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The Effects of Two Different Multivitamins on Aging Mice

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Abstract

Investigating anti-aging factors that is more effective than antioxidants has important theoretical significance and application value. In search for nutritional ingredients that are more effective in anti-aging, two different multivitamins (multivitamin-1 at a dose of 2.5 mg/kg body weight [BW]/day; multivitamin-2 at a dose of 5.4 mg/kg BW/day) were administered to aging mice (N = 40) induced by D-galactose. The content or activity of the biochemical components associated with aging and anti-aging in the brain and the liver of the experimental mice was then determined for analysis of statistically significant difference among the groups. Results showed the mice in the aging model group exhibited obvious senility symptoms. However, the mice in the multivitamin-1 and multivitamin-2 groups were essentially similar to those of the control group, but were obviously better than the mice in the aging model group. Multivitamin-1 and multivitamin-2 decreased significantly the malondialdehyde (MDA) content and monoamine oxidase (MAO) activity ($P < 0.01$), and increased significantly the activity of glutathione peroxidase (GSH-Px), copper/zinc-superoxide dismutase (Cu/Zn-SOD) and manganese-superoxide dismutase (Mn-SOD) ($P < 0.01$) in the brain and the liver of the aging mice. There was no significant difference ($P > 0.05$) between the effects of the two multivitamins on the components associated with aging and anti-aging. In conclusions, this work showed that vitamins B_1, B_2, B_6 and PP (Nicotinic acid or vitamin B_3) play key roles in the anti-aging process of multivitamin-2. Vitamins B_1, B_2, B_6, and PP are more effective nutrients in anti-aging in mice.

Key Words: aging, anti-aging, antioxidases, mechanism, multivitamin

Introduction

In previous studies on the effects of a new multivitamin, consisting of vitamins B_1, B_2, B_6, PP (Nicotinic acid or vitamin B_3) and vitamins A, C and E, on metabolic syndromes and aging, we found that supplementation with this multivitamin could prevent and provide treatment and adjunctive therapy for many chronic metabolic syndromes; the multivitamin also eliminated or attenuated 38 types of symptoms or dysfunctions of the metabolic syndromes investigated, and significantly delayed aging (4, 21). We believed that vitamins B_1, B_2, B_6 and PP might play key roles in the prevention and treatment of chronic metabolic diseases and in anti-aging. Other studies have also shown that vitamins B_1, B_2, B_6 and PP are important components, including thiamine pyrophosphate, flavin mononucleotide, flavin adenine dinucleotide, phosphorylated pyridoxal, nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, of many coenzymes (8, 22, 23). Those coenzymes participate

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in more than 300 enzymatic reactions related to the metabolism of matter-energy and protein. The coenzymes that make up vitamin PP participate in more than 200 enzymatic reactions related to matter-energy metabolism (8) and co-enzymes that make up vitamin B_6 participate in approximately 100 enzymatic reactions related to the metabolism of proteins and fats (22). These coenzymes play important roles in the transfer of hydrogen ions and electrons, decarboxylation, transamination and racemization in their enzymatic reactions, and they can improve matter-energy and protein metabolisms. Furthermore, the co-enzymes serve to prevent the formation and accumulation of components associated with chronic diseases and aging by means of their synergistic effects (23).

In order to verify our conjecture and to further explore the anti-aging matter, we compared in this study the effects and biochemical mechanisms of two different multivitamins on aging mice induced by D-galactose. We found that vitamins B_1, B_2, B_6 and PP played key roles in the anti-aging process of the multivitamin-2 and that they were more effective anti-aging nutrients.

Materials and Methods

Experimental Animals

Healthy male mice were purchased from the Laboratory Animal Department at Central South University, Changsha, Hunan, PRC. At the beginning of the experiments, the mice were about 5 weeks of age, and the body weights (BWs) were 20 ± 2 g. The mice were allowed free access to food and water. The experiments were performed according to the regional Animal Ethics Committee guidelines for animal experimentation and the protocol was reviewed and approved by the Hunan Animal Ethical Committee.

