



Gene Expression of Antioxidant Metabolic Enzymes in Grape Extracts

Huitian Cai^{1,2*}, Yi Zhang², Zhehan Qiu¹, Peihuan Luo¹

¹College of Food, South China Agricultural University, Guangzhou, 510642, China

²Huanong (Chaozhou) Food Research Institute Co., Ltd., Chaozhou, 521000, China

ARTICLE INFO

Original paper

Article history:

Received: August 22, 2021

Accepted: December 04, 2021

Published: December 30, 2021

Keywords:

Fruit; Extract; Antioxidant;

Metabolic Enzyme; Gene;

Expression

ABSTRACT

Fruit can use its structural characteristics to stabilize the excess electrons of free radicals and prevent cell aging, which is the main source of antioxidants supplementation. In the current study, the expression of antioxidant metabolic genes was evaluated in fruit extract. Seventy mice were divided into one control group and six experimental groups. Six experimental groups were fed 0.1g of grape extract daily for 3, 7, 10, 14, 21, 24 and 30 days, respectively. The antioxidant activity of fruit extract was tested by antioxidant metabolic enzyme gene expression in the liver and kidney of different experimental groups. The results showed that grape extract could effectively enhance SOD activity, LPL relative expression and FAS relative expression in mice liver tissue; grape extract could effectively increase SOD relative expression, TrxR2 relative expression and CAT relative expression in mice kidney tissue; grape extract could effectively reduce MDA activity in mice liver tissue, and increase and decrease the extent of MDA activity and the duration of feeding in mice. The proportion of fruit extracts showed that fruit extracts could effectively improve the antioxidant performance of the body.

DOI: <http://dx.doi.org/10.14715/cmb/2021.67.6.27>

Copyright: © 2021 by the C.M.B. Association. All rights reserved.



Introduction

Free radicals are intermediate products of biochemical reactions in the process of an organism's life activities. Under normal circumstances, the production and elimination of free radicals in the body are in a dynamic equilibrium. When it produces too much or eliminates too slowly in the body, it will cause damage to the body at the molecular level, cell level and organ level, attack the unsaturated fatty acids that make up the cells, leading to cell membrane aging, accelerate the aging process of the body, and induce cancer, cardiovascular disease and so on. Under the requirement of advocating nature and returning to nature, great progress has been made in the research and development of natural antioxidants (1). In addition, natural antioxidants have been developed from antioxidants for oil and fat-containing foods to scavengers for oxygen-free radicals in vivo. It has been proved that these natural compounds have physiological functions such as protecting human cell tissues, protecting cardiovascular and cerebrovascular circulation systems, anti-cancer and delaying aging. As we all know, fruits and vegetables not only provide

some important nutrients such as vitamins and minerals but also have the functions of anti-oxidation, anti-mutation, cancer prevention, immunity enhancement, anti-allergy, blood pressure regulation and cholesterol (2). It also provides a variety of basic nutrients such as carbohydrates, minerals, proteins and so on.

Apple is called "all-round healthy fruit" by scientists. Eating apples regularly can reduce blood lipids, control blood pressure, prevent cancer, strengthen bones, maintain acid-base balance and lose weight. In addition to the traditional nutrition of pectin fiber and vitamin group, apple also contains a lot of antioxidants (3). These antioxidants can be hundreds of species and are considered to be the holy products of cancer prevention and aging. Strawberry, also known as red berry, asparagus, raspberry and so on, is the generic name of Rosacea strawberry plants, there are three "kinds in the world. Strawberry nutrients are easily digested and absorbed by the human body. It is healthy food for all ages. The traditional Chinese medicine circles believe that strawberry has the functions of moistening lung,

*Corresponding author. E-mail: caihuitian188@163.com

nourishing the body, clearing heat and cooling blood, invigorating the spleen and eliminating alcohol, and has certain nourishing and regulating effects on the gastrointestinal tract and anemia (4, 5). Strawberry is a plant rich in tannic acid, which can absorb and prevent the absorption of carcinogenic chemicals in the body and has the function of cancer prevention. Grapes are not only delicious but also of high nutritional value. The content of glucose in ripe berries is as high as 10%-30%. Glucose is the main component. Many kinds of fruit acids in grapes help digestion. Eating more grapes properly can strengthen the eyes and stomach. Grapes contain minerals such as calcium, potassium, phosphorus, iron, vitamin B1, vitamin B2, vitamin B6, vitamin C and vitamin P. They also contain a variety of amino acids needed by the human body. Frequent grapes are of great benefit to neurasthenia and fatigue (6). It was found that grapes could prevent thrombosis better than aspirin, reduce serum cholesterol level and platelet cohesion, and play a certain role in preventing cardiovascular and cerebrovascular diseases. Eating a moderate amount of fresh grapes every day not only reduces the risk of cardiovascular disease but also is particularly beneficial to the health of patients with ischemic heart disease and atherosclerotic heart disease (7). Flavonoids in fresh grapes can "clean" blood and prevent cholesterol plaque formation. The more black the grapes are, the more flavonoids they contain. But if grape skin and grape seeds are eaten together, the better the protection of the heart is.

Various endogenous and exogenous factors will produce excess free radicals in the body, causing harm to the body. Some natural phytochemicals in fruits can protect, scavenge and repair excessive free radicals. These natural phytochemicals include carotenoids, phenolic compounds, phytic acid and so on. The antioxidants in grapes mainly include flavonoids such as flavanone and anthocyanin, catechin monomer and oligomeric protoplast anthocyanin, in addition to resveratrol and its glycosides. Catechin can significantly inhibit collagen-induced hydrogen peroxide production (8). Proanthocyanidins can also react directly with hydroxyl radicals and hydrogen peroxide.

The reactive oxygen species (ROS) cluster is an oxidative product produced by organisms during normal aerobic cell metabolism and respiratory burst.

