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Effect of Different Foods on Antioxidant Capacity and Oxidative Damage in Siberian Hamsters

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Abstract

In order to understand whether feeding purple cabbage would affect animals' antioxidant capacity, Siberian hamsters (*Phodopus sungorus*) were utilized and divided into the normal food group (NF, n = 10), normal food plus purple cabbage group (NF + PC, n = 10) and purple cabbage group (PC, n = 10), respectively. The experimental duration was 28 days. We found that SOD activities in the brain, and liver were higher in the NF group than in the NF + PC and PC groups, suggesting that feeding purple cabbage reduced antioxidant capacity in these two organs. In addition, SOD activities in the heart, kidney and testis were higher in the NF and NF + PC groups than in the PC group, suggesting feeding purple cabbage alone reduced antioxidant capacity in these three organs. Lung SOD activity was higher in the NF + PC group than in the NF group, implying feeding purple cabbage increased antioxidant capacity. MDA contents in the brain, heart, liver and kidney were higher in the PC group than in the NF and NF + PC groups, indicating that feeding purple cabbage alone increased lipid peroxidation in these organs. However, MDA contents in the lung and testis were not different among the three groups, implying that no oxidative damages occur in these two organs. In summary, feeding purple cabbage exerted different effects on antioxidant capacity and oxidative damage in different organs.

Keywords

Siberian Hamsters (*Phodopus sungorus*), Purple Cabbage, Superoxide Dismutase (SOD), Malondialdehyde (MDA)

1. Introduction

The antioxidant system plays an important role in scavenging free radicals and

hence is crucial to animals' health [1] [2]. It is clear that aerobic cell respiration inevitably produces reactive oxygen species (ROS), and ROS may impair biomolecules including lipids, proteins, and DNA [3] [4]. However, organisms have the antioxidant defense system to reduce potential oxidative damage [4] [5]. This antioxidant system is composed of exogenous diet-derived antioxidants such as vitamin E and selenium, and endogenous molecules including superoxide dismutase (SOD), catalase, and so on [6] [7]. The antioxidant defense network functions to convert ROS into less reactive molecules [8]. When ROS is not eliminated by the organism's antioxidant defenses, oxidative stress occurs and is deleterious to cell and tissue structure and function, which is related to many diseases [1] [9]. Therefore, animals' antioxidant capacity is important to maintain their health [2]. Moreover, oxidative stress can also affect animals' fitness, such as sexually selected traits [2] [7] [10] [11]. However, the influence of environmental factors on oxidative balance has received little attention [2].

Food is one of the most important factors affecting animals' antioxidant defense. Purple cabbage (*Brassica oleracea var. Capitata F. rubra*) is one of the world's most widely consumed vegetables. Purple cabbage contains anthocyanins, polyphenolic compounds, vitamins, and minerals, which have antioxidant and anti-inflammatory activities [12] [13]. Some researchers have found that feeding purple cabbage could increase SOD activity in female fruit flies (*Drosophila melanogaster*) and reduce MDA concentrations in male fruit flies [14]. Moreover, blue cabbage (*Brassica Oleracea var acephala*) ethanol extracts (BOEE) could increase the activity of SOD and decrease the cellular levels of ROS and MDA in intestinal epithelial Caco-2 cells [15]. Our previous research has shown that feeding purple cabbage alone could reduce body mass and total body fat mass, but did not affect cellular immunity in Siberian hamsters (*Phodopus sungorus*) [16]. In the present study, we want to know whether purple cabbage would affect antioxidant capacity in this species. We hypothesized that feeding purple cabbage would affect antioxidant capacity, and predicted that feeding purple cabbage alone would decrease antioxidant capacity and increase lipid peroxidation in Siberian hamsters.