Composition of Multivitamins and Feed

In designing the multivitamins, the dosages of various vitamins were first according to the recommended nutrient intake for adult humans, and the amounts of vitamin PP and B-6 were increased based on their importance in enzymatic reactions. Multivitamin-1 was composed of vitamin PP (25 mg), vitamins B_1, B_2 and B_6 (2.5 mg each), and medicinal starch (67.5 mg). Multivitamin-2 consisted of vitamin PP (25 mg); vitamins B_1, B_2 and B_6 (2.5 mg each), vitamin C (25 mg), vitamin E (6.25 mg), vitamin A (0.4 mg) and medicinal starch (35.85 mg). Because previous research has shown that vitamins with an antioxidative effect also have an anti-aging action, the anti-aging action of the antioxidant vitamins was not directly investigated in this work. The basic feed for the mice was composed of flour (20%), rice flour (10%), corn flour (20%), wheat bran (25%), bean (20%), bone meal (2.5%) and fish meal (2.5%). The basic feed was purchased from the Laboratory Animal Department at Central South University in Changsha, Hunan, PRC.

Preparation of Mice for Determining the Minimum Dose of Multivitamin-1 Needed to Achieve a Maximum Anti-Aging Effect

Following a 7-day acclimation period, 50 mice were randomly assigned to five different groups of 10 mice each: three dose experimental groups, one aging model group and one control group. The mice were allowed free access to food and water. The mice in the high-, medium- and low-dose experimental groups and in the aging model group were subcutaneously injected with D-galactose at a dose of 150 mg/kg once daily for 6 weeks, whereas mice in the control group were treated with the same volume of physiological saline (0.5 ml). At approximately the same time, all the aging experimental mice were administered multivitamin-1 at doses of 5, 2.5 or 1.25 mg/kg BW/day by oral gavage, and the mice were designated as the high-, medium- and low-dose experimental groups, respectively. The mice in the aging model group and the control group received the same volume of distilled water (0.4 ml/10 g BW/day) instead of the multivitamin. Weekly adjustments were made for BW changes. Following the multivitamin treatment, clinical observations were made. The general conditions of all animals were recorded every day. At the initiation of the experiments and at the end of every other week, BWs were measured. Throughout the experiments, food consumption was recorded at the end of every week, and the average food consumption per animal was calculated at weekly intervals. At the end of the sixth week, the animals were euthanized to undergo a gross pathological examination. The brain and liver were then removed from each cadaver for biochemical analysis.

Preparation of Mice for Determining the Minimum Dose of Multivitamin-2 Needed to Achieve a Maximum Anti-Aging Effect

The aging mice in the high-, medium- and low-dose experimental groups were administered multivitamin-2 at doses of 10.8, 5.4 or 2.7 mg/kg BW/day by oral gavage every morning. The subsequent procedures were the same as those described above for multivitamin-1.

Comparing the Effects of Two Multivitamins on Aging Mice

Following a 7-day acclimation period, 40 mice were randomly assigned to 4 groups of 10 mice each: two experimental groups, one aging model group and one control group. The mice were allowed free access to food and water. The mice in the experimental-1 and experimental-2 groups and in the aging model group were subcutaneously injected with D-galactose at a dose of 150 mg/kg once daily for 6 weeks, whereas those in the control group were treated with the same volume (0.5 ml) of physiological saline. At approximately the same time, the mice in experimental group 1 were administered multivitamin-1 at a dose of 2.5 mg/kg BW/day by oral gavage. The mice in experimental group 2 were administered multivitamin-2 at a dose of 5.4 mg/kg BW/day; in multivitamin-2, the amounts of vitamins PP, B_1, B_2 and B_6 were equal to those of the experimental group 1. The mice in the aging and the control groups received the same volume of distilled water (0.4 ml/10 g BW/day). Subsequently, the mice were monitored as described above.

