in animals and humans [16,17].

Thus, the dietary supplements of AM containing beta-carotene, vitamins E and C, flavonoid rutin, as well as selenium and zinc at moderate doses increased significantly the longevity of young mice (starting at ages 2 and 9 months), whereas their effect on the survival of aged mice (starting at ages 16 and 23 months) was not significant.

Our findings and literature data cannot directly explain the different effects of dietary AM supplements on the longevity in mice of various age. However, it cannot be excluded that the action of antioxidants on the expression of defense genes is age-dependent. Oxidants and nonenzymatic antioxidants play a significant role in the regulation of expression of defence genes [33,34]. Possibly, there is a definite age step in animal organisms, when the effects of antioxidants on gene expression become limited.

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