2. Materials and Methods

2.1. Animals and Experimental Design

All animal procedures abided by the guidelines of the Biomedical Ethics Committee of Qufu Normal University. It must be noted that the experimental design and animals used were the same as in our previously published paper [16]. Briefly, male hamsters (about 6 weeks) were bought from a pet market in Zou city and then were brought to the animal room in Qufu Normal University. The photoperiod was 12L:12D, and the feeding temperature was 23˚C ± 1˚C. Standard rat pellet chow (Beijing KeAo Feed Co., Beijing, China) and water were provided ad libitum. The bedding material was sawdust in plastic cages (30 cm × 15 cm × 20 cm). After adapting for a week, 30 hamsters were randomly divided into the normal food group (NF, n = 10), normal food plus purple cabbage group (NF + PC, n =

10) and purple cabbage group (PC, n = 10), respectively. One cage (30 cm × 15 cm × 20 cm) had 5 animals. Standard rat pellet chow was provided in the NF group. Besides standard rat pellet chow, fresh purple cabbage was also provided in the NF + PC group. Both normal food and fresh purple cabbage were provided sufficiently to the animals during the experiment. Animals in the PC group were only fed with fresh purple cabbage. The purple cabbage was cut into pieces and was provided sufficiently every three days. In fact, the actual ingredients of purple cabbage used in the present study were not measured. According to the previous research, the energy content of purple cabbage is about 0.89 kJ/g [12]. Food and bedding were changed every 3 days at 9:00 am, and the experimental duration was 28 days. After 16 days, one animal in the PC group died, and one female animal in the NP + PC group was found after dissection. Subsequently, these 2 animals were not included in the statistical analysis.

2.2. Organs

After the experiment, the animals were euthanized. Organ measurement followed previous literature [17]. In brief, brain and the visceral organs including heart, thymus, lungs, liver, spleen, kidneys, adrenal glands, testes, epididymis, seminal vesicals and the digestive organs with contents (*i.e.*, stomach, small intestine, caecum and colon) were dissected and weighed (±1 mg). Six organs including brain, heart, lungs, liver, kidneys and testis were stored at −20˚C for later assays of SOD activity and malondialdehyde content.

2.3. Assays of SOD Activity

Six organs, including brain, heart, lungs, liver, kidneys and testis, were cut into pieces on an ice plate, respectively. Then they were transferred into tubes on ice; saline was added in a ratio of 1 to 10 (*i.e.*, tissue weight (g) to saline volume (mL), W/V), respectively. These tissue pieces with saline were homogenized using a high-speed homogenizer (FSH-2A, Jiangsu Jinyi Instrument Technology Co., Ltd.). These homogenates were transferred into the centrifuge tubes and then centrifuged at 4˚C at a speed of 10,000 rpm for 30 minutes. The supernatants were transferred into the EP tubes and were stored at −20˚C for later assays of SOD activity and MDA content.

SOD activity was measured using the pyrogallol autoxidation method with slight modifications [18]. In brief, 9 mL 40 mmol/L Tris-HCl buffer (pH 8.20) containing 2.5 mmol/L EDTA·Na_2 to tube 1 and tube 2, respectively. Added 8.5 mL 40 mmol/L Tris-HCl buffer (pH 8.20) and 0.5 mL SOD enzyme solution to tube 3. These 3 tubes were shaken well and incubated at 25˚C in a water bath for 5 minutes. 20 μL 4.5 mmol/L pyrogallol hydrochloride solution was transferred into tube 2 and tube 3, respectively, and then transferred into cuvettes quickly. Tube 1 was used as the control, and absorbance was adjusted to zero at a wavelength of 325 nm. Measured the absorbance of tube 2 and tube 3. Reacted for 3 minutes and recorded readings every 30 seconds. Plotted the absorbance at 325 nm on the ver-

tical axis and time on the horizontal axis to determine the slopes K_2 and K_3, respectively. One SOD activity unit was defined as the amount of SOD required to inhibit 50% of the reaction rate per minute per milliliter in the extract. SOD activity was calculated using the following formula:

$$\text{SOD activity (U/mL)} = \frac{\left(K_2 - K_3\right)/K_2}{50\%} * \frac{V_1}{V_2}$$

V_1 indicated the total volume of the supernatants; V_2 indicated the volume of SOD solution (0.5 ml), K_2 and K_3 were the slopes obtained from plotting the data in tube 2 and tube 3, respectively.