Determination and Analysis of Components Associated with Aging and Anti-Aging in the Brain and Liver of Experimental Mice

Malondialdehyde (MDA) content and the activities of monoamine oxidase (MAO), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were determined to assess the severity of body aging and health (6, 10, 11). The brain and liver were homogenized by a standard procedure, and the supernatant of the homogenate was removed for biochemical analysis. All the biochemical parameters associated with aging and anti-aging were determined with biochemical assay kits were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, PRC, according to the instructions of the kits. An UV754N ultraviolet-visible spectrophotometer (Shanghai Exact Scientific Instrument Corporation, Shanghai, PRC) was used for optical density measurements. Total protein (TP) content was determined with a TP quantitative assay kit. MDA content was determined with a MDA assay kit using the thiobarbituric acid test method. Activities of total superoxide dismutase (T-SOD) and copper/zinc-superoxide dismutase (Cu/Zn-SOD) were determined with a SOD typed assay kit using the hydroxylamine method. GSH-Px and MAO activities were determined with a GSH-PX or MAO assay kit, respectively, using the colorimetric method.

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). The significance of the difference among the groups for total BWs, food consumption values for mice, MDA content, GSH-Px activity, MAO activity, T-SOD activity, Cu/Zn-SOD activity and manganese-superoxide dismutase (Mn-SOD) activity was analyzed with the Statistical Product and Service Solutions (SPSS) software, version 11.5 (one-way analysis of variance [ANOVA]). Statistical significance was established at $P < 0.05$.

Results

The aging animal model induced by D-galactose was integrated with the aging animal model design based on the principle of metabolic disturbance in the aging process. Each organ of these aging models exhibited different degrees of senile symptoms and aged-related biochemical parameters in the body changes associated with aging. The D-galactose-induced aging model is an ideal experimental tool for studying anti-aging effects, and has been used widely to study the anti-aging action of drugs and functional foods (15, 20, 24).

Minimum Dose of Multivitamin-1 Needed to Achieve a Maximum Anti-Aging Effect

Through observation and comparison, the mice in the aging model group were found to exhibit obvious senile symptoms such as reduced activity, sparse, dull yellow fur, irritable temperament and thin dejecta. The appearance, behavior, urine and fur of the mice in the three dose groups receiving multivitamin-1 were similar to those in the control group but were obviously better than those in the aging model group. A summary of total BW of the mice is presented in Table 1A. Food consumption values are presented in Table 2A. Comparative analysis showed no statistically significant differences in food consumption and BW between the medium- and the high-dose groups ($P > 0.05$), nor between the medium- and high-dose groups and the control group ($P > 0.05$) at the end of the sixth week. However, food consumption and BW of the medium- and high-dose groups were significantly higher than those of the aging model group ($P < 0.05$). The food consumption and BW of the low-dose group were higher than those of the aging model group but lower than the control group.

The MDA content, protein content, MAO activity, and SOD activity were determined with the biochemical assay kits (Tables 3A and 4A). Analysis showed that the MDA content and MAO activity of the mice in the medium- and high-dose groups were significantly lower ($P < 0.01$) than those of mice in the

Table 1. BW data for mice in all groups for multivitamin-1 and -2.