It participates in many biological processes in vivo, including normal cell growth, programmed cell death and cell senescence. In reactive oxygen species, hydrogen peroxide is a peroxide that easily forms free radicals. The formation and action of singlet oxygen-excited molecular oxygen can also involve free radical reactions. Therefore, many free radical reactions in organisms are related to reactive oxygen species (9). Free radicals are substances with strong oxidation ability and active nature produced by redox reactions in the normal metabolism process of the body. Even a small amount of free radicals can cause great harm to the body. Complex systems of endogenous and exogenous antioxidant sources in vivo are used to alleviate the potential damage of free radicals (10). The production and removal of free radicals are in a dynamic balance. If the body's ability to scavenge free radicals decreases (11), resulting in excessive free radicals, structural abnormalities of cell membranes and mitochondrial membranes, dysfunction and aging may occur, and many chronic and degenerative diseases, including cancer. Atherosclerosis and neurodegenerative diseases are examples of free radical-mediated oxidative stress.

Anything that can oxidize stress all the time and damage it to the target molecule can become an antioxidant. The main function of antioxidants is to inhibit and eliminate free radicals. In order to protect itself from free radical-mediated oxidative stress, different cell antioxidants, such as glutathione, tocopherol and ascorbic acid, as well as peroxidase, superoxide dismutase and catalase, can interact with reactive oxygen species. The body can also alleviate oxidative stress in vivo by supplementing antioxidants. However, some synthetic antioxidants have shown potential adverse reactions such as liver injury, especially long-term use of synthetic antioxidants (12). Therefore, exploring safe and natural antioxidants to resist oxidative stress has become a research hotspot in recent years (13). Epidemiological studies have shown that the intake of fruits and vegetables is proportional to the prevention of diseases caused by oxidative stress. Flavonoids, anthocyanins, lignin and tannins in plant tissues all have good free radical scavenging capacity and belong to the category of natural antioxidants.

According to the relationship between antioxidants and health, Cömert *et al.* (14) draw the following

three main conclusions: many epidemiological studies worldwide agree that a diet rich in vegetables containing antioxidants is an important component of several healthy behaviors related to health and longevity. Two large-scale dietary surveys in the United States have shown that the intake of beta-carotene, VA and VE is associated with lower levels of ischemic heart disease and cancer risk. The concentration of some major antioxidants in plasma is a reliable indicator of the actual antioxidant capacity of the body. Blair et al. have shown that physical exercise and lifelong physical activity contribute to a slow decline in age-related functional capacity and prevent or delay the onset of some degenerative diseases (15). The effect of lifelong physical activity on life expectancy is an additional 2-7 years on the basis of average life expectancy. But it has never been determined whether physical activity can increase maximum life expectancy. Simon et al. have shown that physical exercise can reduce the balance of the body and cause a series of adaptive changes in the stress system. Regular exercise can promote the body to adapt to acute exercise stress and enthusiasm, thus restoring its balance. Zhang *et al.* (16) have shown that aerobic exercise can improve the level of endogenous antioxidant enzymes and enhance the ability of antioxidant defense. Its effect is the same as that of pure exogenous supplementation of antioxidants. Exercise-induced increase in endogenous antioxidant levels and defensive capacity may be the first to resist the modification of LDL by ROS, MDA and endothelial cells without consuming the antioxidants contained in LDL. Studies on gene expression of Pre-oxidation and anti-oxidation states have shown that the regulation of protein and DNA can be promoted through physiological oxidation-antioxidation balance. Many studies reviewed by Sen (17) have shown that exercise-induced changes in glutathione redox status contribute to protein synthesis and produce more antioxidant proteins. In order to effectively detect the antioxidant activity of fruits, grape extracts with high antioxidant activity were selected. The antioxidant activity of fruits was effectively monitored by studying the gene expression of antioxidant metabolic enzymes in 70 experimental mice.

Materials and methods

Laboratory Animals

Seventy C57BL/6 mice aged 7 weeks, half male and half female, average weight 16.9g, SPF grade, certificate number: SCXK (Shanghai) 2007-0005. After being purchased from Shanghai Laboratory Animal Center, the IVC system in the laboratory was routinely kept in cages for one week. All cages, food, water and cushions contacted by mice were sterilized under high pressure.

Reagents

SOD and MDA kits were purchased from Nanjing Jiancheng Bioengineering Company; Molecular biology reagents were purchased from Promega and Sigma Company, nylon membrane was purchased from Amershan Company, and recombinant plasmid PUC13-RCS containing mouse SOD gene (DNA size 0.6kb, Cloningsite: EcoRI) was purchased from Wcyne State University. *E. coli* JM-109 was provided by the Microbiology Teaching and Research Department of the Academy of Life Sciences, Nankai University. The others are domestic and imported analytical pure. A-32P-dCTP was purchased from Beijing Yahui Biomedical Engineering Company.

Preparation of fruit extract

The fresh grapes were washed and dried. The skinned grapes were cut into thin slices about 2 mm thick. The grape pulp was centrifuged at 40,000 rpm for 20 minutes. The volume of sampling liquid was measured and the sediment was discarded. Because there was a lot of pectin in fruits, it is necessary to remove pectin: add absolute ethanol of the same volume as the sample solution, gently stir (18) with a glass rod, and then pour it into the centrifugal cup after 30 minutes, centrifuge for 10 minutes at the speed of 4000 revolutions per minute, and take the supernatant. If a small amount of flocculent precipitation is found in the supernatant, the extract can be obtained by filtration.

Experimental group

70 mice were divided into 1 control group and 6 experimental groups. The mice in 6 experimental groups were fed 0.1g fruit extract at the same time every day. The duration of each group was different.

The specific feeding time and groups were shown in Table 1.

