Journal of Food and Nutrition Research, 2017, Vol. 5, No. 10, 736-741
Available online at http://pubs.sciepub.com/jfnr/5/10/3

DOI:10.12691/jfnr-5-10-3

Black Garlic Ameliorates Obesity Induced by a High-fat Diet in Rats

Wei-Tang Chang[1], Duen-Kai Shiau[2,3], Ming-Ching Cheng[4], Chin-Yin Tseng[4], Cheng-Shih Chen[2], Mei-Fang Wu[2], Chin-Lin Hsu[1,5,*]

[1]School of Nutrition, Chung Shan Medical University, Taichung, Taiwan
[2]Department of Industrial Engineering and Systems Management, Feng Chia University, Taichung, Taiwan,
[3]Department of Dental Technology and Materials Science, Central Taiwan University of Science and Technology, Taichung, Taiwan
[4]Department of Health Food, Chung Chou University of Science and Technology, Yuan-Lin, Changhua, Taiwan
[5]Department of Nutrition, Chung Shan Medical University Hospital, Taichung, Taiwan
*Corresponding author: clhsu@csmu.edu.tw

Abstract Black garlic (also called aged garlic) is a type of fermented garlic product from fresh garlic that is often used as a food ingredient and a functional food in Asian countries. The aim of this study was to investigate whether black garlic ameliorates obesity induced by a high-fat diet in rats. Male Wistar rats were fed a normal diet or a high-fat diet (HFD) (30% lard, w/w) combined with 0, 0.2, 0.6, or 1.2% black garlic (BG) (w/w) for a period of six weeks. The results demonstrated that body weight, tissue weights of liver, peritoneal fat, and epididymal fat, serum triglycerides, and hepatic lipid profiles (total lipids, triglycerides, and cholesterol) in the HFD+BG groups were significantly decreased compared with those in the HFD group. BG also reduced hepatic oxidative stress (reduced GSSG and enhanced TEAC, GSH, GRd, and GPx) in HFD-induced obese rats. These results suggest that black garlic may be useful for the treatment of obesity.

Keywords: *black garlic, high-fat diet, Wistar rat, obesity*

Cite This Article: Wei-Tang Chang, Duen-Kai Shiau, Ming-Ching Cheng, Chin-Yin Tseng, Cheng-Shih Chen, Mei-Fang Wu, and Chin-Lin Hsu, "Black Garlic Ameliorates Obesity Induced by a High-fat Diet in Rats." *Journal of Food and Nutrition Research*, vol. 5, no. 10 (2017): 736-741. doi: 10.12691/jfnr-5-10-3.

1. Introduction

Obesity is a serious health problem and is closely associated with lifestyle-related diseases such as hypertension, arteriosclerosis, hyperlipidemia, dyslipidemia, type 2 diabetes mellitus, cancer, respiratory complications, and osteoarthritis [1]. Excessive amounts of body fat have health complications associated with major risk factors for several serious chronic diseases, such as metabolic syndrome. Obesity is also a public health concern and is associated with an increased risk of morbidity and mortality [2]. Obesity-related dyslipidemia plays a crucial role in the development of atherosclerosis and cardiovascular diseases [3]. Therefore, the prevention and treatment of obesity plays an important role in healthy life and longevity [4]. In antiobesity drugs, some medical treatments for obesity have serious side effect, such as fenfluramine, sibutramine, and rimonabant [5]. Many natural compounds have been used to treat obesity, including gallic acid, epigallocatechin 3-gallate, rutin, *o*-coumaric acid, and garlic [6,7,8,9].

The pharmacological actions of garlic (*Allium sativum* L.) include antiobesity, antibacterial, antiviral, antihypertensive, blood glucose lowering, antithrombotic, antimutagenic, and antiplatelet actions [9,10,11,12,13,14]. Sheen et al. [15] indicated that the garlic oil and its organosulfur compounds can be beneficial for the suppression of high-fat diet (HFD)-induced body weight gain in rats. Moreover, bioconversion technology is a technology in which some organic compounds are modified using the specific reactions to change them into specific compounds for special use. Black garlic (also called aged garlic) is a type of fermented garlic product from raw garlic that is used for food ingredients and functional foods in Asian countries, such as Taiwan, Japan, and Korea. Kang et al. [16] indicated that black garlic powder can be used in the treatment of the atherosclerotic process and the improvement of hyperlipidemia. Seo et al. [17] indicated that aged garlic extract has beneficial effects on reducing weight and visceral fat gain and cholesterol lowering in rats fed with HFD. Ried et al. [18] indicated that aged garlic extract reduced the blood pressure in patients with uncontrolled hypertension. Jung et al. [19] indicated that the intake of fermented aged black garlic can be beneficial for the prevention of HFD-induced diabetic complications. However, the literature indicates that the antiobesity effects of black garlic in high-fat diet-fed rats remains unclear.