Week	Low dose	Medium dose	High dose	Aged model	Control
			A. Multivitamin-1		
1	24.07 ± 3.62	24.11 ± 1.75	24.05 ± 2.34	24.89 ± 2.47	24.93 ± 1.41
2	28.41 ± 2.53	28.11 ± 2.12	28.02 ± 1.73	28.19 ± 1.46	29.01 ± 3.23
3	31.09 ± 2.34	31.07 ± 4.03	30.76 ± 2.56	29.92 ± 3.35	32.31 ± 2.85
4	33.46 ± 3.24	33.78 ± 3.72	33.17 ± 3.23	31.07 ± 2.23	34.48 ± 1.67
5	34.61 ± 2.34	34.57 ± 3.23	34.67 ± 2.14	32.30 ± 1.35	35.71 ± 3.09
6	35.43 ± 2.45	36.16 ± 2.93	35.97 ± 2.64	33.49 ± 1.64	36.82 ± 2.74
WG	14.43 ± 2.55	16.16 ± 2.72†	15.97 ± 2.61†	12.79 ± 2.36‡	16.12 ± 2.44
			B. Multivitamin-2		
1	24.5 ± 1.7	24.4 ± 2.3	24.3 ± 2.3	24.9 ± 2.4	24.7 ± 1.2
2	28.2 ± 2.1	28.5 ± 1.7	28.3 ± 1.7	28.2 ± 1.4	28.1 ± 3.3
3	30.6 ± 4.0	31.7 ± 2.5	31.7 ± 2.6	29.9 ± 3.4	31.8 ± 2.8
4	32.8 ± 3.7	33.8 ± 3.2	33.9 ± 3.2	31.1 ± 2.2	34.5 ± 1.6
5	33.9 ± 3.2	35.5 ± 2.1	35.2 ± 2.1	32.3 ± 1.4	35.7 ± 3.1
6	34.8 ± 2.9	36.6 ± 2.6	36.4 ± 2.6	33.2 ± 1.6	36.8 ± 2.7
WG	14.8 ± 2.6	16.6 ± 2.5†	16.4 ± 2.3†	13.2 ± 2.2‡	16.8 ± 2.2

N = 10 mice, Unit = g/mouse. WG, weight gain. † denotes comparison between the three dose groups and aged model group, $P < 0.05$. ‡ denotes comparison between the aged model group and the control group, $P < 0.05$.

Table 2. Food consumption values for mice in all groups for multivitamin-1 and -2.

Week	Low dose	Medium dose	High dose	Aged model	Control
			A. Multivitamin-1		
1	6.50 ± 0.72	6.32 ± 0.67	6.45 ± 0.72	5.83 ± 0.82	6.47 ± 0.45
2	6.47 ± 0.76	6.54 ± 0.63	6.51 ± 0.81	5.62 ± 0.52	6.63 ± 0.52
3	6.58 ± 0.64	6.62 ± 0.36	6.54 ± 0.81	5.57 ± 0.62	6.57 ± 0.2
4	6.69 ± 0.74	6.81 ± 0.38	6.56 ± 0.66	5.40 ± 0.52	6.82 ± 0.95
5	6.85 ± 0.46	6.95 ± 0.82	6.80 ± 0.64	5.65 ± 0.72	6.91 ± 0.64
6	7.03 ± 0.75	7.12 ± 0.32	7.02 ± 0.93	5.45 ± 0.53	7.15 ± 0.38
Total	36.82 ± 0.73	40.36 ± 0.69†	39.88 ± 0.83†	33.92 ± 0.53‡	40.55 ± 0.58
			B. Multivitamin-2		
1	6.42 ± 0.61	6.52 ± 0.52	6.25 ± 0.63	5.43 ± 0.63	6.45 ± 0.85
2	6.15 ± 0.85	6.22 ± 0.76	6.34 ± 0.51	5.55 ± 0.45	6.96 ± 0.67
3	6.21 ± 0.64	6.75 ± 0.36	6.72 ± 0.82	5.86 ± 0.81	6.82 ± 0.63
4	6.01 ± 0.77	6.65 ± 0.84	6.66 ± 0.41	5.59 ± 0.72	6.69 ± 0.46
5	6.16 ± 0.75	6.70 ± 0.62	6.63 ± 0.93	5.61 ± 0.64	6.79 ± 0.90
6	6.26 ± 0.79	6.68 ± 0.68	6.53 ± 0.83	5.51 ± 0.67	6.89 ± 0.81
Total	37.21 ± 0.7	39.82 ± 0.65†	39.73 ± 0.73†	33.55 ± 0.68‡	40.6 ± 0.70