Table 1. Specific grouping of experimental mice

Group	Quantity	Daily feeding dose/g	Feeding days/d
Control group	10		
Experiment A	10	0.1	3
Experiment B	10	0.1	7
Experiment C	10	0.1	10
Experiment D	10	0.1	14
Experiment E	10	0.1	21
Experiment F	10	0.1	30

Instruments and equipment

Instruments and equipment that used in this study were EXL800 enzyme marker (Biotek Instruments Co., Ltd), QL-861 eddy current mixer (Taicang Science and Education Equipment Factory), HH-S11 electrothermal constant temperature water bath (Guangzhou Huruiming Instrument Co., Ltd), AR1140 electronic analysis balance (Ohaus Instruments Co., Ltd), DGG-9140A electric heating blast dryer (Shanghai Senxin Experimental Instrument Co., Ltd), FW80 high-speed universal crusher (Tianjin Tester Instrument Co., Ltd), RE-85Z rotary evaporator (GongyiYingyuYuhua Instrument Factory), FD-1

freeze dryer (Beijing Detianyou Technology Development Co., Ltd), -80°C cryopreservation box (Qingdao Haier Special Electrical Appliances Co., Ltd), 1200N high-performance liquid chromatography (HPLC), and 7200 triple tandem quadrupole time-of-flight mass spectrometry (MS) instrument Agilent Inc.

Primer design and synthesis

Primers were designed and synthesized according to the gene sequence of mice published by Genbank and references. The primers were synthesized by Shanghai Shengong Bioengineering Technology Service Co., Ltd. Primer information is shown in Table 2.

Experimental steps

Animal treatment: The experiment was carried out according to the above animal groups. Animals were decapitated and killed. Livers and kidneys were quickly taken from ice tables to prepare tissue homogenate and supernatant was taken for detection (19). **Total RNA extraction:** Guanidine isothiocyanate-phenol-chloroform one-step extraction method was used to extract and preserve at -80°C.

Table 2. Primer sequences for Sod/Gpx/Cat genes

Name of genes	Accession number	Primer sequences	Fragment length of genes/bp
Sod1	45597446	S: 5'ATGGCGATGAAAGCGGTGTG3' A: 5'TTACTGCGCAATCCCAATCACTC3'	476
Sod2	76253932	S: 5'ATGTTGTGTCGGGCGGCG3' A: 5'TCACTTCTTGCAAGCTGTGTATCTTTCA3'	679
Sod3	86439990	S: 5'ATGTTGGCCTTCTTGTCTACGG3' A: 5'TTAAGTGGTCTTGCACTCGCTCT3'	767
Gpx1	145275166	S: 5'ATGTGTGCTGCTCGGCTCTCC3' A: 5'TTAGGAGTTGCCAGACTGCTGGG3'	617
Gpx2	145275167	S: 5'ATGGCTTACATTGCCAAGTCG3' A: 5'CTAGATGGCAACTTTGAGGAGCC3'	584
Gpx3	145275178	S: 5'ATGTGGCTGAGCGCTTCGG3' A: 5'TTACTTCCCCCTGGCGCTCAG3'	758
Gpx4	90903234	S: 5'ATGAGCTGGGGCCGTCTGAG3' A: 5'CTAGAGATAGCACGGCAGGTCCT3'	605
Gpx5	6754061	S: 5'ATGGTTACAGAGTTAAGAGTCTTCTATCTTGTT3' A: 5'CTATATGGTTTTGAAATGGCTCAGGTAC3'	677
Gpx6	146260277	S: 5'ATGGCCCAGAAGTTGTGGGG3' A: 5'TTACTGGGTACTGGTTTGGTTTAG3'	677
Cat	6753271	S: 5'GCAGATACCTGTGAACTGTCCCT3' A: 5'TTACAGGTTAGCTTTCCCTTCG3'	483

Qualitative analysis of grape extracts by HPLC-MS

Conditions for HPLC: ODC18 column (4.6mm×250mm, 5µm), mobile phase A was water-

formic acid (97:3, V/V) solution, mobile phase B was acetonitrile solution. Gradient elution conditions were as follows: 0-3 min, 10%-15% B; 3-5 min, 15%-20% B; 5-8 min, 20%-25% B; 8-10 min, 25%-30% B; 10-

20 min, 30%-40% B; 20-30 min, 40%-70% B; 35-40 min, 70%-100% B; 40-41 min, 100% B; 3 min, 10% B. The flow rate was 300 L/min, the column temperature was 30°C, the injection volume was 5 µL, and the detection wavelength was 280 and 520 nm.

MS conditions: an electrospray ion source, ion source temperature 350°C, capillary voltage 32.5kV, taper hole voltage -5V, photoelectric multiplier voltage -1030V; electrospray ion source voltage 5kV; positive ion scanning; mass scanning range $m/z = 100\sim 1000$.

Determination of antioxidant components in fruit extracts

Determination of total phenol content: Folin phenol method was used. A 25 µL gallic acid (GAE) standard or fruit extract was added to a 96-well plate, and a 125 µL Folin phenol reagent was added to the plate. After 10 minutes of reaction at room temperature, a 125 µL saturated sodium carbonate solution was added. The absorbency was determined at 765 nm by an enzyme-labeled instrument after 30 minutes at room temperature. The total phenol content is expressed in mgGAE/g. All samples were determined three times.

The content of total flavonoids was determined by the improved colorimetric method by Chun. The extract was prepared into 200 mg/mL solution for reserve. Mix 2 mL solution with 0.2 mL 5 g/100 mL sodium nitrite solution. After 6 minutes, add 0.2 mL 10g/100 mL $AlCl_3 \cdot 6H_2O$ solution, mix and shake well (20). After 5 minutes, 2 mL 1mol/L sodium hydroxide solution was added, and the reaction solution was fully mixed. The absorbance was determined at 510 nm after 15 minutes. The content of total flavonoids was calculated according to the standard curve of rutin. The total flavonoid content was expressed by rutin (RT) equivalent milligram per gram of dry quality sample material (mgRT/g). All samples were determined three times.

Quantitative fluorescence polymerase chain reaction to detect the relative expression of gene mRNA

QRT-PCR was performed according to SYBR Premix Ex Taq II Kit instructions. The reaction system was 25 µL: SYBR Premix Ex Taq II 12.5 µL, DNA 2 µL, upstream and downstream primers 1 µL, and sterilized water was added to 25 µL.

The reaction conditions were as follows: pre-denaturation at 95°C for 30 seconds, denaturation at 95°C for 5 seconds, annealing at 60°C for 30 seconds, extension at 60 (?) for 30 seconds, 40 cycles, dissolution at 55°C for 10 seconds and 81 cycles (18). ActB was used as an internal reference and $2^{-\Delta\Delta Ct}$ was used to calculate the relative expression of each gene.

