ANTI-OBESITY EFFECTS OF GREEN TEA EGCG, ORANGE PEEL EXTRACT, BLACK TEA EXTRACT AND CAFFEINE IN MICE FED ON A HIGH-FAT DIET

by

YU-WEN HUANG

A dissertation submitted to the Graduate School – New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Master of Science

Graduate Program in Food Science

written under the direction of

Dr. Chi-Tang Ho

and approved by

_____

_____

_____

_____

New Brunswick, New Jersey

May, 2007

ABSTRACT OF THE THESIS

ANTI-OBESITY EFFECTS OF GREEN TEA EGCG,
ORANGE PEEL EXTRACT, BLACK TEA EXTRACT AND CAFFEINE
IN MICE FED ON A HIGH-FAT DIET

by YU-WEN HUANG

Thesis Director:

Dr. Chi-Tang Ho

In this study, we elucidate the anti-obesity effects of EGCG (Epigallocatechin-3-gallate), orange peel extracts (OPE), black tea extracts (BTE), and caffeine (CF) by oral feeding to female CD-1 mice. Female CD-1 mice were fed on high-fat diets containing 0.1% EGCG, 0.2% orange peel extract, 0.2% black tea extract and 0.05% caffeine, singly and in combination for 10 weeks. The body weight gain and weights of abdominal fat and brown adipose tissue were significantly reduced by the diets containing orange peel extract, black tea extract, caffeine, OPE + BTE and OPE + CF. Noticeably, our result also found that mice fed with high-fat diet supplemented daily among the combination of 0.2% OPE + 0.2% BTE + 0.05% CF prevented the body weight gain by 48.8%, the parametrial fat pad weight by 88.2%, retroperitoneal fat pad weight by 82.8% and brown adipose tissue by 63.7% compared to mice fed on high-fat diet. On the basis of these finding, it was supported that oral feeding of orange peel extracts, black tea

extracts and caffeine had anti-obesity effects by suppressing body weigh growth and adipose tissue formation. Moreover, it was shown that orange peel extract, black tea extract and caffeine were synergistic in anti-obesity actions.

To clarify the anti-obesity action of orange peel extract, female CD-1 mice were fed on various degrees of orange peel extracts and different composition of orange peel extract for 17 weeks. Our results showed that feeding of different degrees of orange peel extracts to female CD-1 mice fed high-fat diet results in the reduction of body weight, white adipose tissue, brown adipose tissue as well as lipid profile includes total cholesterol, triglyceride, LDL and HDL in blood. Furthermore, long term consumption of dietary orange peel extracts intake considerably decreases diet-induced obesity, hyperglycemia, and hypercholesterolemia in mice without a dose-dependent manner.

ACKNOWLEDGEMENT

I would like to thank my advisor, Dr. Ho, Chi-Tang, for his guiance, encouragement and support. I am eternally grateful to him for providing me with the opportunity to complete my graduate studies, for his patience, and generous support during this work. Working under his guidance made the lab work more challenging and interesting.

Special thanks also go to my co-advisor, Dr. Huang, Mou-Tuan, for guiding my thesis project during these years, invaluable suggestions, constructive criticisms, and sharing the knowledge in science. Most of the work for this thesis has been done cooperatively with him. My gratitude extends to my dissertation committed members, Dr. Qingrong Huang and Dr. Shengmin Sang, for their invaluable advice and help.

Finally, I would like to express my deepest appreciation to my families for their unconditional love and support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

1. INTRODUCTION

Obesity is one of the major public health problems in the United States and other developed countries. It is believed to be associated with several major chronic diseases such as cardiovascular diseases, diabetes, and cancers. Although one of the national health goals for the year 2010 is to reduce the prevalence of obesity among adults to less than fifteen percent, current data indicates that the situation is worsening rather than improving(1). Anti-obesity has become an important issue for food and drug research in which that tea and tea polyphenols has been intensively researched for this purpose.

Although obesity is one of the major health problems in the United States, there is not an effective drug to treat obesity because they all have undesirable side effects. However, it is believed that botanicals provide a safer and natural way to human body in both pharmaceutical and nutraceutical aspect. Under the guidelines of the US Food and Drug Administration, botanical drugs can be developed much faster and less costly than conventional single-entity pharmaceuticals (Raskin *et al*., 2002). Therefore, the present research is to investigate the anti-obesity effects of tea polyphenols both in individual and their combinations.

1.1. Obesity and human health

Obesity results from energy imbalance between energy intake and energy expenditure over a period of time. Increased energy intake (calories) with the decline of physical activity promotes weight gain, body fat storage and adiposity growth in a pathologic direction (Jequier, 2002).

Studies have shown that the obesity contributes to an increasing risk of major depression, emotional disorders, early death, disability, menstrual disorders, infertility, miscarriage, and poor pregnancy. It is also concluded that there is strong evidence that obesity is associated with increased morbidity and mortality. Several chronic diseases are demonstrated related to obesity which including the following: (Grundy, 2007; Lawrence *et al*.; 2004; Kopelman, 2000):

- Hypertension
- Dyslipidemias (high total cholesterol or high levels of triglycerides)
- Type 2 diabetes
- Coronary heart disease
- Metabolic syndrome
- Stroke
- Gallbladder disease
- Osteoarthritis
- Sleep apnea and respiratory problems
- Some cancers (endometrial, breast, and colon)

In addition, obesity has been predicted to be the number one health problem

globally by the year 2025 and thought to be overtaking cigarette smoking soon to become the leading cause of death in the USA (Vaidya, 2006; Low *et al*., 2006). Even though, the consequence of obesity is so severe, there is not an effective method to prevent and treat obesity. We have, therefore, dedicated ourselves to develop a more successful method to prevent obesity.

Medical Complications of Obesity

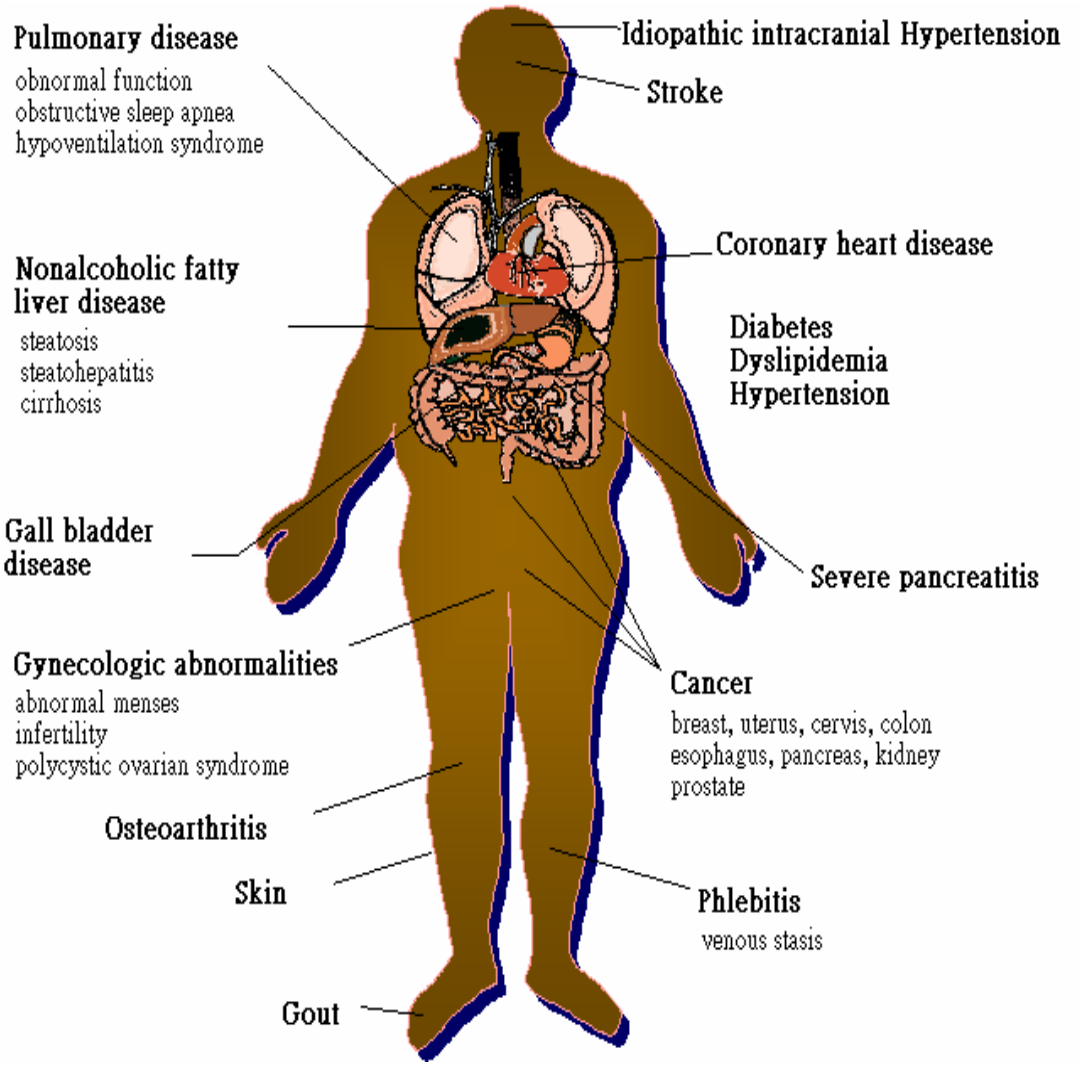


Figure 1.1. Medical complication of obesity

Adapted from NAASO the Obesity Society

1.2. Adipose tissue and obesity

Adipocyte, so called the fat cell, is the major component of adipose tissue that is known as loose connective tissue or fat tissue that function as an energy storage site in the form of triglyceride (Cryer *et al*., 1985). Adipose tissue plays an important role in maintaining the free fatty acid levels and triglycerides in circulation. It has been demonstrated that an increased amount of adipose tissue is related to obesity by hyperplasia or hypertrophy of the adipocyte. Hyperplasia, which is an increase in the number of adipocytes, this occurs by pre-adipocyte differentiating into adipocyte. Adipose tissue mass can also increase by hypertrophic growth, which is an increase in the size of adipocyte (Wolfram *et al*., 2006).

Although obesity is associated to increase of body weight, the definition of obesity is not dependent on body weight but on the amount of body fat, specifically adipose tissue. In other word, obesity is a condition of abnormal large amount of fat stored in adipose tissue and an increase in bodyweight is generally associated with an increased risk of excessive fat-related metabolic diseases (EFRMD) and chronic diseases, including Type 2 diabetes mellitus, hypertension and dyslipidemia (Bays, 2006; Mazzucotelli, 2006; Reeves *et al*., 2006).