2.4. Assays of MDA Contents

MDA content was determined using the thiobarbituric acid method [19]. Briefly, take 2 clean test tubes and number them as 1 and 2. Add 3 ml 0.05 mol/L phosphate-buffered saline (PBS) (pH 7.8) and 5 mL 0.5% thiobarbituric acid (TBA) plus 20% trichloroacetic acid (TCA) solution to tube 1 as the control. Add 3 mL MDA extraction solution (*i.e.*, the supernatant obtained by organ centrifugation) and 5 mL of 0.5% TBA plus 20% TCA to tube 2, shake thoroughly, and place tube 1 and tube 2 in a boiling water bath for 15 minutes. After cooling, centrifuge for 5 minutes at a speed of 3000 rpm, and obtain the supernatant. Tube 1 was considered the control and the absorbance was adjusted to zero. Then, measure the absorbances of tube 2 at the wavelengths 600 nm, 532 nm, and 450 nm to obtain A600, A532 and A450, respectively. MDA content was calculated using the following formula:

$$\text{MDA content (μ mol/g)} = \frac{\left[6.45*\left(A532 - A600\right) - 0.56*A450\right]*V_1}{m*V_2}$$

V_1 indicated total volume of the supernatants, V_2 indicated the volume of MDA solution (3 ml), and m indicated wet mass of organ, respectively.

2.5. Statistical Analysis

Data were analyzed using SPSS 27.0 software (SPSS Inc., Chicago, IL, USA). The differences in SOD activities and MDA contents in a certain organ among the NF, NF + PC and PC groups were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests. Group differences in organ masses with final body mass as the covariate were analyzed by General Linear Model multivariate analysis followed by Bonferroni post hoc tests. Results are presented as Means ± SE, and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Organs

The wet masses of brain, heart, lungs, liver, kidneys, testis, epididymis, and seminal vesicles did not differ among the NF, NF + PC and PC groups (**Table 1**). How-

ever, small intestine and its contents were higher in the PC groups than in the NF and NF + PC groups. Cecum and its contents were the highest in the PC group and were the lowest in the NF group among the three groups (Table 1).

Table 1. Influence of different foods on organ masses in male Siberian hamsters.

Parameters	NF	NF + PC	PC	Statistical summary	
Sample size	10	9	9	$F_{2,25}$	P
Brain (mg)	437 ± 23	438 ± 24	425 ± 27	0.088	0.916
Heart (mg)	223 ± 6	239 ± 15	198 ± 17	2.284	0.123
Lung (mg)	395 ± 27	449 ± 41	325 ± 12	1.350	0.277
Liver (mg)	1834 ± 101	2073 ± 125	1723 ± 104	1.099	0.349
Kidneys (mg)	373 ± 40	440 ± 28	407 ± 19	1.522	0.252
Testis (mg)	828 ± 134	1020 ± 70	470 ± 109	1.301	0.291
Epididymis (mg)	143 ± 24	143 ± 12	61 ± 14	2.770	0.084
Seminal vesicles (mg)	238 ± 56	263 ± 44	70 ± 29	1.876	0.177
Stomach and its contents (mg)	1318 ± 56	1264 ± 82	1536 ± 107	3.240	0.056
Small intestine and its contents (mg)	2130 ± 63^{b}	2378 ± 106^{b}	2540 ± 133^{a}	8.503	0.002
Cecum and its contents (mg)	1157 ± 90^{b}	1524 ± 93^{c}	2303 ± 132^{a}	30.819	<0.001
Colon and its contents (mg)	924 ± 213	820 ± 85	1077 ± 363	1.968	0.161

Data are mean ± SE. Values are significantly different at $P < 0.05$, determined by General Linear Model multivariate analysis with final body mass as the covariate, followed by Bonferroni post hoc tests. Different superscript letters in the same row indicate significant group differences.