The aim of this study was to investigate the anti-obesity effect of black garlic using HFD-induced obese rats. In addition, growth parameters, serum biochemical parameters, organ and adipose tissue weights, histology, and the antioxidant defense system were measured in rats fed a normal diet (ND) and a HFD with or without black garlic powder.

2. Materials and Methods

2.1. Materials

The powders of black garlic were provided by Professor Chin-Yin Tseng (Chung Chou University of Science and Technology, Changhua County, Taiwan). All other chemicals used were of the highest pure grade available.

2.2. Animals, Diets, and Experimental Design

Ten-week-old male Wistar rats were purchased from the BioLASCO Taiwan Corp., Ltd (Ilan, Taiwan). Animals were housed individually in stainless steel cages in an air-conditioned room at 23±2°C, 55-60% relative humidity, and a 12 h light/dark cycle and were given a laboratory rodent chow diet for 1 week. The rats were divided into normal and obese groups and then fed normal diets (NDs) and high fat-diets (HFDs), respectively. The HFD group (30% lard, w/w) (n=8/group) was then divided into four groups according to whether the animals received supplemental black garlic (BG) for six weeks: the HFD group, HFD+BG-LD (low dose, LD), HFD+BG-MD (medium dose, MD), and HFD+BG-HD (high dose, HD) at levels of 0%, 0.2%, 0.6 %, and 1.2% (w/w), respectively. These animals should normally be able to consume 5% of their body weight daily. The diets were stored in a 4°C cold chamber. Body weights, food intakes, and food efficiency were measured every day for six weeks. After overnight fasting, blood was withdrawn from the abdominal aorta under carbon dioxide anesthesia, and serum was harvested. The visceral tissues were immediately excised, rinsed, weighed, and frozen in liquid nitrogen. All experimental procedures involving animals were conducted in accordance with the guidelines of the National Institutes of Health (NIH). This experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of Chung Shan Medical University (IACUC Approval No: 894) in Taichung, Taiwan.

2.3. Measurement of Serum Parameters

Blood was placed into a sterile Vacutainer plastic tube (BD Vacutainer, Plymouth, UK). Serum was separated by centrifugation (4000×g, 10 min) and transferred to Eppendorf tubes. The serum concentrations of triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, creatinine, Na^+, K^+, and Cl^- were measured with commercial kits (Bayer Corporation, Tarrytown, NY, USA). The concentration of ketone bodies was measured with an abundant ketone body kit (Randox Laboratories Ltd., UK).

2.4. Hematoxylin and Eosin (H&E) Staining

Liver and fat tissue samples were collected following euthanasia, fixed in 10% formalin buffered solution, and cut into 5-μm sections. Hematoxylin and eosin (H&E) staining was performed using standard techniques.

2.5. Hepatic Lipid Analysis

Hepatic lipid was extracted according to the methods used by [20], and concentrations of triglycerides and cholesterol were measured using a TG assay kit (Teco Diagnostics, USA) and a cholesterol commercial kit (Randox Laboratories Ltd., UK), respectively.

2.6. Trolox Equivalent Antioxidant Capacity (TEAC) Assay

Determination of TEAC was conduction using the method of Arnao et al. [21]. $ABTS^{\bullet+}$ is generated by the interaction of ABTS (100 μmol/L), H_2O_2 (50 μmol/L), and peroxidase (4.4 U/mL). To measure antioxidant activity, 0.25 mL of serum was mixed well with an equal volume of ABTS, H_2O_2, peroxidase, and 1.5 mL of deionized water. The absorbance was measured at 734 nm after interacting with the sample solution for 10 min. The decrease in absorption at 734 nm after the addition of the reactant was used to calculate the TEAC value. A dose-response curve was plotted for trolox, and antioxidant ability was expressed as the TEAC. The higher the TEAC value of a sample, the stronger the antioxidant activity.

2.7. Determination of GSH and GSSG in the Liver

The levels of GSH and GSSG were determined by a GSH assay kit (Cayman Chemical Company, Ann Arbor, MI) according to the procedure of the manufacturer. Absorbance was measured spectrophotometrically in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at 405 nm, and the concentrations of GSH and GSSG were calculated as nanomoles per milligram of protein.

2.8. Determination of Antioxidant Enzymes in the Liver

All antioxidant enzyme activities were determined after the hepatic tissue was homogenized with phosphate-buffered saline at a pH of 7.0. The GSH peroxidase (GPx) activity was determined according to the method of Lawerence and Burk [22]. Liver homogenate solution (100 μL) was mixed with 800 μL of 100 mmol/L potassium phosphate buffer (pH 7.4) containing 1 mmol/L EDTA, 1 mmol/L NaN_3, 0.2 mmol/L NADPH, 1 U/mL GSH reductase, and 1 mmol/L GSH. After 5 min, 2.5 mmol/L H_2O_2 (100 μL) was added to start the reaction. The absorbance change at 340 nm was recorded over the course of 3 min. Enzyme activity was calculated by E_{340}=6220/M per cm, and the result is expressed as units of nmol NADPH/mg protein/min.