There are two types of adipose tissue, brown adipose tissue and white adipose tissue. White adipose tissue can compose up to 25% of body weight in men and women and its main purpose is the storage site for fat in the form of triglycerides and cholesterol ester. On the other hand, brown adipose tissue is found mainly in newborn or hibernating mammals because its primary purpose is to generate body heat. Brown adipocytes contain several smaller vacuoles while white adipocytes

contain a single large vacuole. The brown adipocytes also contain large number of mitochondria. It also contains more capillaries for its need of oxygen. As for white adipocytes, it serves for three functions such as heat insulation, mechanical cushion and source of energy.

White adipose tissues can be found mostly in perivascular, intermuscular, peritoneal, retroperitoneal, and subcutaneous. It also secretes resistin, adiponectin and leptin. In male mouse, adjacent to the epididymis and testes, there deposited large amount of intra-abdominal white adipocytes. Adipocytes stores along the uterine horns in female mouse are known as the parametrial fat pads. When mice grow into adulthood, its brown fat is best easily observed. Two noticeable, lobulated masses of brown fat on the dorsal of the thorax between the scapulae can be easily seen when dissecting an adult mouse. Another location with great development of brown adipocytes is around the aorta of the heart and also in the hilus of the kidney (Hausman, 1987).

Abdominal fat, or parametrial fat pads in mouse, is believed to be more related to health risk than is whole-body fat. Research has shown that obesity people who have more abdominal fat are more threat of getting cardiovascular disease, diabetes and metabolic syndrome (Saelens *et al.*, 2007; Rezende *et al.*, 2006). Although the physiological role of brown adipose tissue in humans is debated, it is reported that brown adipose tissue in rodents has an important role in the prevention and therapy of obesity (Cinti, 2006).

.

In theory, to prevent pre-adipocyte from differentiating into mature adipocyte, to

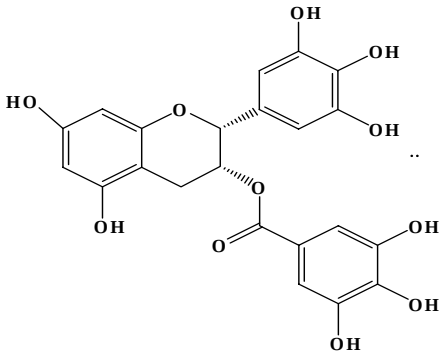
stimulate apoptosis or to morphologically reverse pre-adipocyte to pre-adipocyte is feasible. In conclusion, it is possible to inhibit adipose tissue mass by decreasing the adipose tissue mass as well as adipocyte number (Naslund *et al*., 1988). In addition, to decrease body weight and lower the risk of several chronic diseases- especially metabolic syndrome can also be achieved by lowering the abdominal fat pad.

2. LITERATURE REVIEW

2.1. Green tea EGCG and obesity

For many centuries, tea has been the most wildly consumed beverages in Asian countries. Today, the health benefits of tea have gained more attention from consumers and scientists. Tea has been investigated for its ability to prevent several chronic diseases, including cancer, neurodegenerative diseases and obesity (Higdon, 2003). Reduction of serum cholesterol level, prevention of arteriosclerosis and effects such as protection of blood vessels, were reported as integrated pharmacological effects by tea (Yang *et al*., 1997; Yang, 2001).

EGCG (Epigallocatechin-3-gallate) is one the major catechins in green tea. It provides strong antioxidant property, which is believed to contribute to the possible health benefits of tea consumption. EGCG is also considered a chemopreventive agent for cancer, obesity, and cardiovascular diseases (Yang *et al*., 1993; Yang, 1999; Zheng *et al*., 2004; Murase *et al*., 2002).



Epigallocatechin-3-gallate (EGCG)

Figure 2.1. Chemical structure of green tea catechin- epigallocatechin-3-gallate

Evidences show that green tea and its catechins, especially EGCG, reduce body

weight as well as adipose tissue and blood lipid level (Kao *et al*., 2000; Wolfram *et al.*, 2005; Klaus *et al*., 2005; Vinson, 1998; Muramatsu, 1986). The mechanism of action of EGCG involve certain pathways, including (1) decrease in the energy intake (Kao *et al*., 2000), (2) increase in energy expenditure (Dulloo *et al*., 1999; Dulloo *et al*., 2000), and (3) alterations in the activities of fat, liver, muscle, and intestinal cells (Kao *et al*., 2000; Liao *et al*., 2001).

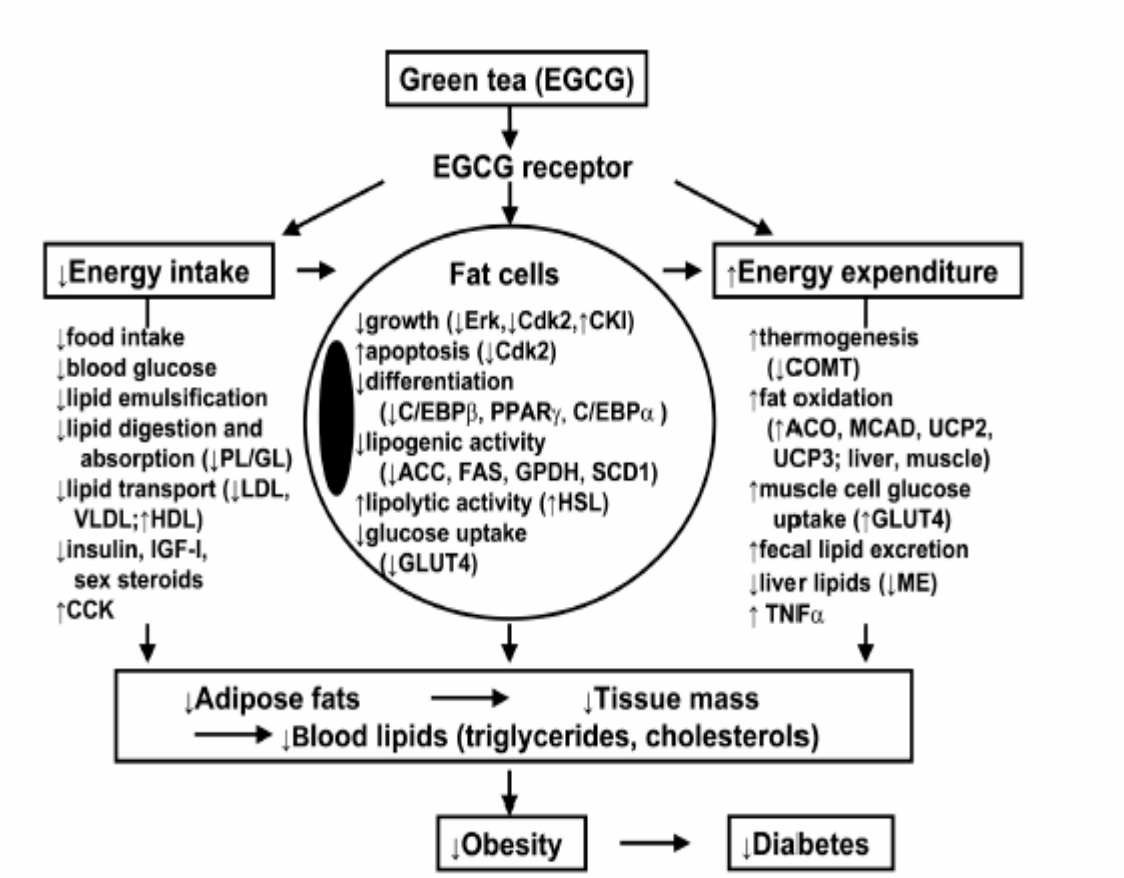


Figure 2.2. Mechanisms of the action of green tea EGCG on obesity and diabetes. Adapted from Kao *et al.* (2000)

2.2. Black tea extract – bioactivity and structure

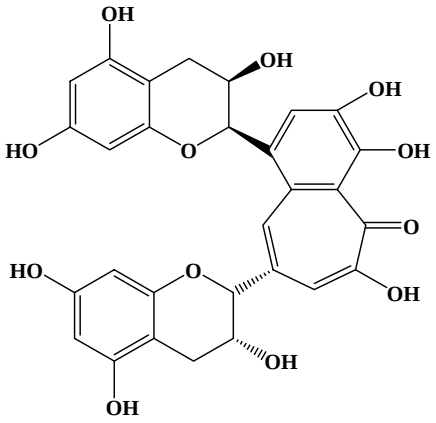
Black tea represents approximately 78% of total tea consumed in the world, whereas green tea accounts for approximately 20% of tea consumed (Siddique *et al*., 2004). Green tea catechins have been intensively investigated for its life-time benefits for decades, while black tea extracts have not been studied until recent years. Even though, green tea has the effect of reducing the risk of several chronic diseases, the effect of black tea is more controversial (Cheng, 2006).

The anti-oxidant ability in green tea is due to tea catechins. These catechins are transformed into theaflavins and thearubigins during production of black tea. Thus, black tea contains less catechins compared to green tea. The content of theaflavins in black tea ranges from 0.3% to 2% and consists of theaflavin (TF-1), theaflavin-3-gallate (TF-2a), theaflavin-3′-gallate (TF-2b) and Theaflavin-3,3′-digallate (TF-3). Other constituents of tea include alkaloids (such as caffeine and theobromine), amino acids, pigments, enzymes and vitamins (Robertson, 1992).

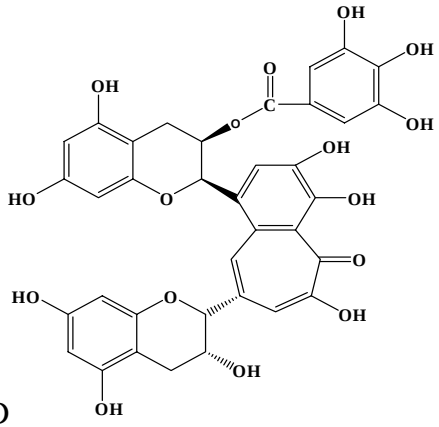
It was previous demonstrated that the antioxidant activity of black tea theaflavins are comparable to green tea catechins (Leung *et al*., 2001). On the other hand, Lee *et al*. (2002) reported that the antioxidant capacity of green tea is higher than that of black tea. When tea supplementation is targeted at human concentration to animals for a short-term basis, positive improvements in the lipid profile of both normal and cholesterol-fed animals are observed (Lin, 2006). Similar results were conducted in Japan when green tea catechins were given to cholesterol-fed rats (Hara *et al*., 1994). In epidemiological studies, it is found that both green and black tea consumptions are

correlated with the decrease of plasma cholesterol and atherogenic index. (Ito *et al*., 1994; Davies *et al*., 2003; Vinson *et al*., 1998).