N = 10 mice, Unit = g/day/mouse. † denotes comparison between the three dose groups and the aged model group, $P < 0.05$. ‡ denotes comparison between the aged model group and the control group, $P < 0.05$.

aging model group. On the other hand, the SOD activity of mice in the medium- and high-dose groups was significantly higher than that of mice in the aging model group ($P < 0.01$). The MDA content and the MAO and SOD activities of the mice in the medium- and high-dose groups did not significantly differ from those of mice in the control group ($P > 0.05$). This finding demonstrated the remarkable anti-aging effects of multivitamin-1. In conclusions, the minimum dose at which multivitamin-1 achieved a maximum anti-aging effect was 2.5 mg/kg BW/day.

Minimum Dose of Multivitamin-2 Needed to Achieve a Maximum Anti-Aging Effect

Observation showed that the mice in the aging model group exhibited obvious senile symptoms. The appearance, behavior, urine and fur of the mice in the three dose groups receiving multivitamin-2 were essentially similar to those in the control group but were obviously better than those in the aging model group. A summary of total BWs is presented in Table 1B. Food consumption values are presented in Table 2B.

Comparative analysis showed no statistically significant differences in food consumption and BW between the medium- and high-dose groups ($P > 0.05$), nor between the medium- and high-dose groups and the control group ($P > 0.05$) at the end of the sixth week. However, food consumption and BW of the medium- and high-dose group were significantly higher than those of the aging model group ($P < 0.05$). The food consumption and BW of the low-dose group were higher than those of the aging model group ($P > 0.05$) but lower than those of the control group ($P > 0.05$).

The protein and MDA contents and the MAO and SOD activities in the brain and the liver were determined with the biochemical assay kits (Tables 3B and 4B). Analysis showed that the MDA content and the MAO activity of mice in the medium- and high-dose groups were very significantly lower ($P < 0.01$) than those of mice in the aging model group. However, the SOD activity of mice in the medium- and high-dose groups was very significantly higher than those of mice in the aging model group ($P < 0.01$). The MDA content and the MAO and SOD activities of mice in the medium- and high-dose groups did not significantly differ from those of mice in the control group ($P > 0.05$). This finding demonstrated the remarkable anti-aging effect of multivitamin-2. In conclusion, the minimum dose at which multivitamin-2 achieved a maximum anti-aging effect was 5.4 mg/kg BW/day.

Comparison of Appearance, Behavior, Urine, Fur, BW and Food Consumption for the Mice

Comparative observations showed that the mice in the aging model group exhibited obvious senility symptoms such as reduced activity, sparse, dull yellow fur, irritable temperament and thin dejecta. The appearance, behavior, urine and fur of mice in the two experimental groups were essentially similar to those of the control group but were obviously better than those in the aging model group. A summary of total BWs is presented in Table 5A. Food consumption values are presented in Table 5B. Analysis showed no significant differences in food consumption and BW at the end of the sixth week between the two experimental groups, nor between the experimental and the control groups ($P > 0.05$); however, food consumption and BW were significantly higher than those of the aging model group ($P < 0.05$).

Comparison of Effects of the Two Multivitamins on the Components Associated with Aging and Anti-Aging in the Brain and Liver of the Mice

The MDA content, MAO and GSH-Px activity in the brain and liver were determined with MDA, MAO and GSH-Px assay kits. Results are shown in Table 6. The T-SOD and CuZn-SOD activity were similarly determined with the SOD typed assay kit, in which T-SOD activity minus CuZn-SOD activity is equal to Mn-SOD activity. Results are shown in Tables 7A and 7B.