The GSH reductase (GRd) activity was determined according to the method of Bellomo et al. [23]. The liver homogenate solution (100 μL) was mixed with 900 μL of 100 mmol/L potassium phosphate buffer (pH 7.0) containing 1 mmol/L $MgCl_2.6H_2O$, 50 mmol/L GSSG, and 0.1 mmol/L NADPH and was incubated for 3 min at room temperature. The absorbance change at 340 nm was recorded over the course of 3 min. The enzyme activity was calculated by E_{340}=6220/M per cm, and the result is expressed as units of nmol NADPH/ mg protein/min.

Superoxide dismutase (SOD) activity was determined by a SOD assay kit-WST (Dojindo Molecular Technologies Inc., Maryland, USA) as specified by the manufacturer. The absorbance was measured spectrophotometrically in a

VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm. The value (%) is expressed as SOD activity of the ND group at 100%.

2.9. Statistical Analysis

The data were analyzed using analysis of variance (ANOVA). Differences were considered significant at the 0.05 probability level, and differences between treatments were evaluated using the Least Significant Difference (LSD) test. All statistical analyses were performed using SAS (SAS Institute Inc., 2002).

3. Results

3.1. Effect of Black Garlic on Body Weight, Food Intake, and Energy Intake of Rats with Obesity Induced by a HFD

In the present study, the HFD groups (high fat-diet containing 30% lard, w/w) were divided into four groups and supplemented with black garlic [0, 0.2, 0.6, and 1.2% (w/w)] for six weeks. After six weeks of feeding, body weights in the HFD groups supplemented with black garlic (0.2-1.2% black garlic in diet) were significantly decreased compared with those in the HFD group (Table 1). Moreover, after six weeks, food intake was significantly higher in the HFD group compared with the ND group. Food intake and energy intake in the HFD groups supplemented with black garlic were not significantly different (p>0.05).

3.2. Effect of Black Garlic on the Weights of Organs and Adipose Tissue of Rats with Obesity Induced by a HFD

The organ and adipose tissue weights of the five groups are depicted in Table 2. There were no significant differences in the weights of the heart, spleen, lung, and kidney among the five groups. The organ weights of liver (0.2, 0.6, and 1.2% black garlic in diet) and adipose tissue [peritoneal fat (0.2, 0.6, and 1.2% black garlic in diet) and epididymal fat (0.6 and 1.2% black garlic in diet)] in the HFD groups supplemented with black garlic was significantly decreased compared with those of the HFD group. The changes of liver and adipose tissue morphology are depicted in Figure 1 and Figure 2. As demonstrated in Figure 1, hematoxylin and eosin staining revealed hepatic macrovesicular fat accumulation in the HFD group. The HFD groups supplemented with black garlic (0.2-1.2% black garlic in diet) exhibited microvesicular fat accumulation. As shown in Figure 2, the sizes of the peritoneal fat in the HFD groups supplemented with black garlic (0.2-1.2% black garlic in diet) were smaller than those in the HFD group.

Table 1. Effect of black garlic on body weights, food intake, and energy intake of rats with obesity induced by a high-fat diet

Body weight *	ND	HFD supplemented with black garlic (%, w/w)			
		0	0.2	0.6	1.2
Initial body weight (g)	354.3±4.8^{a}	354.0±5.0^{a}	354.4±8.6^{a}	354.0±3.9^{a}	354.7±8.9^{a}
Final body weight (g)	421.1±10.9^{c}	485.1±12.1^{a}	462.0±17.2^{ab}	447.2±9.0^{b}	441.8±14.9^{b}
Food intake (g/rat/day)	25.0±0.3^{a}	19.0±0.5^{b}	18.6±0.4^{b}	19.0±0.3^{b}	19.3±0.2^{b}
Energy intake (kcal/rat/day)	99.0±1.3^{a}	103.7±2.8^{a}	101.7±2.4^{a}	103.9±1.6^{a}	105.6±1.1^{a}

*The reported values are the mean ± SEM (n=8). Mean values with different letters were significantly different (p < 0.05). ND, normal diet; HFD, high-fat diet.