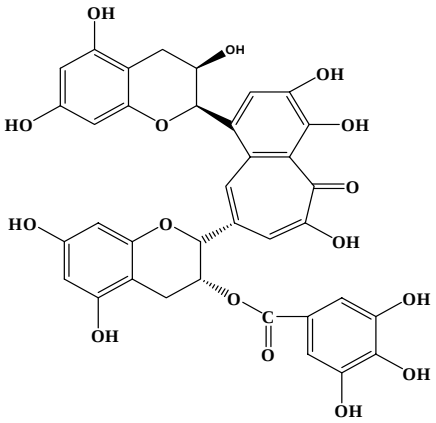
A



B



C



D

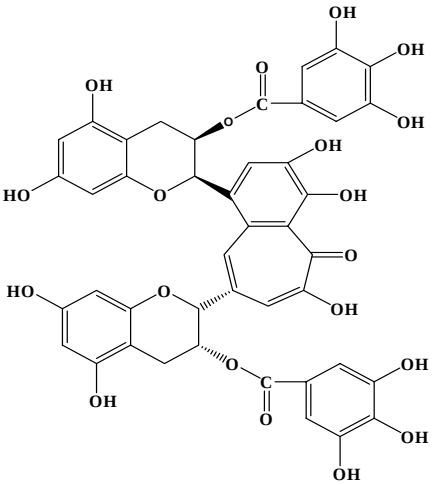


Figure 2.3. Major component of black tea extracts. A: Theaflavin (TF-1);

B: Theaflavin 3-gallate (TF-2a); C: Theaflavin 3′-gallate (TF-2b);

D: Theaflavin 3,3-digallate (TF-3)

2.3. Orange peel extract – bioactivity and structure

The major component of orange peel is flavonoids, especially the polymethoxyflavones (PMFs). Other components include the terpenoids, such as limonene, linalool; and volatile oils. Since PMFs are the major components, more than 20 polymethoxylated flavonoids have been identified, including tangeretin and nobiletin, which are relative abundant PMFs compared to other PMFs exist in orange peel. PMF are considered to contribute to the chemoprevention activity of orange peel extracts (Li *et al*., 2006).

PMFs, especially tangeretin and nobiletin, have been found to have health benefits, including anti-inflammatory, anti-carcinogenic, anti-tumor, anti-viral, anti-oxidant, anti-thrombogenic and anti-atherogenic properties (Middleton *et al*., 2000). Other reported also suggested that the other PMF from orange named hesperetin can lower the risk of coronary heart disease by the effect of hypolipidemia (Borradaile *et al*., 1999; Kurowska and Manthey, 2002; 2004; Whitman *et al*., 2005).

Recent research done at Rutgers University shows that hydroxylated polymethoxyflavones formed in orange peel extract have higher anti-inflammatory activity compared to polymethoxyflavones (Li *et al*., unpublished results). These newly identified hydroxylated polymethoxyflavones might give a new lead for nutraceutical research. In addition, due to the lipophilicity of PMFs, they may have higher permeability through small intestine and easier to be absorbed into the blood circulation system.

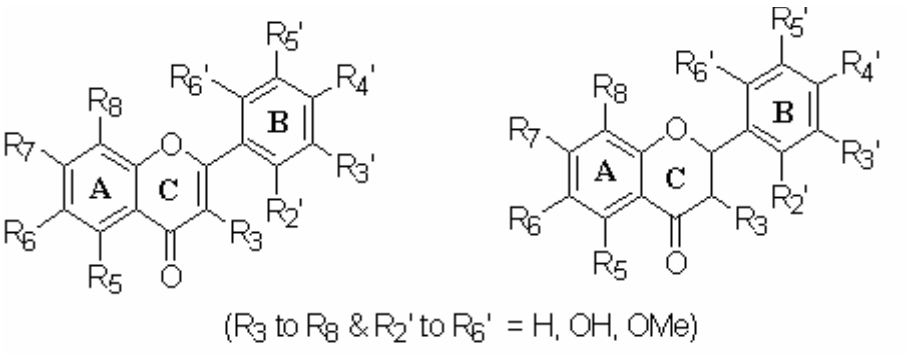


Figure 2.4. Polymethoxylated flavonoids (PMFs)

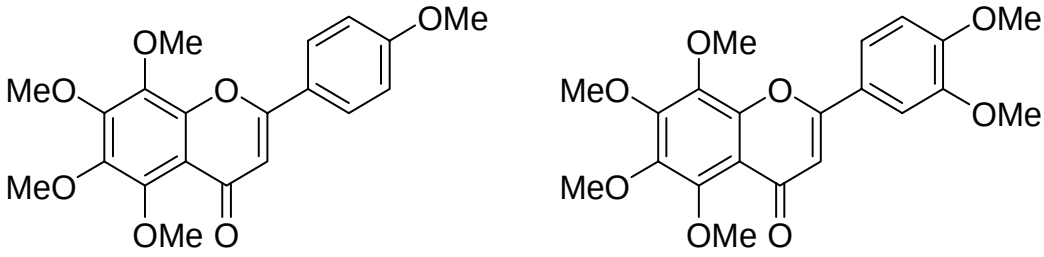


Figure 2.5. Structure of tangeretin and nobiletin

2.4. Caffeine – anti-obesity effect and structure

Caffeine, a methylxanthine, can be found in many plant species, such as coffee, tea and cocoa. It is a central nervous system stimulant that can have an effect of restoring awareness. While, it is the world most widely consumed psychoactive substance, its use is legal and unregulated in most countries. Caffeine can be used in the form of drug or beverages to treat obesity patients.

Possible mechanism by which caffeine affects body weight reduction has been intensively investigated and reported:

1. Stimulate thermogenesis involves inhibiting the phosphodiesterase-induced degradation of intra-cellular cyclic AMP (cAMP)and extend the sympathetic stimulation (Dulloo, 1993; Dulloo *et al*., 1999; 2000; Leblanc *et al*., 1995; Dipvens *et al.*, 2005; Westerterp-Plantenga *et al.*, 2007).
2. Stimulate substrate cycles- Cori cycle and the FFA-triglyceride cycle (Astrup *et al*., 1993; Astrup *et al*., 1990; Yoshida *et al*., 1994).
3. Suppress the accumulation of body fat and reduce the adipose tissues mass and body fat percentage (Kazuo *et al*., 2005; Hasegawa *et al.*, 2000; Bukowiecki *et al*., 1983; Chen *et al*., 1994; Michna *et al*., 2003).
4. Reduce food intake for short term consumption of caffeine (Racotta *et al*., 1994; Tremblay *et al*., 1988); insensitivity of the effect might occur if long term consumption of caffeine (Westerterp-Plantenga *et al.*, 2005; Pasman *et al*., 1997).

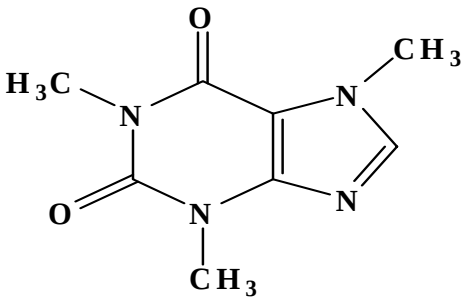


Figure 2.6. Structures of caffeine

3. MATERIALS AND METHODS

3.1. Experiment 1 - animal and diets

Female CF-1 mice, 6 weeks old, were purchased from Charles River Breeding Laboratories (Kingston, New York). The mice were randomly divided into 10 groups (10 mice per group) except the caffeine group had 9 mice. The average of initial body weight for each group ranged from 19 to 23 g. Mice were housed in plastic cages, in an air conditional room with temperature at 22 to 24°C under a 12 h light – dark cycle (the light period is form 06:00 to 18:00) and humidity 50 ±10%.

Mice in different group were fed with different diets for 10 weeks. Low-fat diet is 5% corn oil in AIN-76A diet, and high-fat diet is 20% corn oil in AIN-76A diet. Other groups that contained specific amount of plant extracts are in the high-fat diet with equal amount of calorie in each diet per gram. Diets were provided by Wellgen Inc. (Piscataway, New Jersey) and the composition of different diets is shown in Table 3.1. The experiment was terminated after 10 weeks.

Product #	5 % corn oil N		20 % corn oil H		0.2 % OPE H		0.2 % BTE 0.2 % OPE H		0.2 % OPE 0.1 % EGCGN H	
	gm%	kcal%	gm%	gm%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	20.3	21	24.1	24.0	24.0	21	24.0	21	24.0	21
Carbohydrate	66.0	68	44.9	44.7	44.8	39	44.7	39	44.7	39
Fat	5.0	12	20.7	20.7	20.7	40	20.7	40	20.7	40
Total		100				100.0		100		100
kcal/gm	3.90		4.62	4.60	4.61		4.60		4.61	
Ingredient	gm	kcal	gm	gm	gm	kcal	gm	kcal	gm	kcal
Casein	200	800	200	200	200	800	200	800	200	800
DL-Methionine	3	12	3	3	3	12	3	12	3	12
Maltodextrin	0	0	75	75	75	300	75	300	75	300
Corn starch	150	600	75	75	75	300	75	300	75	300
Sucrose	500	2000	218.8	218.8	218.8	875	218.8	875.2	218.8	875.2
Cellulose, BM200	50	0	50	50	50	0	50	0	50	0
Corn Oil	50	450	175	175	175	1575	175	1575	175	1575
Ethoxyquin	0.01	0	0.01	0.01	0.01	0	0.01	0	0.01	0
Mineral Mix S10001	35	0	35	35	35	0	35	0	35	0
Vitamin Mix V10001	10	40	10	10	10	40	10	40	10	40
Choline Bitartrate	2	0	2	2	2	0	2	0	2	0
Orange Peel Extract	0	0	0	1.8	1.8	0	1.8	0	1.8	0
Black tea Extract	0	0	0	1.8	0	0	1.8	0	0	0
Epigallocatechin Gallate(EGCG)	0	0	0	0	0	0	0	0	0.9	0
Caffeine	0	0	0	0	0	0	0	0	0	0
Total	1000	3902	843.81	847.41	845.61	3902	847.41	3902	846.51	3902

Table 3.1.1. Diet composition. AIN-76A Diet with 0.01 gm/kg Ethoxyquin and 5 % or 20.7 gm% fat and same with varying concentrations of orange peel extracts, black tea extracts, caffeine, or EGCG N: low-fat diet; H: high-fat diet.