3.2. SOD Activity

SOD activities in the brain ($F_{2,26} = 10.578$, $P < 0.001$), liver ($F_{2,26} = 5.668$, $P = 0.009$) and were higher in the NF group than in the NF + PC and PC groups (Figure 1(A), Figure 1(D)). Similarly, SOD activities in the heart ($F_{2,26} = 17.573$, $P < 0.001$), kidney ($F_{2,26} = 9.252$, $P < 0.001$) and testis ($F_{2,26} = 19.565$, $P < 0.001$) were lower in the PC group than in the NF and NF + PC groups (Figure 1(B), Figure 1(E), Figure 1(F)). Lung SOD activity was lower in the NF group than in the NF + PC group ($F_{2,26} = 4.832$, $P = 0.016$) (Figure 1(C)).

3.3. MDA Content

MDA contents in the brain ($F_{2,26} = 5.374$, $P = 0.011$), heart ($F_{2,26} = 41.756$, $P < 0.001$), liver ($F_{2,26} = 5.045$, $P = 0.014$) and kidney ($F_{2,26} = 7.956$, $P = 0.002$) were higher in the PC group than in the NF and NF + PC groups (Figure 2(A), Figure 2(B), Figure 2(D), Figure 2(E)). However, MDA contents in the lung ($F_{2,26} = 2.891$, $P = 0.074$) and testis ($F_{2,26} = 2.665$, $P = 0.089$) did not differ among the three groups (Figure 2(C), Figure 2(F)).

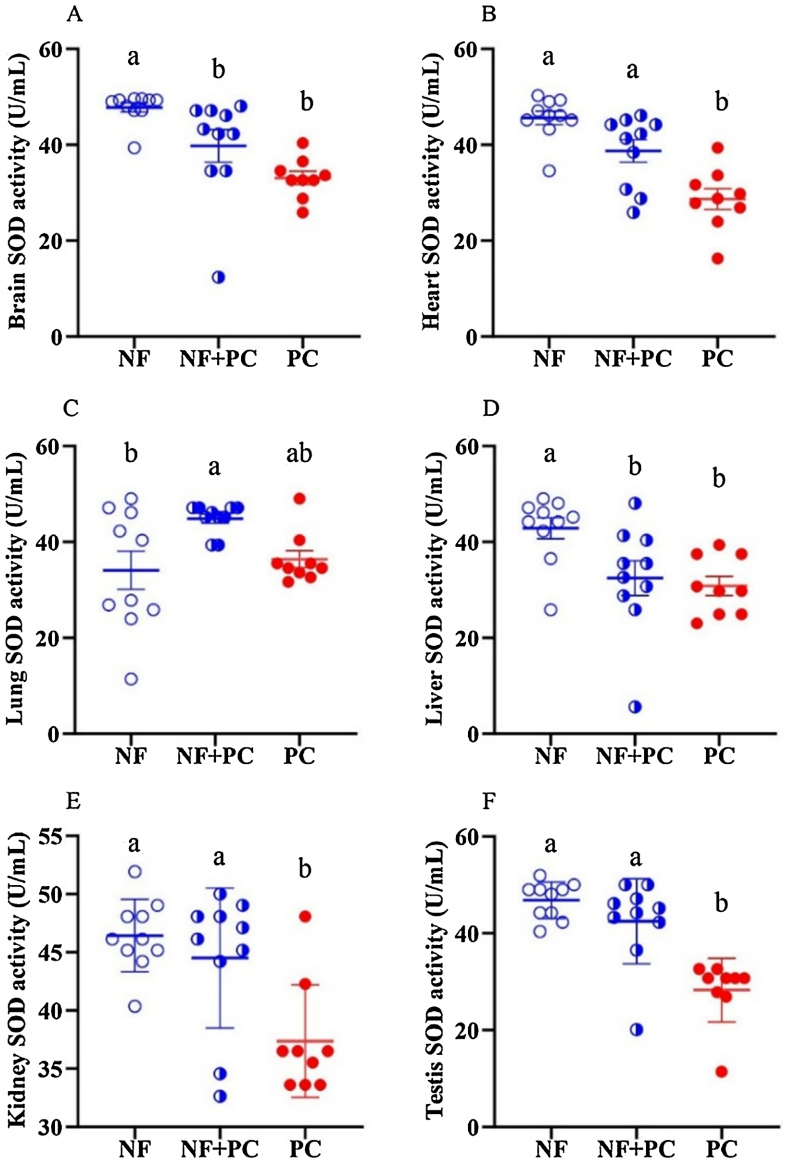
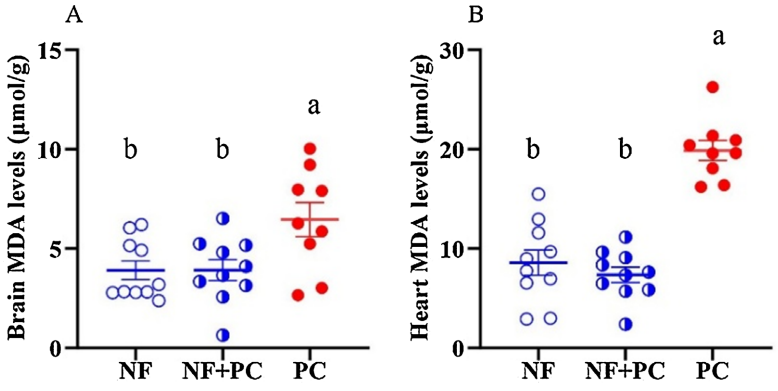