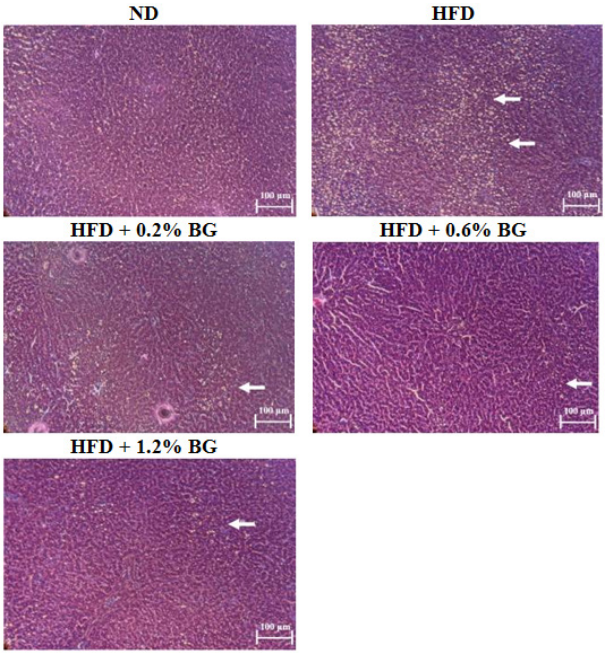

Figure 1. Effect of black garlic (BG) on hepatosteatosis in rats with obesity induced by a high-fat diet. Livers were stained with hematoxylin and eosin (H&E). Original magnification: 200 ×

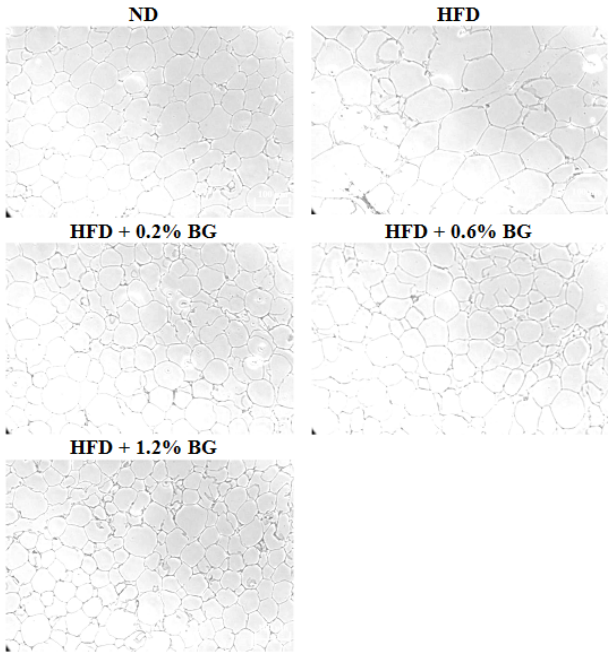

Figure 2. Effect of black garlic (BG) on the size of adipocytes of rats with obesity induced by a high-fat diet. Original magnification: 200 ×

Table 2. Effect of black garlic on the weights of organs and adipose tissue of rats with obesity induced by a high-fat diet

Tissue weights (mg/g rat) *	ND	HFD supplemented with black garlic (%, w/w)			
		0	0.2	0.6	1.2
Heart	2.94±0.08^{a}	2.97±0.05^{a}	2.97±0.06^{a}	3.05±0.10^{a}	3.14±0.12^{a}
Liver	25.4±0.5^{b}	26.8±0.6^{a}	24.7±0.8^{b}	24.6±0.5^{b}	24.5±0.4^{b}
Spleen	2.13±0.10^{a}	2.18±0.05^{a}	2.13±0.10^{a}	2.04±0.10^{a}	2.15±0.11^{a}
Lung	4.08±0.15^{a}	4.20±0.23^{a}	4.13±0.39^{a}	4.13±0.15^{a}	4.21±0.13^{a}
Kidney	6.38±0.12^{a}	6.40±0.09^{a}	6.41±0.11^{a}	6.36±0.11^{a}	6.36±0.12^{a}
Peritoneal fat	20.4±1.3^{c}	28.9±1.6^{a}	24.0±2.9^{b}	23.7±3.1^{b}	22.3±3.0^{bc}
Epididymal fat	15.1±0.9^{d}	24.0±1.2^{a}	22.6±2.0^{ab}	21.0±1.4^{b}	18.1±1.4^{c}

*The reported values are the mean ± SEM (n=8). Mean values with different letters were significantly different (p <0.05).