Product #	0.2 % OPE 0.1 % CF H		0.2 % BTE 0.2 % OPE 0.1 % CF		0.2 % BTE H		0.1 % EGCG H		1.1 % CF H	
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	24.0	21	23.9	21	24.0	21	24.0	21	24.0	21
Carbohydrate	44.8	39	44.7	39	44.8	39	44.8	39	44.9	39
Fat	20.7	40	20.6	40	20.7	40	20.7	40	20.7	40
Total		100		100		100		100		100
kcal/gm	4.61		4.60		4.61		4.62		4.62	
Ingredient	gm	kcal	gm	kcal	gm	kcal	gm	kcal	gm	kcal
Casein	200	800	200	800	200	800	200	800	200	800
DL-Methionine	3	12	3	12	3	12	3	12	3	12
Maltodextrin	75	300	75	300	75	300	75	300	75	300
Corn starch	75	300	75	300	75	300	75	300	75	300
Sucrose	218.8	875.2	218.8	875.2	218.8	875.2	218.8	875.2	218.8	875.2
Cellulose, BM200	50	0	50	0	50	0	50	0	50	0
Corn Oil	175	1575	175	1575	175	1575	175	1575	175	1575
Ethoxyquin	0.01	0	0.01	0	0.01	0	0.01	0	0.01	0
Mineral Mix S10001	35	0	35	0	35	0	35	0	35	0
Vitamin Mix V10001	10	40	10	40	10	40	10	40	10	40
Choline Bitartrate	2	0	2	0	2	0	2	0	2	0
Orange Peel Extract	1.8	0	1.8	0	0	0	0	0	0	0
Black tea Extract	0	0	1.8	0	1.8	0	0	0	0	0
Epigallocatechin Gallate(EGCG)	0	0	0	0	0	0	0.9	0	0	0
Caffeine	0.45	0	0.45	0	0	0	0	0	0.45	0
Total	846.06	3902	847.86	3902	845.61	3902	844.71	3902	844.26	3902

Table 3.1.2. (continued) Diet composition. AIN-76A Diet with 0.01 gm/kg Ethoxyquin and 5% or 20.7 gm% fat and same with varying concentrations of orange peel extracts, black tea extracts, caffeine, or EGCG N: low-fat diet; H: high-fat diet.

3.2. Experiment 2 – animal and diets

Female CD-1 mice, 5 weeks old, were purchased from Charles River Breeding Laboratories (Kingston, New York). The mice were randomly divided into 14 groups (10 mice per group). After 17 weeks on the special AIN76A diet, the mice were sacrificed and blood samples were collected by pooling blood together from 3 or 4 mice. Total cholesterol levels, triglyceride levels and HDL levels were determined by a CardioChek, Lipid Panel Test (Polymer Technology System Inc, (Indanapolis, IN). Effects of various diets on body weight gain, abdominal fat weight (size), total cholesterol levels, triglyceride levels and blood glucose levels were summary in Table 2.

The average of initial body weight for each group ranged from 25 to 26 g. These five groups were housed in plastic cages, windowless room at 22 to 24°C under a 12 h light – dark cycle (the light period is form 06:00 to 18:00) and humidity 60%.

All mice were fed different diets: low-fat diet (5% corn oil in AIN-76A diet), high-fat diet (20% corn oil in AIN-76A diet), and specific amount of orange peel extracts in the high-fat diet with equal amount of calorie in each diet per gram for 17 weeks. Groups include: (1) low-fat diet (5% corn oil in AIN-76A diet);

(2) high-fat diet (20% corn oil in AIN-76A diet);

(3) 0.01%; 0.03%; 0.05%; 0.1%; 0.2% OPE (WG 361) in high-fat diet;

(4) 0.01%; 0.03%; 0.1% OPE (WG 362) in high-fat diet;

(5) 0.01%; 0.03%; 0.1% OPE (WG 363) in high-fat diet

WG361 is an orange peel extract (OPE) containing 40% polymethoxyflavones, WG362 is an orange peel extract containing 70% polymethoxyflavones, and WG363 is HCl-hydrolyzed product from WG361 rich in 5-hydroxy-polymethoxyflavones. They were provided by Wellgen, Inc. (Piscataway, New Jersey). The experiment was terminated after 17 weeks.

3.3. Body weight, food and water intake

Body weight of each mouse was monitored weekly. Food and water intake was measured on a per-cage (10 mice per cage) basis 3 times per week and the average of food and water consumed were calculated weekly every week. Unit for food consumption is gram/day/mouse (g/d/m) and unit for water consumption is ml/day/mouse (ml/d/m).

.

3.4. Sample collections

The mice were sacrificed at the day that mice have been given the specific diets for 10 weeks (experiment 1) and 17 weeks (experiment 2). Blood was collected from the jugular vein for the determination of total blood cholesterol, triglyceride, HDL, LDL, and glucose levels. Serum was separated immediately from blood samples by centrifugation at 1200 g for 30 minutes at 4°C. The supernatant fraction was used for examination of leptin levels in serum. Parametrical fat pad, retroperitoneal fat pad, brown adipose tissue, liver and spleen were weighed and then frozen and kept in -80°C freezer.

3.5. Determination of serum leptin levels

The Enzyme-Linked ImmunoSorbent Assay, or ELISA, was used to measure

mouse leptin level in serum. The ELISA kit for leptin assay was purchased from R&D Systems, Inc. (Minneapolis, MN).

First, coat the 96 wells plate with 100 μL goat anti-mouse leptin (capture antibody: 2.0 μg/mL) and incubate overnight at room temperature. Aspirate each well and wash with wash buffer (0.05% Tween 20 in phosphate buffered saline) three times and then block plated by adding 300 μL of block buffer (5% Tween-20 in phosphate buffered saline with 0.05% NaN_3) for one hour at room temperature. Repeat the aspiration/wash step for three times and add 100 μL sample or standard into each well. After incubating the plate for two hours at room temperature, add 100 μL of detection antibody (200 ng/mL biotinylated goat anti-mouse leptin) and incubate another two hours at room temperature. Wash the plate for three times, add 100 μL Streptavidin-HRP (streptavidin conjugated to horseradish-peroxidase), and let the plate sits for 20 minutes in dark place. Wash again and treat with 100 μL substrate solution (1:1 mixture of H_2O_2 and Tetramethylbenzidine). Avoid placing the plated in direct light for 20 minutes, the reaction is stopped by adding 50 μL stop solution (2 N H_2SO_4) per well. Measure the optical density immediately by using microplate reader set to 450 nm.

All samples were assayed within one month and measured in triplicate.

3.6. Triglyceride assay

Blood triglyceride levels were measured by the CardioChek- PTS PANELS Lipid Panel Test (Polymer Technology Systems, Inc. Indianapolis, IN). The method is based on enzymatic reactions that produce color that is read by the analyzer using

photometry.

Principle:

$$\text{Triglyceride} + 3\ H_2O \xrightarrow{\text{lipoprotein lipase}} \text{Glycerol} + 3\ \text{fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{Mg}]{\text{glycerol kinase}} \text{Glycerol - 3-}PO_4 + \text{ADP}$$

$$\text{Glycerol - 3-}PO_4 + O_2 \xrightarrow{\text{glycerophosphate oxidase}} \text{Dihydroxyacetone - }PO_4 + H_2O_2$$

$$2\ H_2O_2 + \text{4-AAP} + \text{N,N-disubstituted aniline} \xrightarrow{\text{peroxidase}} \text{Quinoneimine (red)} + 4H_2O$$

The sensitivity of the assay is ranged from 50-500 mg/dL. All samples were assayed immediately and measured in triplicate.

3.7. High-Density Lipoprotein (HDL) assay

Blood HDL level was measured by the CardioChek- PTS PANELS Lipid Panel Test (Polymer Technology Systems, Inc. Indianapolis, IN). The method is based on enzymatic reactions that produce color that is read by the analyzer using photometry.

Principle:

$$\text{VLDL, LDL, HDL plasma} \longrightarrow \text{VLDL, LDL, depleted plasma}$$

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acid}$$

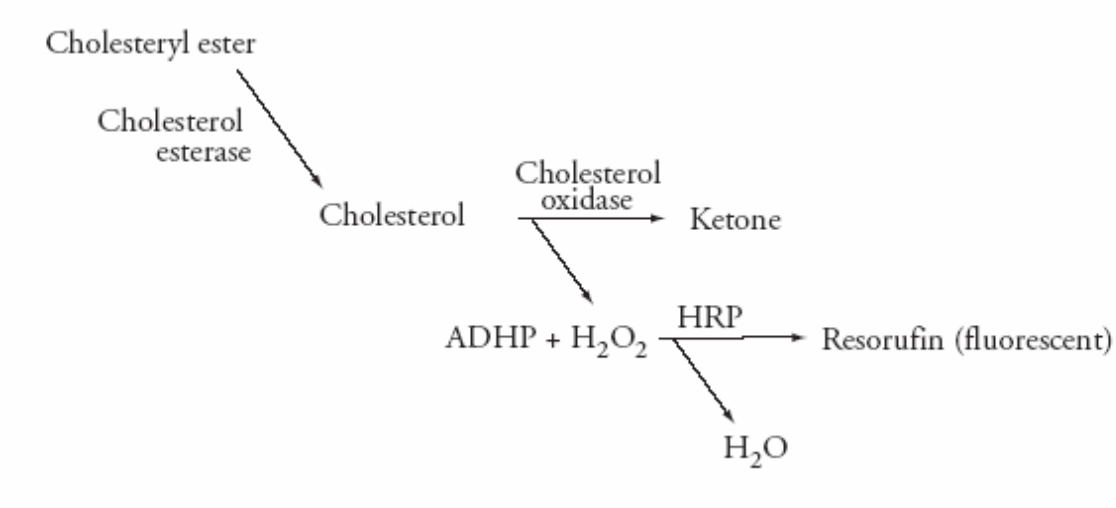
$$\text{Cholesterol} + H_2O + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{Cholesterol-4-en-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{4-AAP} + \text{Dosibstotited Aniline} \xrightarrow{\text{peroxidase}} \text{Quinoneimine (red)} + 4H_2O$$

The sensitivity of the assay is ranged from 15- 100 mg/dL. All samples were assayed immediately and measured in triplicate.

3.8. Cholesterol assay

Cholesterol level was detected by Cholesterol Assay Kit provided by Cayman Chemical Company (Ann Arbor, MI). The assay is based on an enzyme-coupled reaction that detects both free cholesterol and cholesteryl esters. Cholesteryl esters are hydrolyzed by cholesterol esterase into cholesterol, which is then oxidized by cholesterol oxidase to yield hydrogen peroxide and the corresponding ketone product. Hydrogen peroxide is then detected using ADHP (10-acetyl-3,7-dihydroxyphenoxazine). In the presence of horseradish peroxidase, DHP reacts with H_2O_2 to produce fluorescent resorufiin (assay protocol adapted from Cayman Chemical).