Statistical analysis

The experimental data were expressed by mean±standard deviation). The results of LSD multiple comparisons were analyzed by the single factor variance method using SPSS 17.0. When $P < 0.05$, there is a significant difference in the experimental results. When $P < 0.01$, there is a significant difference in the experimental results. The above two cases show that the experimental results have statistical significance.

Results and Discussions

Component identification of grape extract

The high-performance liquid chromatography (HPLC) image of grape extract at 280 nm is shown in Figure 1.

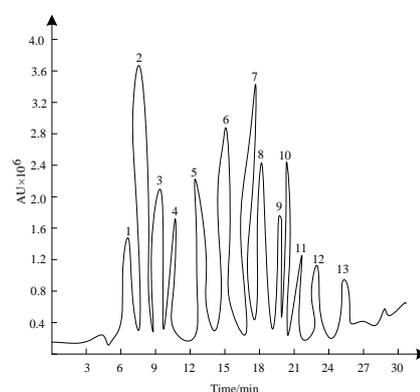


Figure 1. HPLC chromatogram at 280 nm of ethanol extract from grape

The results of HPLC-MS analysis of grape extracts are shown in Table 3.

The results of Figure 1 and Table 3 show that the main components of the grape extract absorbed at 280 nm are trans-resveratrol, hexose protocatechuate, quercetin-3-O-rhamnoside, hexose vanillin, dimethylanthocyanidin-3,5-diglucoside and anthocyanin-3,5-diglucoside, which contain a large number of antioxidants, which effectively illustrates the effectiveness of the grape extract and can be

applied to anti-oxidation. Then, studying on gene expression of oxidative metabolic enzymes is useful.

Table 3. HPLC-MS analysis of ethanol extract from grape

Peak number	Reserved Time/min	Maximum absorption Wavelength/nm	MS/m/z	Component identification
1	7.22	280	326	Protocatechuic acid hexose
2	8.11	280	238	Anti-resveratrol
3	8.99	280	458	Quercetin-3-O-rhamnoside
4	10.93	280	331	Vanillic acid hexose ester
5	12.49	280	145	Ellagic acid
6	17.95	280、520	612 (314)	Anthocyanin coumaricglucoside
7	19.51	280、530	666	Dimethylglycine-3,5-diglucoside
8	19.91	280、520	622	Anthocyanin-3,5-diglucoside
9	21.52	280、520	828	Dimethyl anthocyanin-3-Trans-caffeylated glucoside
10	21.91	280、520	798	3'-methanin-3-coumamidation Glucoside-5-glucoside
11	22.52	280、520	812	Dimethyl anthocyanin-3-trans-coumaroylated glucoside-5-glucoside
12	25.63	280	611	Quercetin-3-rutinoside
13	28.19	280、520	641	Dimethyl anthocyanin trans-coumaroylatedglucoside

Antioxidant activity of fruit extracts

The antioxidant activity, total phenolic content and total flavonoid content of grape extract are shown in Table 4.

Table 4. Antioxidant activity of the grape extract

index	Grape extract
Total phenolic content/(mg GAE/g)	19.64±0.99
Total flavonoids/(mg RT/g)	33.12±2.35

According to the relevant research results, the total phenolic content of 1 g mango extract is about 1.0 mg; 1 g strawberry extract is about 2.8 mg; 1 g mango extract is about 5.3 mg; and 1 g strawberry extract is about 7.1 mg. The results of Table 4 showed that the total phenolic content of the grape extract was (19.64±0.99) mg GAE/g) and total flavonoid content was (33.12±2.35) mg RT/g. The total phenolic content and total flavonoid content of grape extract were much higher than those of mango and strawberry. Phenolic compounds were the main antioxidant components of fruit extract. The experimental results showed that the antioxidant activity of the grape extract was obvious. The antioxidant activity of the grape extract was higher than that of mango and strawberry, which verified the validity of selecting grape extract to study the gene expression of antioxidant metabolic enzymes.

Effect of fruit extract on SOD activity in liver tissue of mice

The comparison of SOD activity in liver tissues of experimental mice in each group is shown in Table 5.

Table 5. Comparison results of SOD activity

Group	Quantity	SOD activity/u/mg
Control group	10	54.94±0.65
Experiment A	10	55.18±0.87
Experiment B	10	56.81±0.97
Experiment C	10	58.64±1.05
Experiment D	10	61.57±1.18
Experiment E	10	64.25±1.28
Experiment F	10	67.54±1.42

Superoxide Dismutase Orgotein (SOD), alias liver protein. SOD is an active substance derived from living organisms, which can eliminate harmful substances produced by organisms in the process of metabolism. Continuous supplementation of SOD has a special anti-aging effect on the human body. The experimental results of each group in Table 5 were all $P < 0.05$, with statistical significance. The results of Table 5 showed that the SOD activity in liver tissue of mice fed with grape extract was significantly higher than that of control mice, and the duration of feeding was proportional to the SOD activity. The experimental results showed that the SOD activity of mice fed with fruit extract could be effectively enhanced.

Effect of a grape extract on MDA activity in liver tissue of mice

The comparison of MDA activity in liver tissues of experimental mice in each group is shown in Table 6.

Table 6. Comparison results of MDA activity

Group	Quantity	MDA activity/nmol·mg ⁻¹
Control group	10	4.31±1.86
Experiment A	10	4.25±1.89
Experiment B	10	4.18±1.76
Experiment C	10	4.04±1.61
Experiment D	10	3.91±1.55
Experiment E	10	3.85±1.43
Experiment F	10	3.78±1.33

Malondialdehyde (MDA), an end product of lipid oxidation, affects the activity of mitochondrial respiratory chain complexes and key enzymes in mitochondria in vitro. MDA is one of the most important products of membrane lipid peroxidation, and its production can also aggravate membrane damage. Therefore, the content of MDA is a commonly used index in plant senescence physiology and resistance physiology research. The degree of membrane lipid peroxidation can be understood by MDA to indirectly measure the degree of membrane system damage and plant stress resistance. The experimental results of each group in Table 6 were all $P < 0.05$, with statistical significance. The results of Table 6 showed that the activity of MDA in liver tissue of mice fed with grape extract was significantly lower than that of control mice, indicating that grape extract could slow down the aging rate of mice, and the duration of feeding was inversely proportional to the activity of MDA. The results showed that the activity of MDA in mice fed with fruit extract could be effectively reduced.