Table 3. Effect of black garlic on the serum biochemical parameters of rats with obesity induced by a high-fat diet

Biochemical parameters *	ND	HFD supplemented with black garlic (%, w/w)			
		0	0.2	0.6	1.2
Triglyceride (mg/dL)	70.0±4.0^{b}	95.1±5.1^{a}	71.0±4.7^{b}	75.0±3.6^{b}	63.3±5.7^{b}
Total cholesterol (mg/dL)	53.0±3.4^{a}	52.7±2.7^{a}	53.0±4.2^{a}	50.2±7.6^{a}	49.0±4.1^{a}
HDL-cholesterol (mg/dL)	51.2±2.5^{a}	38.4±3.4^{c}	44.3±2.8^{b}	44.1±3.5^{b}	46.3±2.3^{b}
AST (U/L)	65.4±3.0^{b}	69.7±2.7^{a}	63.2±5.0^{b}	61.2±1.9^{b}	61.0±4.3^{b}
ALT (U/L)	60.6±4.8^{a}	63.3±2.3^{a}	57.8±4.7^{a}	60.7±4.5^{a}	62.8±1.9^{a}
Uric acid (mg/dL)	4.32±0.28^{a}	4.63±0.38^{a}	4.35±0.30^{a}	4.10±0.24^{a}	4.28±0.49^{a}
Creatinine (mg/dL)	1.01±0.03^{a}	1.00±0.03^{a}	0.98±0.03^{a}	0.93±0.04^{a}	0.95±0.03^{a}
Na^{+} (mmol/L)	161.1±2.8^{a}	158.3±1.6^{a}	160.9±2.8^{a}	158.7±2.6^{a}	157.2±1.7^{a}
K^{+} (mmol/L)	6.86±0.26^{a}	6.77±0.30^{a}	6.93±0.23^{a}	6.78±0.18^{a}	7.15±0.26^{a}
Cl^{-} (mmol/L)	103.3±1.2^{a}	102.2±1.0^{a}	104.9±1.7^{a}	102.2±1.6^{a}	103.4±0.8^{a}
Ketone body (mmol/L)	0.76±0.01^{c}	1.15±0.08^{a}	1.14±0.15^{a}	0.99±0.15^{b}	0.93±0.12^{b}

*The reported values are the mean ± SEM (n=8). Mean values with different letters were significantly different (p < 0.05). HDL, high-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 4. Effect of black garlic on the hepatic lipid profiles and antioxidant enzymes of rats with obesity induced by a high-fat diet

Contents (mg/g tissue)*	ND	HFD supplemented with black garlic (%, w/w)			
		0	0.2	0.6	1.2
Hepatic lipid profiles					
Hepatic total lipid	70.9±2.2^{c}	110.7±3.0^{a}	82.2±2.7^{b}	80.9±3.9^{b}	85.6±1.7^{b}
Hepatic triglyceride	11.3±0.5^{c}	22.3±1.6^{a}	19.4±2.6^{a}	16.6±2.2^{b}	14.1±1.4^{b}
Hepatic cholesterol	4.69±0.14^{c}	7.74±0.89^{a}	6.19±0.94^{b}	6.09±0.92^{b}	5.68±0.58^{b}
Hepatic antioxidant enzymes					
TEAC (nmol/mg protein)	0.69±0.02^{a}	0.36±0.07^{c}	0.53±0.04^{b}	0.58±0.05^{b}	0.57±0.03^{b}
GSH (μmol/mg protein)	3.05±0.09^{a}	2.34±0.05^{c}	2.39±0.13^{c}	2.80±0.26^{b}	2.21±0.06^{c}
GSSG (μmol/mg protein)	1.20±0.05^{b}	1.38±0.05^{a}	1.13±0.06^{b}	1.02±0.15^{b}	1.11±0.04^{b}
GRd (nmol/mg protein)	31.7±1.4^{a}	17.0±0.5^{b}	18.8±2.9^{b}	19.4±2.1^{b}	28.4±2.7^{a}
GPx (nmol/mg protein)	1.43±0.21^{a}	0.59±0.06^{b}	1.18±0.17^{a}	1.39±0.13^{a}	1.42±0.10^{a}
SOD (U/mL)	4.92±0.27^{a}	4.18±0.24^{b}	4.10±0.57^{b}	4.14±0.39^{b}	4.04±0.26^{b}

*The reported values are the mean ± SEM (n=8). Mean values with different letters were significantly different (p < 0.05). TEAC, Trolox equivalent antioxidant capacity; GSH, glutathione; GSSG, glutathione disulfide; GRd, glutathione reductase; GPx, glutathione peroxidase; SOD, superoxide dismutase.

3.3. Effect of Black Garlic on the Serum Biochemical Indicators of Rats with Obesity Induced by a HFD

As shown in Table 3, serum levels of triglycerides (0.2-1.2% black garlic in diet), AST (0.2-1.2% black garlic in diet), and ketone bodies (0.6 and 1.2% black garlic in diet) in the HFD groups supplemented with black garlic were significantly decreased compared with those in the HFD group. Serum HDL-cholesterol levels in the HFD groups supplemented with black garlic at 0.2-1.2% were significantly increased compared with those in the HFD group. Moreover, there were no significant differences in the serum levels of total cholesterol, ALT, uric acid, creatinine, Na^{+}, K^{+}, or Cl^{-} among the five groups.