All samples were assayed within one month of the day that the experiment was terminated and measured in triplicate. The fluorescence was read by a fluorometric

plate reader reader at excitation wavelength of 550 nm and emission wavelength of 590 nm.

3.9. LDL determination

The LDL level was used Friedewald's formula to calculate. The Friedewald formula: [LDL-C = total cholesterol - (HDL-C + TG/5].

3.10. Glucose assay

Blood glucose level was measured by the CardioChek- PTS PANELS Glucose Test (Polymer Technology Systems, Inc. Indianapolis, IN). The method is based on the analyzer reading light reflected off a test strip that has changed color after blood has been placed on it. The darker the color, the higher the glucose level (protocol adapted from CardioChek, Polymer Technology Systems, Inc.).

All samples were analyzed immediately and measured in triplicate. The sensitivity of the glucose assay ranged from 20-600 mg/dL.

3.11. Statistical analysis of the data

The values obtained were expressed as the mean ± SE (standard error). Statistical comparisons of two groups were determined by the Student's t-test. Statistical significance was defined as $p<0.05$.

4. RESULTS AND DISCISSION

4.1. Experiment 1

4.1.1. Effect of feeding various plant extracts on food intake

Figure 4.1 shows the effect of different compounds on the food intake by mice fed on the high-fat diet and low-fat diet (experiment 1), there is no significant difference among all groups, including 0.1% EGCG; 0.2% orange peel extracts (OPE); 0.2% black tea extracts (BTE); 0.05% caffeine (CF); 0.2% OPE + 0.1% EGCG; 0.2% OPE + 0.2% BTE; 0.2% OPE + 0.05%CF; 0.2% OPE + 0.2% BTE + 0.05% CF in high-fat diet. In other word, these modulated diets, compounds that added into diet, do not influence the mice appetite as well as energy intake for long term consumption.

4.1.2. Effects of high-fat diet and low-fat diet on body weight gain, and abdominal fat size

Table 4.1 shows the effects of high-fat diet and low-fat diet on body weight gaining, and abdominal fat size in CF-1 mice. Although no significant difference was apparent in the food intake between the high-fat diet (20% corn oil in AIN-76A diet) group and the low-fat diet group (5% corn oil in AIN-76A diet), there were significant increase in the level of body weight gain, parametrial fat pad weight, retroperitoneal fat pad weight, and brown adipose tissue in mice fed on the high-fat diet compared to mice fed on the low-fat diet for 10 weeks. The results clearly demonstrated that body weight gain and fat accumulation in mice were remarkably increased by the high-fat diet (Table 4.1).

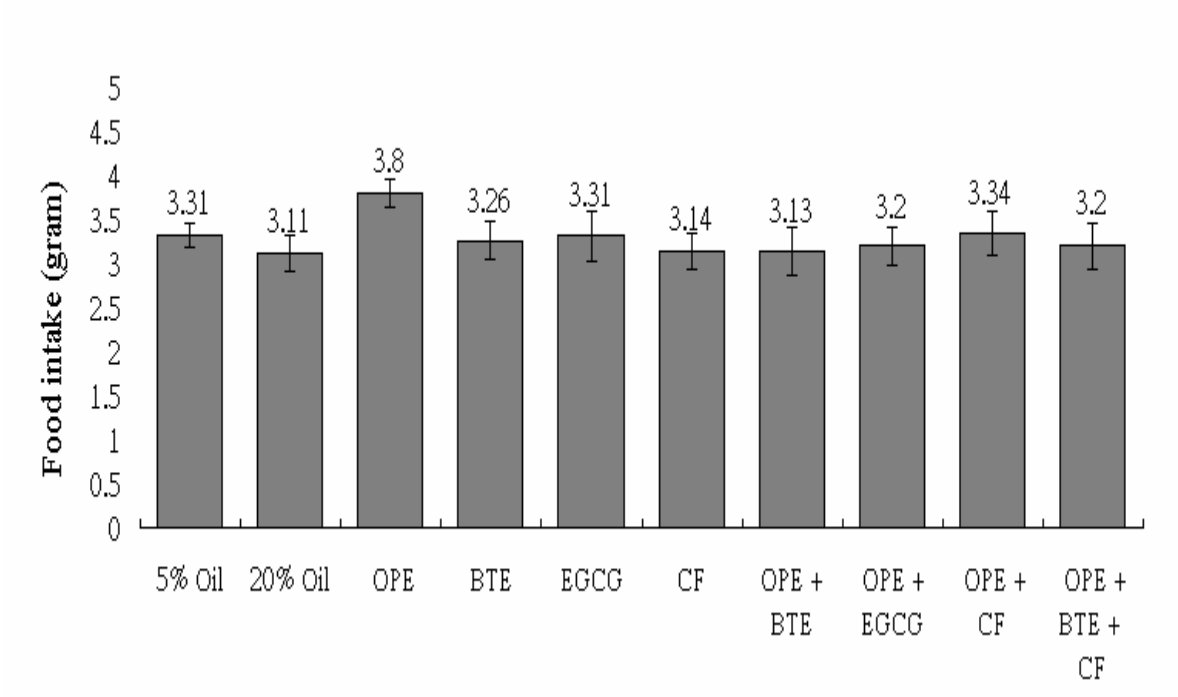


Figure 4.1. Monitoring daily food intakes in mice fed with various plant extracts in the high-fat diets

Female CF-1 mice (6 weeks old; 10 mice r per group) were given a low-fat diet (AIN-76A 5% corn oil), a high fat-diet (AIN-76A 20% corn oil), 0.2% OPE, 0.2% BTE, 0.05%CF in high-fat diet or in combination of 0.2% OPE + 0.2% BTE, 0.2%OPE + 0.1%EGCG, 0.2%OPE +0.05%CF or 0.2%OPE + BTE + 0.05%CF in high-fat diet and water *ad lititum* for 10 weeks. Food intake was monitored weekly. Data are mean±SE (n=10). *significant different ($p<0.05$).

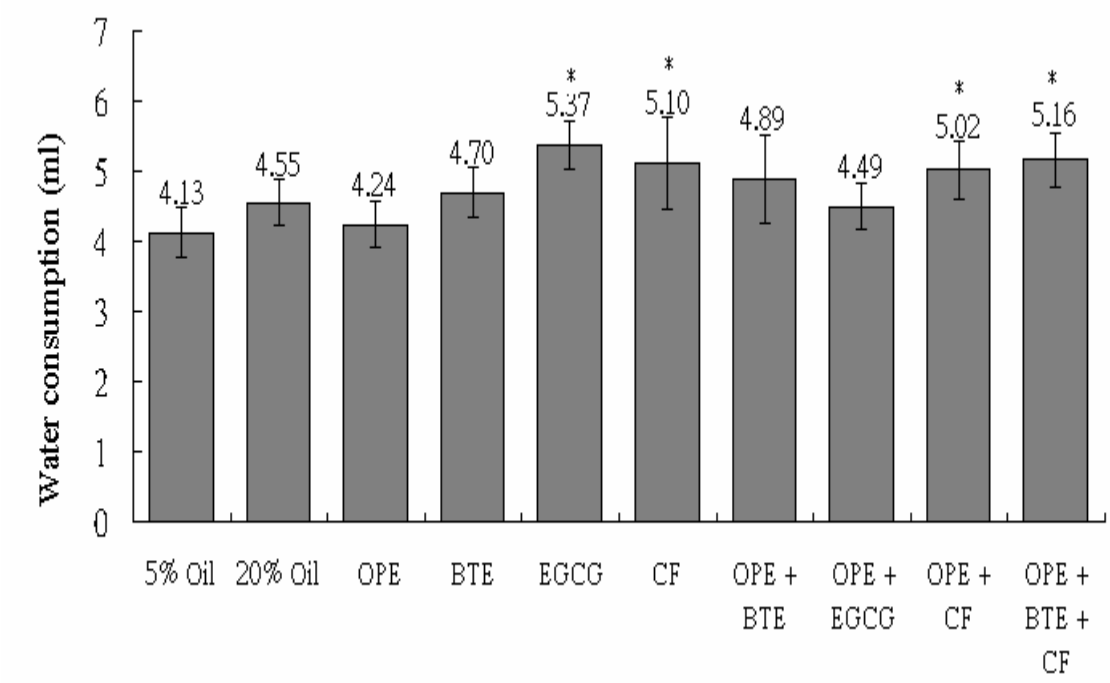


Figure 4.2. Monitoring daily water consumption in mice fed with various plant extracts in the high-fat diets

Female CF-1 mice (6 weeks old; 10 mice r per group) were given a low-fat diet (AIN-76A 5% corn oil), a high fat-diet (AIN-76A 20% corn oil), 0.2% OPE, 0.2% BTE, 0.05%CF in high-fat diet or in combination of 0.2% OPE + 0.2% BTE, 0.2%OPE + 0.1%EGCG, 0.2%OPE +0.05%CF or 0.2%OPE + BTE + 0.05%CF in high-fat diet and water *ad lititum* for 10 weeks. Water consumption was monitored weekly. Data are mean±SE (n=10). *significant different ($p<0.05$).

	5% Corn oil AIN-76A diet	20% Corn oil AIN-76A diet
Initiated body weight (g)	21.89 ± 1.03	20.94 ± 0.82
End body weight (g)	31.29 ± 2.17	35.90 ± 3.42
Body weight gaining (g)	9.40	14.96
Food intake (g/mouse/day)	3.31 ± 0.14	3.11 ± 0.21
Parametrial fat pad weight (g)	0.557 ± 0.040	1.598 ± 0.163
Retroperitoneal fat pad weight (g)	0.163 ± 0.002	0.378 ± 0.003
Brown adipose tissue (g)	0.199 ± 0.005	0.344 ± 0.043
Liver weight (g)	1.501 ± 0.187	1.303 ± 0.077
Spleen weight (g)	0.132 ± 0.013	0.148 ± 0.005

Table 4.1. Effects of high-fat diet and low-fat diet on body weight gain, and abdominal fat size in CF-1 mice

Female CF-1 mice (6 weeks old; 10 mice per group) were given a low-fat diet (AIN76A 5% corn oil) or a high-fat diet (AIN76A 20% corn oil) and water *ad libitum* for 10 weeks. Food and water consumption, body weight were monitored weekly. After 10 weeks on the special diets, the mice were sacrificed. Parametrical fat, retroperitoneal fat and brown fat as well as spleen and liver were removed and weighed. Data are mean ±SE (n=10).