Normality test showed that the biochemical parameters of each group were subject to the normal distribution ($P > 0.1$). Homogeneity of the variance test showed that there was no significant difference in the homogeneity of variances among the groups ($P > 0.1$); the biochemical parameters among the groups met the homogeneity of variance. One-way ANOVA analysis showed that the MDA content and MAO activity of the brain and liver of the mice in the aging model group were significantly higher than those of the control group ($P < 0.01$). The GSH-Px, T-SOD, CuZn-SOD and Mn-SOD activities of mice in the aging model group were all significantly lower than that of the control group ($P < 0.01$). This finding shows that the injection of D-galactose into the mice induced changes related to aging and confirmed that the construction of the mouse aging model was successful.

The MDA content and the MAO, GSH-Px, T-SOD, CuZn-SOD and Mn-SOD activities of the mice in experimental groups 1 and 2 did not significantly differ from those of mice in the control group ($P > 0.05$), but the results significantly differed from those of mice in the aging model group ($P < 0.05$ or $P < 0.01$). This finding demonstrated the anti-aging

Table 3. Effects of the multivitamin-1 and -2 on MDA content and MAO activity of the brain and liver of aging mice

	MDA (*nmol/mgprot*)		MAO (*U/mgprot*)	
Group	Brain	Liver	Brain	Liver
		A. Multivitamin-1		
Low dose	1.69 ± 0.25	1.62 ± 0.12	9.76 ± 1.46	2.43 ± 0.11
Medium dose	1.47 ± 0.24†	1.34 ± 0.13 †	8.16 ± 1.23†	1.93 ± 0.31†
High dose	1.41 ± 0.11†	1.44 ± 0.15 †	8.20 ± 1.00†	1.99 ± 0.28†
Aged model	2.03 ± 0.22‡	1.94 ± 0.18‡	10.75 ± 1.36‡	2.85 ± 0.35‡
Control	1.40 ± 0.14	1.46 ± 0.21	8.42 ± 1.62	1.86 ± 0.23
		B. Multivitamin-2		
Low dose	1.66 ± 0.18	1.64 ± 0.14	9.56 ± 1.37	2.37 ± 0.22
Medium dose	1.38 ± 0.21†	1.33 ± 0.16†	8.65 ± 1.21†	1.86 ± 0.17†
High dose	1.41 ± 0.21†	1.35 ± 0.19†	8.70 ± 1.10†	1.92 ± 0.19†
Aged model	2.17 ± 0.3‡	2.12 ± 0.18‡	11.98 ± 2.12‡	2.89 ± 0.13‡
Control	1.36 ± 0.15	1.28 ± 0.15	8.51 ± 1.74	1.88 ± 0.16

N = 10. † denotes comparison between the three dose groups and the aging model group, $P < 0.01$. ‡ denotes comparison between the aged model group and control group, $P < 0.01$.

Table 4. Effects of the multivitamin-1 and -2 on SOD activity of the brain and liver of aging mice.

Group	Brain	Liver
	A. Multivitamin-1	
Low dose	191.4 ± 16.3	138.0 ± 14.6
Medium dose	219.7 ± 17.1†	151.5 ± 10.7†
High dose	216.4 ± 10.8†	155.4 ± 16.3†
Aged model	175.1 ± 13.6‡	125.4 ± 13.5‡
Control	226.7 ± 13.8	156.4 ± 15.8
	B. Multivitamin-2	
Low dose	191.5 ± 14.5	136.7 ± 11.3
Medium dose	225.6 ± 13.6†	153.8 ± 10.0†
High dose	223.9 ± 17.6†	151.9 ± 11.9†
Aged model	176.7 ± 17.83‡	123.4 ± 9.2‡
Control	228.1 ± 14.75	155.8 ± 14.6

N = 10; Unit = U/mgprot. † denotes comparison between the three dose groups and the aging model group, $P < 0.01$. ‡ denotes comparison between the aged model group and control group, $P < 0.01$.

effects of multivitamin-1 and -2. The MDA content and the five enzymatic activities of the aging mice in experimental group 1 did not significantly differ from those of the aging mice in experimental group 2 ($P > 0.05$), thus indicating that their anti-aging effects were not significantly different. This finding also suggested that the antioxidant vitamins included in multivitamin-2 did not enhance an anti-aging effect. On the other hand, vitamins B_1, B_2, B_6 and PP played key roles in the anti-aging process of multivitamin-2 and were more effective ant-aging nutrients.