Effect of a grape extract on LPL expression in liver tissue of mice

The relative expression of LPL in liver tissues of experimental mice in each group was compared as shown in Table 7.

Table 7. Comparison results of relative expression of LPL

Group	Quantity	LPL
Control group	10	0.58±0.17
Experiment A	10	0.73±0.13
Experiment B	10	0.93±0.11
Experiment C	10	1.27±0.18
Experiment D	10	1.47±0.22
Experiment E	10	1.69±0.19
Experiment F	10	1.87±0.23

LPL is a key enzyme for lipid deposition in animal tissues. It is a rate-limiting enzyme for the degradation of triglycerides to glycerol and free fatty acid (FFA), and plays an important role in lipid metabolism and transport. By controlling the expression of LPL in adipose tissue and other tissues and organs, it directly determines the relative amount of lipid substrates in adipose tissue and other tissues and organs, thus indirectly deciding the metabolic future of lipid intake from food: stored in the form of body fat or consumed as energy substrates, and ultimately has a decisive impact on body fat accumulation. The experimental results of each group in Table 7 were all $P < 0.05$, with statistical significance. The results of Table 7 showed that the relative expression of LPL in liver tissue of mice fed with grape extract was significantly higher than that of control mice, and the duration of feeding was proportional to the relative expression of LPL. The experimental results showed that the relative expression of LPL in mice fed with fruit extract could be effectively increased.

Effect of a grape extract on the expression level of FAS in liver tissue of mice

The relative expression of FAS in liver tissues of experimental mice in each group was compared as shown in Table 8.

Table 8. Comparison results of relative expression of FAS

Group	Quantity	LPL
Control group	10	1.18±0.08
Experiment A	10	1.26±0.38
Experiment B	10	2.08±0.64
Experiment C	10	2.84±0.78
Experiment D	10	3.29±0.89
Experiment E	10	3.85±0.94
Experiment F	10	4.38±0.87

FAS can catalyze the synthesis of palmitic acid (C16:0) from acetyl coenzyme A and malonate monoacyl coenzyme A. The amount and activity of FAS are of great significance to animal body fat deposition, thus playing an important role in animal body fat deposition. Lower FAS content affects the activation of insulin signaling cascade factors, which may mimic, enhance or interfere with the regulation of insulin on carbohydrate and fat metabolism, affecting body fat deposition and reducing human antioxidant capacity. The

experimental results of each group in Table 8 were all $P < 0.05$, with statistical significance. The results of Table 8 showed that the relative expression of FAS in liver tissue of mice fed with grape extract was significantly higher than that of control mice, and the duration of feeding was proportional to the relative expression of FAS. The experimental results showed that the relative expression of FAS in mice fed with fruit extract could be effectively increased.

Effect of a grape extract on SOD expression in kidney tissue of mice

The relative expression of SOD in kidney tissue of experimental mice in each group was compared as shown in Table 9.

Table 9. Comparison of SOD expression levels in kidney tissues

Group	Quantity	SOD1	SOD2
Control group	10	0.65±0.08	0.43±0.11
Experiment A	10	0.71±0.11	0.51±0.18
Experiment B	10	0.79±0.12	0.59±0.21
Experiment C	10	0.81±0.21	0.62±0.19
Experiment D	10	0.88±0.24	0.73±0.21
Experiment E	10	0.92±0.32	0.85±0.26
Experiment F	10	0.99±0.35	0.98±0.29

In the SOD-catalyzed disproportionation of O_2^- , H_2O_2 was produced, and the accumulation of H_2O_2 inhibited SOD. Normally, H_2O_2 produced by SOD disproportionation can be transformed into a non-toxic substance by CAT in time, thus regulating the balance of O_2^- and H_2O in the body. The experimental results of each group in Table 9 were all $P < 0.05$, with statistical significance. The results of Table 9 showed that the relative expression of SOD1 and SOD2 in kidney tissue of mice fed with grape extract was significantly higher than that of control mice, and the duration of feeding was proportional to the relative expression of SOD. The experimental results showed that the relative expression of SOD in kidney tissue of mice fed with fruit extract could be effectively increased.

Effect of grape extract on TrxR2 expression in kidney tissue of mice

The relative expression of TrxR2 in kidney tissues of mice in each group was compared as shown in Table 10.

Table 10. Comparison of TrxR2 expression levels in kidney tissues

Group	Quantity	TrxR2
Control group	10	0.58±0.11
Experiment A	10	0.63±0.13
Experiment B	10	0.68±0.16
Experiment C	10	0.71±0.19
Experiment D	10	0.86±0.23
Experiment E	10	0.92±0.28
Experiment F	10	0.99±0.31

TrxR2 is an oxidoreductase that mainly exists in mitochondria. It can maintain the reductive form of Trx and regulate oxidative stress in mitochondria. Therefore, it is important to detect the relative expression of TrxR2 in kidney tissues of mice in each group for studying the gene expression of antioxidant metabolic enzymes in fruit extracts. The experimental results of each group in Table 10 were all $P < 0.05$, with statistical significance. The results of table 10 showed that the relative expression of TrxR2 in kidney tissue of mice fed with grape extract was significantly higher than that of control mice, and the duration of feeding was proportional to the relative expression of TrxR2. The experimental results showed that the relative expression of TrxR2 in kidney tissue of mice fed with fruit extract could be effectively increased.

Effect of a grape extract on CAT expression in kidney tissue of mice

The expression of CAT in kidney tissues of experimental mice in each group was compared as shown in Table 11.