4.1.3. Dietary OPE, BTE and CF suppress body weight gain in CF-1 mice

The effects of dietary orange peel extract (OPE), (-)-epigallocatechin gallate (EGCG), black tea extract (BTE) and caffeine (CE) on body weight changing is shown Figure 4.3. To elucidate the anti-obesity effect of OPE, BTE, EGCG and CF, we measured the parametrial fat pad, retroperitoneal fat pad and brown adipose tissue weights after feeding 0.2% OPE, 0.2% BTE, 0.01% EGCG or 0.05% CF diet for 10 weeks (Figure 4.5; Figure 4.6; Figure 4.7). The body weight gained was significantly reduced by 0.2% OPE (32%), 0.2% BTE (35.7%) and 0.05% CF (41.1%) diets from the initiated week until the end of feeding, but not by the 0.1% EGCG diet.

4.1.4. The anti-obesity effect of EGCG on abdominal fat and brown adipose tissue

It has been reported catechins, especially EGCG, have anti-obesity effect in mice by lowering the food intake and preventing body weight gain (Kao *et al*., 2000; Murase *et al*., 2002). Moreover, previous research also shows that EGCG reduced body fat accumulation in human (Nagao *et al*., 2001). Although the diet containing 0.1% EGCG did not influence the body weight and food intake in mice, it significantly inhibited the P-fat pad, R-fat pad and brown adipose tissue accumulation by 34.6%, 12.7% and 39%, respectively, compared to mice fed on high-fat diet (Figure 4.4; Figure 4.5; Figure 4.6; Figure 4.7). Study has been done suggests that the dose dependent manner on anti-obesity action of EGCG (Zheng *et al*., 2004). Thus, we can conclude that 0.1% EGCG might have anti-obesity effect potential on prevention of white adipose tissue and brown adipose tissue. Furthermore, the anti-obesity effect of EGCG on body weight reduction might be at a higher dose.

4.1.5. The anti-obesity effects of black tea extract on abdominal fat and brown adipose tissue

The diet containing 0.2% black tea extract (BTE) significantly suppressed body weight gain by 35.7%, reduced P-fat pad weight, R-fat pad weight and brown adipose tissue by 40.6%, 24.1% and 46.5%, respectively, compared to mice fed on high-fat diet (Figure 4.4; 4.5; 4.6; 4.7). However, it was reported that rats fed a high-cholesterol diet supplemented daily with black tea extract (100-200 mg/kg bw) by oral drinking did not influence the food intake and body weight in rats (Yokozawa *et al*., 1998). Calculating the average intake of BTE in our experiment is around 326 mg/kg bw daily. Thus, the anti-obesity effect of BTE might be at a higher dose.

4.1.6. The anti-obesity effects of orange peel extract on abdominal fat and brown adipose tissue

Addition of 0.2% orange peel extract (OPE) to the high-fat diet decreased the body weight by 32%. Our result also found that mice fed with high-fat diet supplemented daily among 0.2% OPE prevented the P-fat pad weight by 22.8%, R-fat pad weight by 22.5% and brown adipose tissue by 11.3% in comparison to that mice fed on high-fat diet (Figure 4.4;4.5; 4.6; 4.7). These results indicated that orange peel extracts had anti-obesity effects on body weight reduction, white adipose tissue and brown adipose tissue suppression. Although the anti-obesity effect of OPE has been demonstrated by our research, the effects of dietary OPE on body weight gaining and formation of white and brown adipose tissues are not dose-dependent according to our results of different concentrations of OPE added in high-fat diet.

4.1.7. The anti-obesity effects of caffeine on abdominal fat and brown adipose tissue

High-fat diet that contained 0.05% caffeine (CF) significantly suppressed body weight gain by 41.1%, reduced P-fat pad weight, R-fat pad weight and brown adipose tissue by 44.2%, 22.5% and 38.4%, respectively, compared to mice fed on high-fat diet (Figure 4.4; 4.5; 4.6; 4.7, respectively). These results suggested that mice fed with high-fat diet supplemented daily with caffeine had anti-obesity effect by suppressing the accumulation of adipose tissue and body weight gain in mice. The relation of caffeine and its effect on body fat under the influence of a high-fat diet has been intensively investigated. It has been reported that caffeine ingestion stimulates the metabolic rate and fat oxidation in vivo in fat cells (Higdon *et al.*, 1981; Arciero *et al*., 1995). In addition, the percentage of body fat can also be reduced by supplementing caffeine in a dose-dependent manner (Kazuo *et al*., 2005). This decrease in body fat percentage accompanied by the reduction in body fat mass strongly supports the anti-obesity effect of caffeine (Kazuo *et al*., 2005; Zheng *et al*., 2004; Chen *et al*., 1994; Hongu *et al*., 2000). Although other studies showed that caffeine ingestion could reduce food intake, as well as energy intake, the results from our experiment suggest that 0.05% caffeine does not have influence on food intake in mice fed with high-fat diet for 10 weeks (Racotta *et al*., 1994; Tremblay *et al*., 1988).

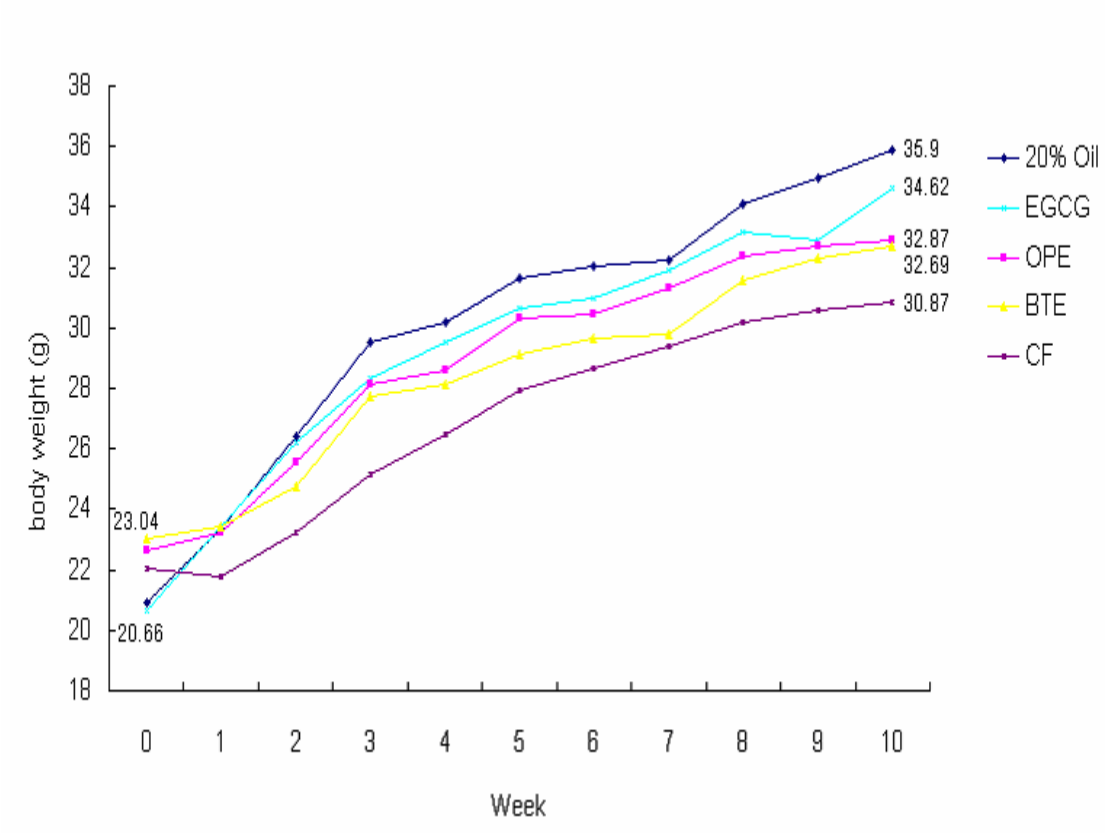


Figure 4.3. Effect of EGCG, OPE, BTE and CF on body weight changing in CF-1 mice

Female CF-1 mice (6 weeks old; 10 mice per group) were given a high-fat diet (AIN-76A 20% corn oil); 0.2% OPE, 0.2% BTE, or 0.05% CF in high-fat diet and water *ad libitum* for 10 weeks. Body weights were monitored weekly for 10 weeks. Data are mean±SE (n=10).

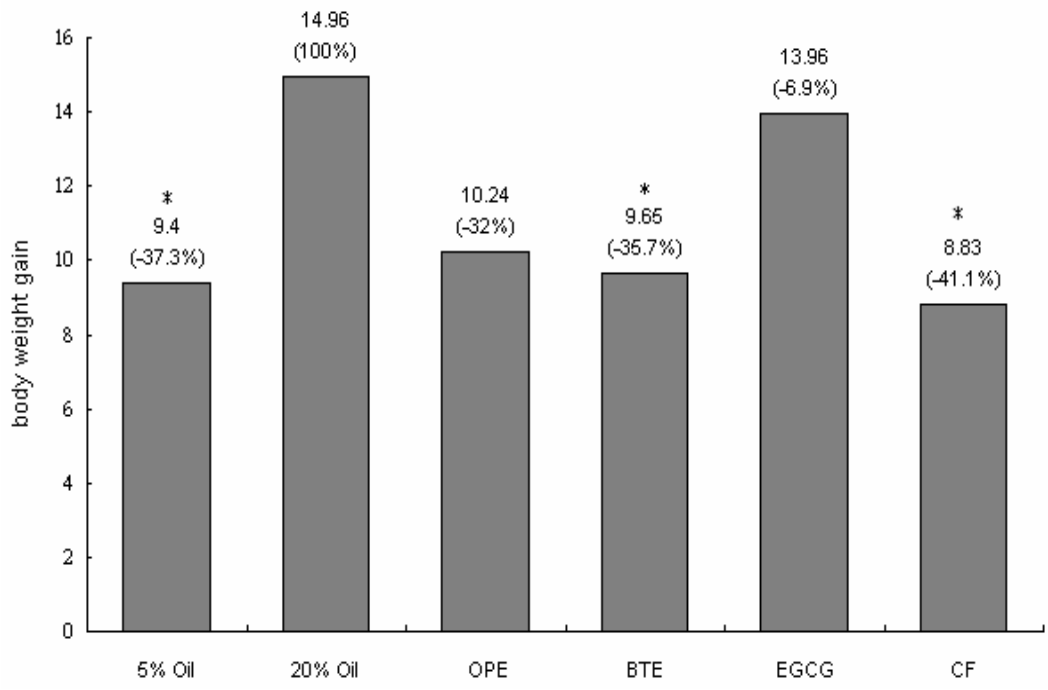


Figure 4.4. Dietary OPE, BTE and CF suppress body weight gain in CF-1 mice

Female CF-1 mice (6 weeks old; 10 mice per group) were fed with high-fat diet (AIN-76A 20% corn oil); 0.2% OPE, 0.2% BTE, or 0.05% CF in high-fat diet and water *ad libitum* for 10 weeks. Body weights were monitored weekly for 10 weeks. Data are mean±SE (n=10). Body weight gain= (Body weight at 10^{th} week – body weight at 0 week). "- %" represents the percentage of inhibition on body weight gain compared to high-fat diet = (1- weight gained of selected group/weight gained of 20% oil group) X100. *significant different ($p<0.05$).