3.4. Effect of Black Garlic on Hepatic Lipid Profiles and Antioxidant Enzymes of Rats with Obesity Induced by a HFD

Table 4 presents the effect of black garlic on the hepatic lipid profiles and antioxidant enzymes of rats with obesity induced by a HFD. Hepatic lipid profiles of total lipids (0.2-1.2% black garlic in diet), triglycerides (0.6 and 1.2% black garlic in diet), and cholesterol (0.2-1.2% black garlic in diet) in the HFD groups supplemented with black garlic were significantly decreased compared with those in the HFD group. The levels of TEAC (0.2-1.2% black garlic in diet), GSH (0.6 and 1.2% black garlic in diet), GRd (1.2% black garlic in diet), and GPx (0.2-1.2% black garlic in diet) in the HFD groups supplemented with black

garlic were significantly increased compared with those in the HFD group. The level of GSSG in HFD groups supplemented with black garlic at 0.2-1.2% was significantly decreased compared with those in the HFD group. There were no significant differences in the levels of SOD among the HFD groups supplemented with black garlic.

4. Discussion

The protective effects of black garlic have been investigated in the context of the immune system, oxidative stress, and inflammation. Dietary fat is an important environmental factor contributing to obesity-related diseases, such as hyperlipidemia, hypertension, arteriosclerosis, type 2 diabetes mellitus, cancer, respiratory complications, and osteoarthritis [1]. However, we examined the effect of black garlic on growth parameters, serum biochemical parameters, organ and adipose tissue weights, histology, and the antioxidant defense system in HFD-induced obese rats. Many studies have reported that obesity is induced in mice and rats by feeding the animals a high-energy diet containing 30% lard [24,25]. Black garlic was then given as a supplement at levels of 0, 0.2, 0.6, or 1.2% in diets for a period of six weeks. Our data indicated diets containing black garlic provided over six weeks suppressed the increases in body weight and tissue weights of liver, peritoneal fat, and epididymal fat induced by a HFD (Table 1 and Table 2). The reports indicated that aged garlic extract can reduce body weight gain, visceral fat, and liver weight gain in high-fat diet-induced obese rats [17]. Obesity is associated with a high incidence of steatosis, such as non-alcoholic fatty liver disease (NAFLD), and has been recognized as one of the most common causes of chronic liver disorders [26]. Over-accumulation of hepatic lipids and the oxidation of fatty acids are important sources of reactive oxygen species in fatty liver [27]. Morphologically, the livers of HFD rats exhibited abundant and large lipid droplets and an obvious increase in liver derangement compared with those of ND rats. The HFD groups supplemented with black garlic exhibited microvesicular fat accumulation in liver and small adipocyte sizes in peritoneal fat (Figure 1 and Figure 2).

Previous research demonstrated that the oral administration of phytochemicals (such as gallic acid, epigallocatechin gallate, rutin, *o*-coumaric acid, and curcumin) prevents HFD-induced dyslipidemia in animal models and clinical trials [6,8,28,29]. We found that a HFD supplemented with black garlic resulted in significantly decreased serum levels of triglyceride, AST, and ketone bodies (Table 3). In addition, serum level of HDL-cholesterol in a HFD group supplemented with black garlic was significantly increased compared to those in the HFD group. Lavie and Milani [30] indicated that obesity has adverse effects on health and adversely affects the levels of plasma lipids, including high levels of triglyceride and low levels of HDL-cholesterol. *In vivo* studies indicated that obesity is associated with decrease antioxidant activities and increased oxidative stress in plasma and organ tissue [31,32]. Oxidative stress plays an important role in the risk factors of hyperlipidemia and atherosclerosis [33]. Antioxidant enzymes can scavenge free radicals, balance of oxidative stress, and prevent reactive oxygen species formation [34]. Our data indicated that the HFD group supplemented with black garlic exhibited significantly decreased levels of hepatic lipid profiles (total lipid, triglyceride, and cholesterol) and GSSG and increased levels of antioxidant enzymes (TEAC, GSH, GRd, and GPx) (Table 4).

5. Conclusion

Rats fed diets containing black garlic for six weeks suppressed the increases in body weight, the tissue weights of liver, peritoneal fat, and epididymal fat, the levels of serum lipid triglycerides, and the hepatic lipid profiles induced by a HFD. We also found that the HFD supplemented with black garlic exhibited significantly decreased level of GSSG and increased levels of antioxidant enzymes (TEAC, GSH, GRd, and GPx). These results demonstrate that the intake of black garlic can be beneficial for the suppression of HFD-induced obesity in rats.

Acknowledgements

This research work was partially supported by the grant from National Science Council, Taiwan (NSC98-2622-B-235-001-CC1) and Council of Agriculture, Executive Yuan, Taiwan (105AS-16.4.1-ST-a7).