Table 11. Comparison of CAT expression levels in kidney tissues

Group	Quantity	CAT
Control group	10	0.49±0.13
Experiment A	10	0.57±0.16
Experiment B	10	0.63±0.18
Experiment C	10	0.78±0.21
Experiment D	10	0.83±0.27
Experiment E	10	0.98±0.26
Experiment F	10	0.99±0.24

CAT is also an important antioxidant enzyme, which is indispensable in the regulation of superoxide anion free radicals and hydrogen peroxide. SOD and CAT play a protective role through mutual regulation.

In Table 11, the experimental results of each group were all $P < 0.05$, with statistical significance. The results of Table 11 showed that the relative expression of CAT in kidney tissue of mice fed with grape extract was significantly higher than that of control mice, and the duration of feeding was proportional to the relative expression of CAT. The experimental results showed that continuous feeding of fruit extract could effectively increase the relative expression of CAT in the kidney tissue of mice.

The results showed that fruit extract could effectively improve the function of the antioxidant system and inhibit lipid peroxidation in mice kidneys. Too little antioxidant leads to the decrease of TrxR2 synthesis, the increase of oxidative stress in mice kidneys, and the oxidative damage of mitochondria. Mitochondrial damage leads to the disorder of cellular aerobic metabolism and the increase of O_2^- production, which affects the activity and expression of SOD and CAT. The supplementation of fruit extracts can protect animal kidneys and organisms better by inhibiting lipid peroxidation, reducing mitochondrial damage and scavenging oxygen free radicals.

Antioxidants are any substance that prevents oxidation of lipids, carbohydrates, proteins, DNA and other oxidizable substrates. Many enzymes such as superoxide dismutase, catalase, and some non-enzymatic substances such as vitamin E, vitamin C, beta-carotene and glutathione have antioxidant effects (21). Among them, the antioxidant enzymes with important physiological functions are SOD, CAT and GSH. Antioxidants, such as VE, VC and GSH, can synergize with various compounds to scavenge free radicals or interrupt free radical reactions. The role of antioxidant enzymes in health has attracted close attention in part because of the anti-aging effect of the mass antioxidant market.

Free radicals are atoms, clusters or molecules with unpaired electrons in outer orbits. According to statistics, in every 25 oxygen molecules used for metabolism, one oxygen can produce unstable forms of oxygen, called superoxide anion free radicals. During the oxidative phosphorylation of mitochondria, most of the oxygen is reduced to water, but at the same time a small amount of oxygen (2-5%) is transformed into an intermediate state substance with active and unpaired electrons, called reactive

oxygen groups (ROS). When oxygen is not completely reduced to water through the transfer of four hydrogens and four electrons, a superoxide anion radical or some other ROS will be produced. To restore atomic and molecular stability, superoxide anion radicals actively seek to strip an electron from various cell sources containing one hydrogen. In order to pair an electron with the lone electron in its outer orbit, superoxide anion radicals attack sugar, protein, DNA, polyunsaturated fatty acids and other biological molecules. ROS is a general name used to describe oxygen-centered free radicals and some non-free radical oxygen derivatives. ROS is also a relative term for atoms or molecules that have more activity than other atoms or molecules in the search for electrons. There is a lot of evidence that ROS products can cause cell and tissue damage through a series of physiological and biochemical reactions, as well as some diseases and even aging. Usually, whatever ROS attacks, it will be changed in some way (22). In this way, it will no longer play an appropriate role. However, recent evidence suggests that some types of ROS can act as signal transduction messengers. In fact, this may be an important adaptive signal in muscle tissue that promotes enzyme repair and protein synthesis.

While superoxide anion radicals and ROS are produced, the body also has a complete antioxidant system to scavenge superoxide anion radicals and ROS. The antioxidant system protects the body against the harmful effects of superoxide anion radicals and ROS by providing electrons that stabilize free radical molecules or by regulating the transformation of superoxide anion radicals and ROS into lower harmful forms. Antioxidants play an independent role in cells and tissues and cooperate with each other to some extent to scavenge superoxide anion free radicals and ROS. The three main antioxidant enzymes are SOD, CAT and glutathione Superoxidase (GPX). SOD scavenges superoxide anion radicals by converting two oxygen radicals and two hydrogens into hydrogen peroxide. The hydrogen peroxide produced by SOD has potential toxicity to cells (23). Previous studies have shown that H_2O_2 can indirectly induce HIV-1 gene expression in the human immunodeficiency virus. Although the transformation of oxygen free radicals into H_2O_2 is temporarily beneficial to the body, the removal of H_2O_2 is

essential for the survival of cells. Cat helps to remove H_2O_2 and generates harmless water in the process. GPX can also remove H_2O_2 from cells by converting GSH to oxidized glutathione using H_2O_2 . When the body is in a quiet state or slightly exercising, the oxidation and antioxidant systems are in a balanced state, but with the increase of exercise intensity, the oxidation and antioxidant state will be unbalanced. If the unbalanced relationship between superoxide anion free radicals and ROS production and antioxidants is beneficial to the former, oxygen stress will occur, which is related to many chronic diseases (such as cancer, diabetes mellitus and hypertension) related toxicity (5, 24).

A large number of convincing epidemiological studies support the relationship between antioxidants, health and disease status. Epidemiological studies from many countries have shown a negative correlation between increased risk of heart disease and stroke and reduced antioxidant intake. These studies have shown that some of the main antioxidant concentrations in the body depend mainly on the dietary supply. The WHO cardiovascular disease surveillance project and vitamin sub-project studies show that among comparable risk factors for heart disease, some of the major antioxidants measured show a statistically significant negative correlation between the age of cardiac death and the absolute concentration of vitamin E. In addition to the fruit studied in this paper has certain antioxidant properties, physical exercise is also an important way to improve the body's antioxidant properties.