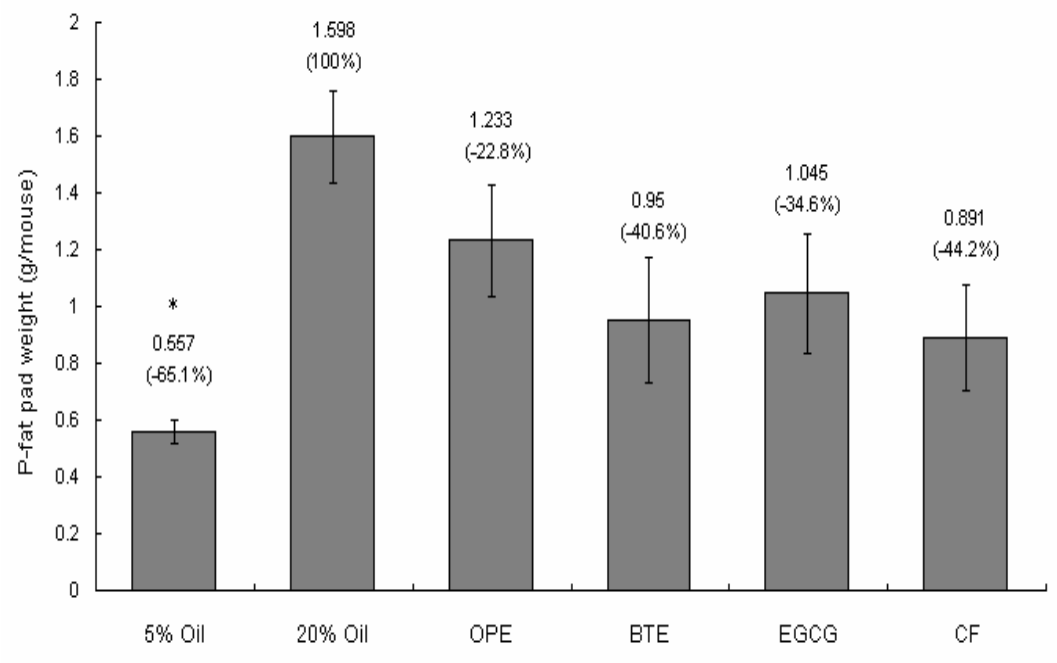


Figure 4.5. Dietary OPE, BTE, EGCG and CF decrease parametrial fat in CF-1 mice

Female CF-1 mice (6 weeks old; 10 mice per group) were fed with high-fat diet (AIN-76A 20% corn oil); 0.2% OPE, 0.2% BTE, 0.1% EGCG, or 0.05% CF in high-fat diet for 10 weeks. The mice were sacrificed and abdominal fats (parametrial fats) were removed and weighed. Data are average weight of parametrial fat for 10 mice (mean±SE). "- %" represents the percent of inhibition of parametrical fat weight in comparison to high-fat diet = (1- parametrial fat weight of selected group/parametrial fat of 20% oil group) X100. *significant different ($p<0.05$).

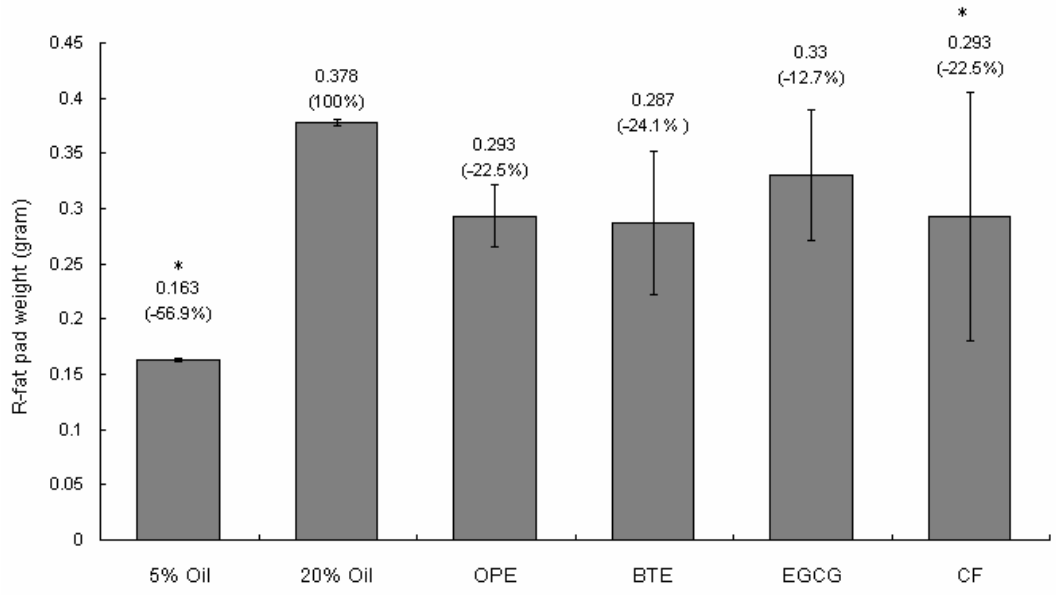


Figure 4.6. Dietary OPE, BTE, EGCG and CF decrease retroperitoneal fat in CF-1 mice

Female CF-1 mice (6 weeks old; 10 mice per group) were fed with high-fat diet (AIN-76A 20% corn oil); 0.2% OPE, 0.2% BTE, 0.1% EGCG, or 0.05% CF in high-fat diet for 10 weeks. The mice were sacrificed and retroperitoneal fats (R-fat) were removed and weighed. Data are average weight of parametrial fat for 10 mice (mean±SE). "- %" represents percent of inhibition of retroperitoneal fat weight in comparison to high-fat diet = (1- R-fat weight of selected group/R-fat weight of 20% oil group) X100. *significant different ($p<0.05$).

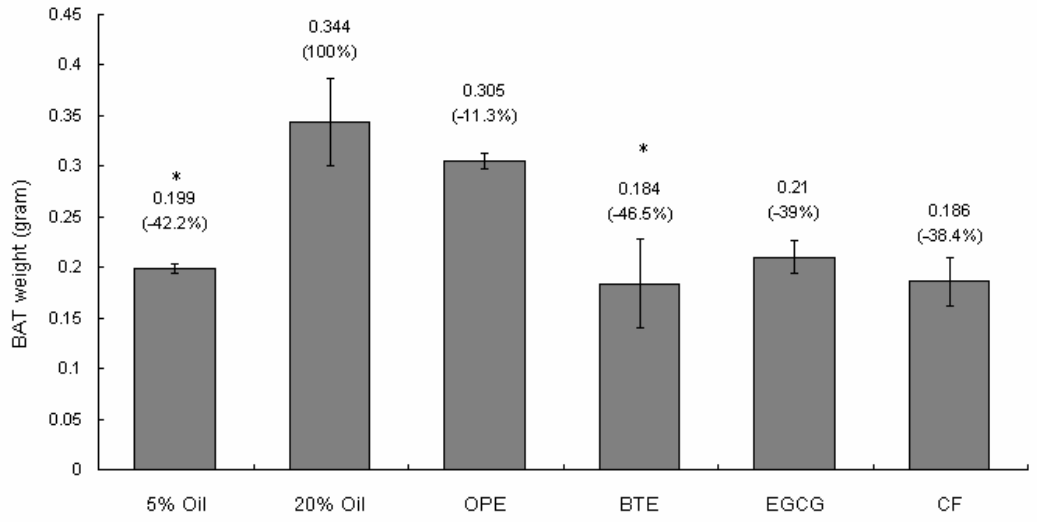


Figure 4.7. Dietary OPE, BTE, EGCG and CF decrease brown adipose tissue in CF-1 mice

Female CF-1 mice (6 weeks old; 10 mice per group) were fed with high-fat diet (AIN76A 20% corn oil); 0.2% OPE, 0.2% BTE, 0.1% EGCG, or 0.05% CF in high fat diet for 10 weeks. The mice were sacrificed and brown adipose tissue (BAT) were removed and weighed. Data are average weight of parametrial fat for 10 mice (mean±SE). ↓ represents percent of inhibition of brown adipose tissue weight in comparison to high-fat diet = (1- BAT weight of selected group/BAT weight of 20% oil group) X100. *significant different ($p<0.05$).

4.1.8. Effects of combinations plant extracts on the body weight gain in CF-1 mice

The body weights of mice administered with different combinations: 0.2% OPE+0.2% BTE, 0.2% OPE+0.1% EGCG, 0.2% OPE+0.05% CF and 0.2% OPE+0.2% BTE+0.05% CF are shown in Figure 4.8 and 4.9. The parameter of parametrial fat pad weight, retroperitoneal fat pad weight and brown adipose tissue weight were measured to elucidate the anti-obesity action of these combinations groups (Figure 4.10; 4.11; 4.12). The body weight gained was significantly decreased by 0.2% OPE + 0.2% BTE, 0.2% OPE + 0.05% CF and 0.2% OPE + 0.2% BTE + 0.05% CF diets from the first week until the end of the experiment, but the 0.2% OPE+0.1% EGCG diet did not inhibit the body weight gain but slightly promoted body weight gain.

4.1.9. The anti-obesity effects of the combination of 0.2% OPE and 0.1% EGCG on the abdominal fat and brown adipose tissue

From our previous result, it showed that 0.2% orange peel extracts has significantly reduced body weight gain by 32%, while 0.1% EGCG also reduced body weight gain by 6.9% (Figure 4.4). To our surprise, the combinations of 0.2% OPE and 0.1% EGCG did not reduce the body weight in mice rather it stimulated body weight gain (Figure 4.8; 4.9). Similar result also shown that the parametrial fat pad weight was increased by 8.2%, while retroperitoneal fat pad weight were increased by 8.2% in mice that were fed with 0.2% OPE + 0.1% EGCG diet (Figure 4.10; 4.11). These results supported that the combinations of orange peel extracts and green tea EGCG may have the antagonist activity on anti-obesity effect.