Figure 1. Influence of different foods on the SOD activity in brain (A), heart (B), lungs (C), liver (D), kidneys (E), and testis (F) in male Siberian hamsters. NF, normal food; NF + PC, normal food plus purple cabbage; PC, Purple cabbage. Different letters above the scattered dots in a figure indicated significant group differences.



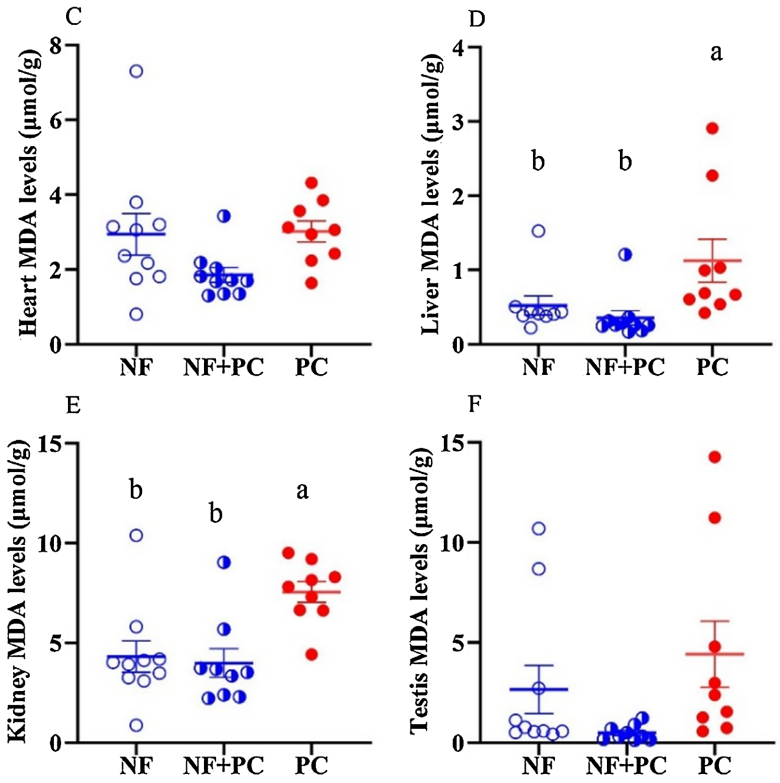


Figure 2. Influence of different foods on the contents of brain (A), heart (B), lungs (C), liver (D), kidneys (E), and testis (F) in male Siberian hamsters. NF, normal food; NF + PC, normal food plus purple cabbage; PC, Purple cabbage. Different letters above the scattered dots in a figure indicated significant group differences.