References

[1] Kopelman, P.G, "Obesity as a medical problem," *Nature,* 404 (6778). 635-643. Apr. 2000.

[2] Medrikova, D., Jilkova, Z.M., Bardova, K., Janovska, P., Rossmeisl, M. and Kopecky, J, "Sex differences during the course of diet-induced obesity in mice: adipose tissue expandability and glycemic control," *International Journal of Obesity,* 36 (2). 262-272. Feb. 2012.

[3] Poirier, P., Giles, T.D., Bray, G.A., Hong, Y., Stern, J.S., Pi-Sunyer, F.X., Eckel, R.H., American Heart Association., Obesity Committee of the Council on Nutrition., Physical Activity and Metabolism "Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. American Heart Association; Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism," *Circulation,* 113 (6). 898-918. Feb. 2006.

[4] Hofbauer, K.G., Nicholson, J.R. and Boss, O, "The obesity epidemic: current and future pharmacological treatments," *Annual Review of Pharmacology and Toxicology,* 47. 565-592. 2007.

[5] Dietrich, M.O. and Horvath, T.L, "Limitations in anti-obesity drug development: the critical role of hunger-promoting neurons," *Nature Reviews Drug Discovery,* 11 (9). 675-691. Sep. 2012.

[6] Hsu, C.L. and Yen, G.C, "Effect of gallic acid on high fat diet-induced dyslipidemia, hepatosteatosis, and oxidative stress in rats," *British Journal of Nutrition,* 98 (4). 727-735. Oct. 2007.

[7] Bose, M., Lambert, J.D., Ju, J., Reuhl, K.R., Shapses, S.A. and Yang, C.S, "The major green tea polyphenol, (-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice," *The Journal of Nutrition,* 138 (9). 1667-1683. Oct. 2008.

[8] Hsu, C.L., Wu, C.H., Huang, S.L. and Yen, G.C, "Phenolic compounds rutin and *o*-coumaric acid ameliorate obesity induced by high-fat diet in rats," *Journal of Agricultural and Food Chemistry,* 57 (2). 425-431. Feb. 2009.

[9] Joo, H., Kim, C.T., Kim, I.H. and Kim, Y, "Anti-obesity effects of hot water extract and high hydrostatic pressure extract of garlic in rats fed a high-fat diet," *Food and Chemical Toxicology,* 55. 100-105. May. 2013.

[10] Agarwal, K.C. "Therapeutic actions of garlic constituents," *Medicinal Research Reviews,* 16 (1). 111-124. Jan. 1996.

[11] Rahman, K, "Historical perspective on garlic and cardiovascular disease," *The Journal of Nutrition,* 313 (3s). 977S-979S. Mar. 2001.

[12] Gorinstein, S., Jastrzebasi, Z., Namiesnik, J., Leontowicz, H., Leontowicz, M. and Trakhtenberg, S, "The atherosclerotic heart disease and protecting properties of garlic: contemporary data," *Molecular Nutrition & Food Research,* 51 (11). 1365-1381. Oct. 2007.

[13] Morihara, N., Nishihama, T., Ushijima, M., Ide, N., Takeda, H. and Hayama, M, "Garlic as an anti-fatigue agent," *Molecular Nutrition & Food Research,* 51 (11). 1329-1334. Nov. 2007.

[14] Rahman, K, "Effects of garlic on platelet biochemistry and physiology," *Molecular Nutrition & Food Research,* 51 (11). 1335-1344. Nov. 2007.

[15] Sheen, L.Y., Chen, H.W., Kung, Y.L., Liu, C.T. and Lii, C.K, "Effects of garlic oil and its organosulfur compounds on the activities of hepatic drug-metabolizing and antioxidant enzymes in rats fed high- and low-fat diets," *Nutrition and Cancer,* 35 (2). 160-166. 1999.

[16] Kang, M.J., Lee, S.J., Shin, J.H., Kang, S.K., Kim, J.G. and Sung, N.J, "Effect of garlic with different processing on lipid metabolism in 1% cholesterol fed rats," *Journal of the Korean Society of Food Science and Nutrition,* 37 (2). 162-169. Feb. 2008.

[17] Seo, D.Y., Lee, S., Figueroa, A., Kwak, Y.S., Kim, N., Rhee, B.D., Ko, K.S., Banq, H.S., Baek, Y.H. and Han, J, "Aged garlic extract enhances exercise-mediated improvement of metabolic parameters in high fat diet-induced obese rats," *Nutrition Research and Practice,* 6 (6). 513-519. Dec. 2012.

[18] Ried, K., Frank, O.R. and Stocks, N.P, "Aged garlic extract reduces blood pressure in hypertensives: a dose–response trial," *European Journal of Clinical Nutrition,* 67 (1). 64-70. Jan. 2013.