Studies have shown that moderate exercise can increase some antioxidant concentrations, while physical activity restriction can reduce antioxidant activity. Guo Lin and others have shown that endurance exercise can improve the activity of antioxidant enzymes and the ability to fight free radicals in tissues, and reduce the damage caused by free radicals. Endurance training can lead to the doubling of mitochondrial components such as cytochrome c and a. Cytochrome in the electron transfer system helps to completely convert four electrons and hydrogen into oxygen to form water. If no electrons are leaked from the formation of ROS from ETS, the body will weaken its need for antioxidants. Therefore, the decreased regulation of antioxidant activity during quiet time in exercise-

trained people may be due to the more smooth ETS function (25). The effects of plant and fruit extracts on gene expression have been investigated in many studies (26-32).

During exercise, it is easy to increase the total body oxygen consumption by more than 10 times. Increased energy demand and calorific output related to exercise can make ETS strive to completely reduce oxygen to water, resulting in the formation of hydrogen peroxide due to the leakage of some electrons. Studies have shown that the increase in antioxidant levels is proportional to the demand for exercise. Participation in regular physical activity usually results in a proportional change in antioxidants and oxygen stress. That is to say, when the current oxidation activity is low (e.g., when the oxygen consumption is at baseline level at rest), the antioxidant level is also low. When the current oxidation activity is high (e.g. during exercise), the antioxidant level increases correspondingly with the pre-oxidation activity. Many studies have reported that long-term physical activity can improve antioxidant levels after exercise. Long-term training did not significantly affect antioxidant levels at rest, perhaps due to the increase in mitochondrial number or volume, and the rare occurrence of interruptive reactions during oxygen reduction to water, resulting in an increase in the body's natural resistance to oxygen stress. There is a close relationship between the level of cardiovascular quality and the ability of oxidation and antioxidant at rest. The higher a person's cardiovascular quality level is, the higher the activity of oxidase (e.g., cytochrome a and c), and the lower the level of antioxidants at rest. This suggests that less oxygen stress may be due to the reduction of ROS products by promoting the ability of electrons to convert to oxygen. Relative to the percentage of maximum aerobic capacity, exercise-induced oxygen stress can affect oxidation and antioxidant status. Untrained people have less antioxidant and oxidative capacity than trained people. It is speculated that individuals who are physically active may be better trained to deal with acute oxygen stress associated with exercise, or some other metabolic stressors. Regular physical activity is essential to naturally improve the ability of endogenous antioxidants to resist superoxide anion radicals and ROS, as well as to reduce the risk of some aging-related diseases or delay the onset of these

diseases. If health and longevity are significantly affected by oxygen stress, then exogenous and endogenous antioxidants can affect health and longevity. If endogenous antioxidants cannot effectively eliminate superoxide anion radicals and ROS, antioxidant supplements can provide a second line of defense for scavenging superoxide anion radicals and ROS. Regular physical activity can not only increase muscle quality and oxidative capacity, but also enhance immune function, enhance the ability of anti-oxidation to resist oxygen free radicals, and promote the ability of tissues to resist oxygen stress.

Conclusions

More and more studies have shown that antioxidant is an important step to prevent aging because free radicals or oxidants can decompose cells and tissues, affect metabolic function, and cause different health problems. If excessive oxidative free radicals can be eliminated, many free radical-induced and aging-related diseases can be prevented. For example, common cancers, arteriosclerosis, diabetes, cataracts, cardiovascular diseases, Alzheimer's disease, arthritis and so on, these diseases are considered to be associated with free radicals. Adequate antioxidants should be ingested to slow down the speed of body degradation, prevent skin aging, and keep a youthful spirit at all times. Fruit has more antioxidants, which is the main source of antioxidants supplementation. 70 mice were fed with grape extract. The antioxidant activity of fruit extract was tested by detecting the gene expression level of antioxidant metabolic enzymes in liver and kidney of experimental mice. The results showed that the fruit extract could enhance the antioxidant level of the body, and had a certain anti-aging effect.

Acknowledgements

The research is supported by: 2018 Special fund for scientific and technological innovation strategy of Guangdong Province (No.2018B090902010).

Interest conflict

None.

References

1. Huang L, Wen Y, Guo W et al. Mobility pattern analysis of ship trajectories based on semantic transformation and topic model. *Ocean Eng* 2020; 201: 107092.
2. Ji H, Sun J, Huang M, Wei L, Zha X. A nonlinear large-signal model for DC-DC converters. Paper presented at: 2016 IEEE 8th International Power Electronics and Motion Control Conference (IPEMC-ECCE Asia), 2016.
3. Podder AK, Chakraborty O, Islam S, Kumar NM, Alhelou HH. Control Strategies of Different Hybrid Energy Storage Systems for Electric Vehicles Applications. *IEEE Access* 2021.
4. Zenghui G. The Expression of Architectural Decoration Engineering Design Effect Based on Computer Aided Software. *J World Archit* 2017; 1(1).
5. Guo X, Yin B, Wang C, Huo H, Aziziaram Z. Risk assessment of gastric cancer in the presence of *Helicobacter pylori* cagA and hopQII genes. *Cell Mol Biol* 2021; 67(4): 299-305.
6. Huo Y, Wan X, Ling T, Wu J, Wang W, Shen S. Expression and purification of norovirus virus like particles in *Escherichia coli* and their immunogenicity in mice. *Mol Immunol* 2018; 93: 278-284.
7. Ou W, Liu S, He X, Cao Z. Study on intelligent recognition model of enemy target's tactical intention on battlefield. *Comput Simul* 2017; 34(9): 10-14.
8. Wiczorek E, Jablonowski Z, Tomasik B et al. Different gene expression and activity pattern of antioxidant enzymes in bladder cancer. *Anti Cancer Res* 2017; 37(2): 841-848.
9. Pío-León JF, Montes-Avila J, López-Angulo G et al. Melanins of *Vitex mollis* fruit with differences in water-solubility show high inhibition of carbohydrate digestive enzymes and antioxidant activity. *J Food Biochem* 2018; 42(3): e12509.
10. Rani M, Mahato AK, Sinha SK, Dalal M, Singh NK, Mandal PK. Homeologue specific gene expression analysis of two vital carbon metabolizing enzymes—citrate synthase and NADP-isocitrate dehydrogenase—from wheat (*Triticum aestivum* L.) under nitrogen stress. *Appl Biochem Biotechnol* 2019; 188(3): 569-584.
11. Cheng H, Huang C, Tang G et al. Emerging role of EPHX1 in chemoresistance of acute myeloid leukemia by regulating drug-metabolizing enzymes and apoptotic signaling. *Mol Carcinog*