Discussion

Studies have shown that aging causes a decline in body metabolism, which in turn accelerates the

Table 5. Comparing BW data and food consumption values for mice in each group.

Week	Experiment-1 group	Experiment-2 group	Aged model group	Control group
		A. BW (g/mouse)		
1	24.5 ± 1.7	24.4 ± 2.3	24.3 ± 2.4	24.6 ± 1.2
2	28.6 ± 2.1	28.4 ± 1.7	28.2 ± 1.4	28.7 ± 3.3
3	31.3 ± 4.0	31.1 ± 2.5	29.9 ± 3.4	32.3 ± 2.8
4	33.8 ± 3.7	33.2 ± 3.2	31.1 ± 2.2	34.5 ± 1.6
5	34.9 ± 3.2	34.8 ± 2.1	32.3 ± 1.4	35.7 ± 3.1
6	36.2 ± 2.9	36.3 ± 2.6	33.2 ± 1.6	36.8 ± 2.7
WG	16.2 ± 2.6†	16.3 ± 2.2†	13.2 ± 2.3‡	16.8 ± 2.5
		B. Food consumption (g/day/mouse)		
1	6.4 ± 0.7	6.3 ± 0.7	5.8 ± 0.8	6.3 ± 0.4
2	6.5 ± 0.8	6.5 ± 0.6	5.7 ± 0.5	6.6 ± 0.5
3	6.5 ± 0.8	6.6 ± 0.4	5.6 ± 0.6	6.6 ± 0.3
4	6.5 ± 0.6	6.8 ± 0.4	5.7 ± 0.5	6.8 ± 0.9
5	6.8 ± 0.6	6.9 ± 0.8	5.7 ± 0.7	6.9 ± 0.6
6	7.2 ± 0.7	7.1 ± 0.3	5.6 ± 0.5	7.1 ± 0.4
total	39.9 ± 0.8†	40.4 ± 0.7†	34.1 ± 0.6‡	40.3 ± 0.6

N = 10 mice. † denotes comparison between experiment groups and aged model group, $P < 0.05$. ‡ denotes comparison between the aged model group and control group, $P < 0.05$.

Table 6. Comparing the MDA content and MAO activity and the GSH-Px activity of the brain and liver of mice in each group.

Group	MDA (nmol/mgprot)		MAO (U/mgprot)		GSH-Px (U/mgprot)	
	Brain	Liver	Brain	Liver	Brain	Liver
Experiment-1	1.43 ± 0.14‡‡	1.48 ± 0.17‡‡	8.37 ± 1.19‡	2.08 ± 0.30‡‡	162 ± 29.2‡‡	436 ± 40.5‡
Experiment-2	1.46 ± 0.15‡‡	1.42 ± 0.16‡‡	8.46 ± 0.93‡	1.99 ± 0.29‡‡	171 ± 26.3‡‡	441 ± 37.4‡
Aged model	2.06 ± 0.25§	1.89 ± 0.28§	10.6 ± 1.46§	2.82 ± 0.35§	107 ± 7.92§	368 ± 57.9§
Control	1.45 ± 0.13	1.41 ± 0.25	8.44 ± 1.52	1.87 ± 2.27	188 ± 19.9	448 ± 60.2

N = 10 mice. ‡ denotes comparison between experiment-1 and -2 groups and the aging model group, $^{‡}P < 0.05$; $^{‡‡}P < 0.01$. § denotes comparison between the aged model group and the control group, $P < 0.01$.