4. Discussion

As expected, feeding purple cabbage alone decreased SOD activities in the heart, kidney and testis in the PC group compared with the NF and NF + PC groups, suggesting feeding purple cabbage alone for a long time reduced antioxidant capacity in these three organs. This result indicates that the enhancing effect of purple cabbage on antioxidant capacity depends on the consumption of normal food. The reason might lie in that purple cabbage had lower energy content, and hence body mass and body fat mass were all reduced in the NF group compared with the other two groups [16]. Although nutritional values of purple cabbage are high because of its composition containing the antioxidants and anti-inflammatory activity of anthocyanins, vitamins, and minerals, animals also require other nutrients such as carbohydrates, proteins and lipids to maintain health [13]. Moreover, lung SOD activity was higher in the NF + PC group than in the NF group, implying that feeding purple cabbage increased antioxidant capacity in this organ. Contrary to our expectations, SOD activities in the brain and liver were lower in the NF + PC and PC groups than in the NF group, indicating that feeding purple cabbage reduced antioxidant capacity in these two organs. The brain and liver are important metabolically active organs that consume a lot of energy during life activities, which may lead to the production of excessive free radicals, which in turn

may inhibit the activity of SOD. The lungs consume less energy in metabolic processes than the brain and liver, so they may produce fewer free radicals, resulting in higher SOD activity. Taken together, the influence of feeding purple cabbage on SOD activities was organ-specific.

MDA contents in the brain, heart, liver and kidney were higher in the PC group than in the NF and NF + PC groups, indicating that feeding purple cabbage alone led to lipid peroxidation in these organs. These results might be due to the lower SOD activities in these organs in the PC group compared with the controls, which could not effectively eliminate free radicals and subsequently increased MDA contents. It is clear that SOD is a protein that needs energy derived from carbohydrates and lipids, and raw materials such as protein and amino acids from food when synthesized. However, purple cabbage contains very little protein, carbohydrates, and lipids. Therefore, feeding purple cabbage alone would suppress protein synthesis, including SOD. It can be seen that the weakening of antioxidant capacity leads to the occurrence of lipid peroxidation. However, MDA contents in the lung and testis did not differ among the three groups, suggesting that no oxidative damages occur in these two organs.

The present research also had some limitations. First, animals were housed in groups but not in individual cages, subsequently, all the measured indices may be influenced by feeding density. In future research, we can redesign an experiment to raise animals in single cages. Second, the activities of other antioxidant enzymes such as catalase and glutathione peroxidase were not detected; therefore, we could not understand antioxidant capacity more comprehensively. In addition, we only measured the MDA content that reflects lipid peroxidation, but did not examine oxidative damage to proteins and nucleic acids. Therefore, more parameters indicative of antioxidant capacity and oxidative damages should be considered in future research.

In summary, feeding purple cabbage alone decreased SOD activities in the heart, kidney, and testis in the PC group compared with the NF and NF + PC groups, which indicated that feeding purple cabbage alone reduced antioxidant capacity in these three organs. Lung SOD activity was higher in the NF + PC group than in the NF group, implying that feeding purple cabbage had an enhancing effect on antioxidant capacity in the lungs. SOD activities in the brain and liver were lower in the NF + PC and PC groups than in the NF group, indicating that feeding purple cabbage reduced antioxidant capacity in these two organs. MDA contents in the brain, heart, liver and kidney were higher in the PC group than in the NF and NF + PC groups, suggesting that feeding purple cabbage alone led to lipid peroxidation in these organs. Taken together, the influence of feeding purple cabbage on SOD activities was organ-specific, and feeding purple cabbage alone would lead to oxidative damage.

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Author Contributions

D.L.X. designed the study and supervised the analyses. Y.F.T wrote the draft paper. C.Y.Y. and S.C.Z. performed the experiment. D.L.X. revised the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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