- 2019; 58(5): 808-819.
12. Zhan T, Pan L, Liu Z et al. Metabolic susceptibility of 2-chlorothioxanthone and its toxic effects on mRNA and protein expression and activities of human CYP1A2 and CYP3A4 enzymes. *Environ Sci Technol* 2018; 52(20): 11904-11912.
 13. Vishwakarma S, Agarwal R, Goel SK et al. Altered expression of sphingosine-1-phosphate metabolizing enzymes in oral cancer correlate with clinicopathological attributes. *Cancer Invest* 2017; 35(2): 139-141.
 14. Cömert ED, Mogol BA, Gökmen V. Relationship between color and antioxidant capacity of fruits and vegetables. *Curr Res Food Sci* 2020; 2: 1-10.
 15. Ahanger MA, Tittal M, Mir RA, Agarwal R. Alleviation of water and osmotic stress-induced changes in nitrogen metabolizing enzymes in *Triticum aestivum* L. cultivars by potassium. *Protoplasma* 2017; 254(5): 1953-1963.
 16. Zhang X-r, Zhou W-x, Zhang Y-x. Improvements in SOD mimic AEOL-10150, a potent broad-spectrum antioxidant. *Military Med Res* 2018; 5(1): 1-10.
 17. Sen CK. Glutathione homeostasis in response to exercise training and nutritional supplements. *Stress Adap Prophyllaxis Treat* 1999: 31-42.
 18. Branisa J, Jomova K, Porubska M, Kollar V, Simunkova M, Valko M. Effect of drying methods on the content of natural pigments and antioxidant capacity in extracts from medicinal plants: a spectroscopic study. *Chem Pap* 2017; 71(10): 1993-2002.
 19. Jelski W, Mroczko B, Szmitkowski M. The diagnostic value of alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) measurement in the sera of colorectal cancer patients. *Dig Dis Sci* 2010; 55(10): 2953-2957.
 20. Roszek K, Wujak M. How to influence the mesenchymal stem cells fate? Emerging role of ectoenzymes metabolizing nucleotides. *J Cell Physiol* 2019; 234(1): 320-334.
 21. Aziziaram Z. C3953T genetic variation in interleukin 1 β and idiopathic male infertility: a systematic review and meta-analysis. *Cent Asian J Med Pharm Sci Innov* 2021; 1(6): 242-249.
 22. Durazzo A, Lucarini M, Novellino E, Daliu P, Santini A. Fruit-based juices: Focus on antioxidant properties—Study approach and update. *Phytother Res* 2019; 33(7): 1754-1769.
 23. Karpets YV, Kolupaev YE, Lugovaya A, Shvidenko N, Yastreb T. Effects of nitrate and L-arginine on content of nitric oxide and activities of antioxidant enzymes in roots of wheat seedlings and their heat resistance. *Russ J Plant Physiol* 2018; 65(6): 908-915.
 24. Ercisli MF, Kahrizi D, Aziziaram Z. Environmental factors affecting the risk of breast cancer and the modulating role of vitamin D on this malignancy. *Cent Asian J Environ Sci Technol Innov* 2020; 2(4): 175-183.
 25. Park D, Park Y, Lee YH et al. A Comparative Study of Phenolic Antioxidant Activity and Flavonoid Biosynthesis-Related Gene Expression Between Summer and Winter Strawberry Cultivars. *J Food Sci* 2017; 82(2): 341-349.
 26. Ghaheri M, Kahrizi D, Bahrami G, Mohammadi-Motlagh HR. Study of gene expression and steviol glycosides accumulation in *Stevia rebaudiana* Bertoni under various mannitol concentrations. *Molecular biology reports*. 2019 Feb;46(1):7-16.
 27. Fallah F, Nokhasi F, Ghaheri M, Kahrizi D, Agha AB, Ghorbani T, Kazemi E, Ansarypour Z. Effect of salinity on gene expression, morphological and biochemical characteristics of *Stevia rebaudiana* Bertoni under in vitro conditions. *Cellular and Molecular Biology*. 2017 Aug 15;63(7):102-6.
 28. Kahrizi D, Ghari SM, Ghaheri M, Fallah F, Ghorbani T, Agha AB, Kazemi E, Ansarypour Z. Effect of KH₂PO₄ on gene expression, morphological and biochemical characteristics of *stevia rebaudiana* Bertoni under in vitro conditions. *Cellular and Molecular Biology*. 2017 Aug 15;63(7):107-11.
 29. Akbari F, Arminian A, Kahrizi D, Fazeli A, Ghaheri M. Effect of nitrogen sources on gene expression of *Stevia rebaudiana* (Bertoni) under in vitro conditions. *Cellular and Molecular Biology*. 2018 Feb 10;64(2):11-6.
 30. Esmaeili F, Ghaheri M, Kahrizi D, Mansouri M, Safavi SM, Ghorbani T, Mohammadi S, Rahmanian E, Vaziri S. Effects of various glutamine concentrations on gene expression and steviol glycosides accumulation in *Stevia rebaudiana* Bertoni. *Cellular and Molecular Biology*. 2018 Feb 10;64(2):1-5.

31. Kahrizi D, Ghaheri M, Yari Z, Yari K, Bahraminejad S. Investigation of different concentrations of MS media effects on gene expression and steviol glycosides accumulation in *Stevia rebaudiana* Bertoni. *Cellular and Molecular Biology*. 2018 Feb 10;64(2):23-7.
32. Eruygur N, Ucar E, Akpulat HA, Shahsavari K, Safavi SM, Kahrizi D. In vitro antioxidant assessment, screening of enzyme inhibitory activities of methanol and water extracts and gene expression in *Hypericum lydium*. *Molecular biology reports*. 2019 Apr;46(2):2121-9.