[19] Jung, Y.M., Lee, S.H., Lee, D.S., You, M.J., Chung, I.K., Cheon, W.H., Kwon, Y.S., Lee, Y, S. and Ku, S.K, "Fermented garlic protects diabetic, obese mice when fed a high-fat diet by antioxidant effects," *Nutrition Research,* 31 (5). 387-396. May. 2011.

[20] Tzang, B.S., Yang, S.F., Fu, S.G., Yang, H.C., Sun, H.L. and Chen, Y.C, "Effects of dietary flaxseed oil on cholesterol metabolism of hamsters," *Food Chemistry,* 114 (4). 1450-1455. Jun. 2009.

[21] Arnao, M.B., Cano, A., Hernandez-Ruiz, J., Garcia-Canovas, F. and Acosta, M, "Inhibition by L-ascorbic acid and other antioxidants of the 2,2′-azino-bis (3- ethylbenzthiazoline-6-sulfonic acid) oxidation catalyzed by peroxidase: a new approach for determining total antioxidant status of foods," *Analytical Biochemistry,* 236 (2). 255-261. May. 1996.

[22] Lawerence, R.A. and Burk, R.F, "Glutathione peroxidase activity in selenium-deficient rat's liver," *Biochemical and Biophysical Research Communications,* 71 (4). 952-958. Aug. 1976.

[23] Bellomo, G., Mirabelli, F., Dimonte, D., Richelmi, P., Thor, H. and Orrenius, C, "Formation and reduction of glutathione-mixed disulfides during oxidative stress," *Biochemical Pharmacology,* 36 (8). 1313-1320. Apr. 1987.

[24] Tsuda, T., Horio, F., Uchida, K., Aoki, H., and Osawa, T, "Dietary cyaniding 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice," *The Journal of Nutrition,* 133 (7). 2125-2130. Jul. 2003.

[25] Park, Y.S., Yoon, Y. and Ahn, H.S, "Platycodon grandiflorum extract represses up-regulated adipocyte fatty acid binding protein triggered by a high fat feeding in obese rats," *World Journal of Gastroenterology,* 13 (25). 3493-3499. Jul. 2007.

[26] Zhang, S., Zheng, L., Dong, D., Xu, L., Yin, L., Qi, Y, "Effects of flavonoids from Rosa laevigata Michx fruit against high-fat diet-induced non-alcoholic fatty liver disease in rats," *Food Chemistry,* 141 (3). 2108-2116. Dec. 2013.

[27] Pettinelli, P., Obregón, A.M. and Videla, L.A, "Molecular mechanisms of steatosis in nonalcoholic fatty liver disease," *Nutrición Hospitalaria,* 26 (3). 441-450. May. 2011.

[28] Ramesh, E., Elanchezhian, R., Sakthivel, M., Jayakumar, T., Senthil Kumar, R.S., Geraldine, P. and Thomas, P.A, "Epigallocatechin gallate improves serum lipid profile and erythrocyte and cardiac tissue antioxidant parameters in Wistar rats fed an atherogenic diet," *Fundamental & Clinical Pharmacology,* 22 (3). 275-284. Jun. 2008.

[29] Mohammadi, A., Sahebkar, A., Iranshahi, M., Amini, M., Khojasteh, R., Ghayour-Mobarhan, M. and Ferns, G.A, "Effects of supplementation with curcuminoids on dyslipidemia in obese patients: a randomized crossover trial," *Phytotherapy Research,* 27 (3). 374-379. Mar. 2013.

[30] Lavie, C.J. and Milani, R.V, "Obesity and cardiovascular disease: the Hippocrates paradox?" *Journal of the American College of Cardiology,* 42 (4). 677-679. Aug. 2003.

[31] Olusi, S.O, "Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotectic enzymes in humans," *International Journal of Obesity and Related Metabolic Disorders,* 26 (9). 1159-1164. Sep. 2002.

[32] Ozata, M., Mergen, M., Oktenli, C., Aydin, A., Sanisoglu, S.Y., Bolu, E., Yilmaz, M.I., Sayal, A., Isimer, A. and Ozdemir, I.C, "Increased oxidative stress and hypozincemia in male obesity," *Clinical Biochemistry,* 35 (8). 627-631. Nov. 2002.

[33] Young, I. S. and McEneny, J, "Lipoprotein oxidation and atherosclerosis," *Biochemical Society Transactions,* 29 (Pt2). 358-362. May. 2001.

[34] Husain, K., Mejia, J., Lalla, J. and Kazin, S, "Dose response of alcoholinduced changes in BP, nitric oxide and antioxidants in rat plasma," *Pharmacology Research,* 51 (4). 337-343. Apr. 2005.