aging of the body. Since the life processes essentially consist of a series of enzymatic reactions, any abnormal enzymatic reaction can affect physiological functions and lead to serious illness and accelerated aging process (1, 4, 8, 21, 22). Improvement in body metabolism can prevent diseases and delay aging. Vitamins B_1, B_2, B_6 and PP supplements can increase the number of coenzymes and active enzymes (17), leading to faster enzymatic reactions and accelerated metabolism of matter-energy and proteins, the oxidative decomposition of lipids and saccharides into carbon dioxide and water to generate a large amount of energy. Faster enzymatic reactions can also help to prevent the production and accumulation of the aging-associated components, including MDA, galactitol, mesostates and other peroxides. Improvements in the metabolism of matter–energy and protein can enhance vitality of the body, promote synthesis of proteins and other functional components in the body, and repair damaged tissue cells and DNA (18). On the cellular level, improvement in the metabolism of matter-energy and proteins can help to maintain the normal structure of histiocytes and to enhance their anti-aging activity by repressing the

Table 7. Comparing T-SOD, CuZn-SOD and Mn-SOD activities of the brain and liver of mice in each group.

Group	T-SOD	CuZn-SOD	Mn-SOD
A. T-SOD, CuZn-SOD and Mn-SOD activities of the brain (U/mgprot)			
Experiment-1	217 ± 31.3‡	160 ± 30.0‡	57.6 ± 9.78‡
Experiment-2	220 ± 30.2‡	160 ± 29.21‡	59.8 ± 10.3‡
Aged model	177 ± 14.9§	132 ± 15.9§	44.9 ± 10.4§
Control	219 ± 28.2	162 ± 17.0	57.5 ± 12.6
B. T-SOD, CuZn-SOD and Mn-SOD activities of the liver (U/mgprot)			
Experiment-1	147 ± 9.02‡	103 ± 4.80‡	44.2 ± 9.76‡
Experiment-2	152 ± 9.03‡	104 ± 4.82‡	48.0 ± 9.78‡
Aged model	122 ± 3.93§	87.4 ± 6.86§	34.6 ± 6.57§
Control	154 ± 13.7	105 ± 8.73	49.2 ± 10.6

N = 10 mice. ‡ denotes comparison between experiment-1 and -2 groups and the aging model group, $P < 0.01$. § denotes comparison between the aged model group and the control group, $P < 0.01$.

expression of the MAO and promoting the expression of anti-oxidases GSH-Px, CuZn-SOD and Mn-SOD. Studies have shown that a decrease in the MAO content may reduce the breakdown of monoamine neurotransmitters, improve brain health and prevent dementia (2, 8, 11), while an increase in the anti-oxidase can accelerate the removal of free radicals and peroxides and delay senescence (12, 13, 16). Improvement in the metabolism of matter-energy and proteins can also promote the ubiquitin-proteasome pathway or system, which is dependent on adenosine triphosphate (ATP) to accelerate the decomposition of lipofuscin and to eliminate age pigment and delay aging (5, 14, 19).

The anti-aging action of antioxidant vitamins A, C and E mainly occurs through the elimination of peroxides formed in the body. Their anti-aging effects were not demonstrated in the absence of peroxides in the bodies of the experimental mice since vitamins B_1, B_2, B_6 and PP can prevent the formation and the accumulation of the peroxides. Therefore, the inclusion of antioxidant vitamins A, C and E in multivitamin-2 did not increase the anti-aging effects of this multivitamin. In addition, Bjelakovic, *et al*. reported that long-term excessive use of vitamins A and E could produce side effects and increased mortality (3).

Substances with antioxidative effects have generally been regarded as having an anti-aging action; hence, many researchers studying anti-aging have focused on antioxidative substances. Vitamins A, C and E, which have remarkable antioxidant activities, have been widely used in preventing diseases and in promoting anti-aging effects (7, 9). However, our study showed that vitamins B_1, B_2, B_6 and PP played key roles in the anti-aging process of multivitamin-2 and were more effective than antioxidant vitamins A, C and E. The present study provided new scientific evidence for better anti-aging drugs and nutrients.

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Conflict of Interests

The authors declare that there are no conflicts of interests.

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