4.1.10. The anti-obesity effects of the combination of 0.2% OPE and 0.2% BTE on the abdominal fat and brown adipose tissue

The body weight increase was remarkably reduced in the 0.2% OPE + 0.2% BTE group by 43.4% among 0.2% OPE (32% reduction) and 0.2% BTE (35.7% reduction) groups along (Figure 4.9; 4.4). Particularly, the parametrial fat pad weight, retroperitoneal fat pad weight and brown adipose tissue were also significantly inhibited by 56.9%, 45.2% and 42%, respectively, in mice fed on the 0.2% OPE + 0.2% BTE- added diet (Figure 4.10; 4.11; 4.12) compared to mice fed on high-fat diet. To conclude the information, these results suggested that not only the orange peel extracts or the black tea extracts along had anti-obesity abilities, but also the combination group of 0.2% OPE and 0.2% BTE had anti-obesity effect by preventing body weight gain, P-fat pad, R-fat pad and brown adipose tissue accumulation. Furthermore, it was shown that the combination of orange peel extracts and black tea extracts acted better in manifestation of anti-obesity activities than orange peel extract or black tea extract alone.

4.1.11. The anti-obesity effects of the combination of 0.2% OPE and 0.05% CF on the abdominal fat and brown adipose tissue

Like 0.2% OPE + 0.2% BTE group, another combination diet, 0.2% OPE + 0.05% CF, also achieved a better result than 0.2% OPE and 0.05% CF along. The body weight gain was significantly reduced in the 0.2% OPE + 0.05% CF group by 43.4% among 0.2% OPE (32% reduction) and 0.05% CF (41.1% reduction) groups along (Figure 4.9; 4.4). In addition, parametrial fat pad weight, retroperitoneal fat pad weight and brown adipose tissue weight in 0.2% OPE + 0.05% CF- fed mice were remarkably lowered after feeding the diet supplemented with orange peel extracts and

caffeine. These results showed that the administration of 0.2% OPE + 0.05% CF in mice fed on high-fat diet suppressed the P-fat pad weight, R-fat pad weight and brown adipose tissue by 62.3%, 58.2%, and 56.7%, respectively, compared to mice fed on high-fat diet (Figure 4.10; 4.11; 4.12). The anti-obesity effect of 0.2% OPE + 0.05% CF diet in mice fed on high-fat diet has not been observed until the three weeks after feeding the diet might due to the temporarily insensitivity to the effect of 0.2% OPE + 0.05% CF (Figure4.8). This decrease in body weight gain accompanied by the reduction in both white adipose tissue and brown adipose mass supports the anti-obesity action of 0.2% OPE + 0.05% CF. To sum up, these results indicated that administration of orange peel extracts and caffeine work concurrently to generate anti-obesity activities in mice fed on high-fat diet.

4.1.12. The anti-obesity effects of the combination of 0.2% OPE, 0.2% BTE and 0.05% CF on the abdominal fat and brown adipose tissue

Among all these groups, including 0.1% EGCG; 0.2% orange peel extracts (OPE); 0.2% black tea extracts (BTE); 0.05% caffeine (CF); 0.2% OPE and 0.1% EGCG; 0.2% OPE and 0.2% BTE; 0.2% OPE and 0.05%CF in high-fat diet, the administration of 0.2% OPE, 0.2% BTE and 0.05%CF gave the most effective result toward anti-obesity activities in mice fed on high-fat diet over 10 weeks. Addition of 0.2% orange peel extracts, 0.2% black tea extracts and 0.05% caffeine to the high-fat diet significantly decreased the body weight gain by 48.4% as early as the first week after feeding the diet compared to the mice fed on high-fat diet (Figure 4.8; 4.9). Our result also found that mice fed with high-fat diet supplemented daily among the combination of 0.2% OPE, 0.2% BTE and 0.05% CF prevented the parametrial fat pad weight by 88.2%, retroperitoneal fat pad weight by 82.8% and

brown adipose tissue by 63.7% compared to mice fed on high-fat diet (Figure 4.10; 4.11; 4.12). These results indicated that the combination of orange peel extracts, black tea extracts and caffeine have anti-obesity activities by reducing body weight gain, preventing accumulation of P-fat pad weight, R-fat pad weight and brown adipose tissue mass. Although mice were temporarily insensitivity to the effect of 0.2% OPE + 0.05% CF, addition of 0.2% BTE to the diet apparently eliminate the predicament. On the other hand, we demonstrated that not just orange peel extracts acted together with black tea extracts in manifestation of better anti-obesity activities, but also caffeine cooperated with these two compounds to achieve the most effective anti-obesity action.

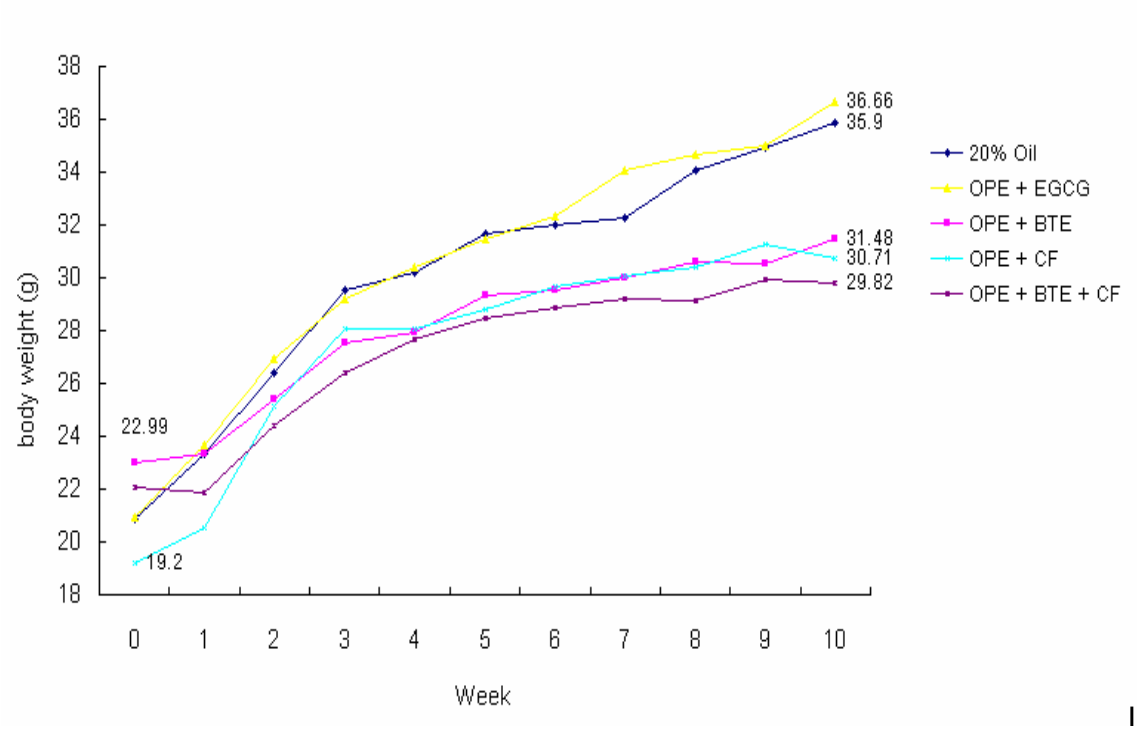


Figure 4.8. Effect of EGCG, OPE, BTE and CF on body weight changing in CF-1 mice

Female CF-1 mice (6 weeks old; 10 mice per group) were given high-fat diet; 0.2% OPE+0.2% BTE, 0.2% OPE+0.1% EGCG, 0.2% OPE+0.05% CF or 0.2% OPE+0.2% BTE+0.05% CF in high fat diet and water *ad libitum* for 10 weeks. Body weights were monitored weekly for 10 weeks. Data are mean±SE (n=10).

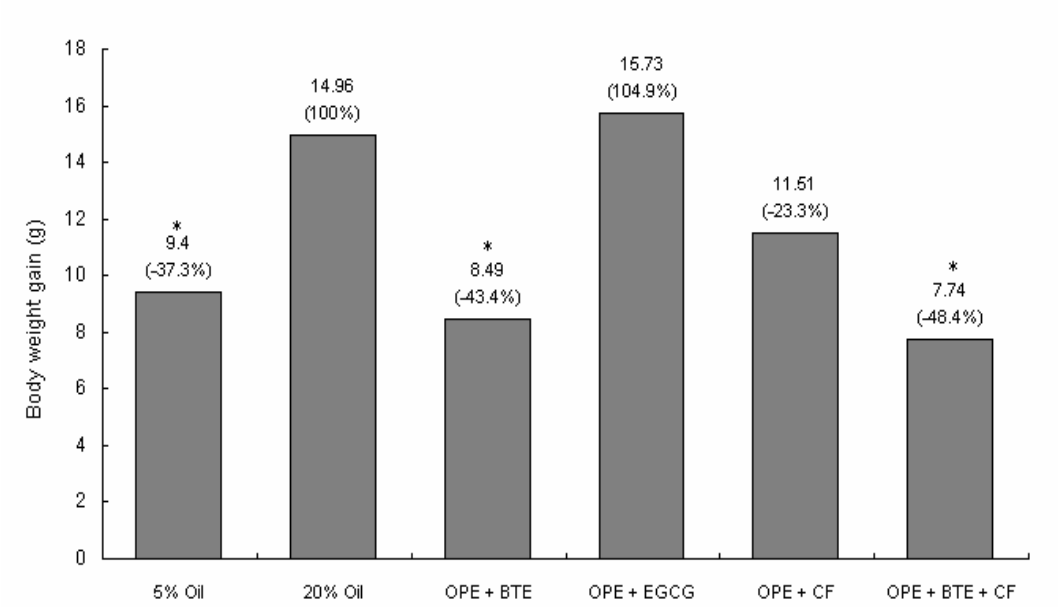


Figure 4.9. Effect of different combinations on body weight gain in mice fed with high-fat diet

Combination groups include 0.2% OPE+0.2% BTE, 0.2% OPE+0.1% EGCG, 0.2% OPE+0.05% CF and 0.2% OPE+0.2% BTE+0.05% CF. Values are mean±SE (n=10 per group). "- %" represents the percentage of inhibition on body weight gain compared to high-fat diet = (1- weight gained of selected group/weight gained of 20% oil group) X100. *significant different ($p<0.05$).

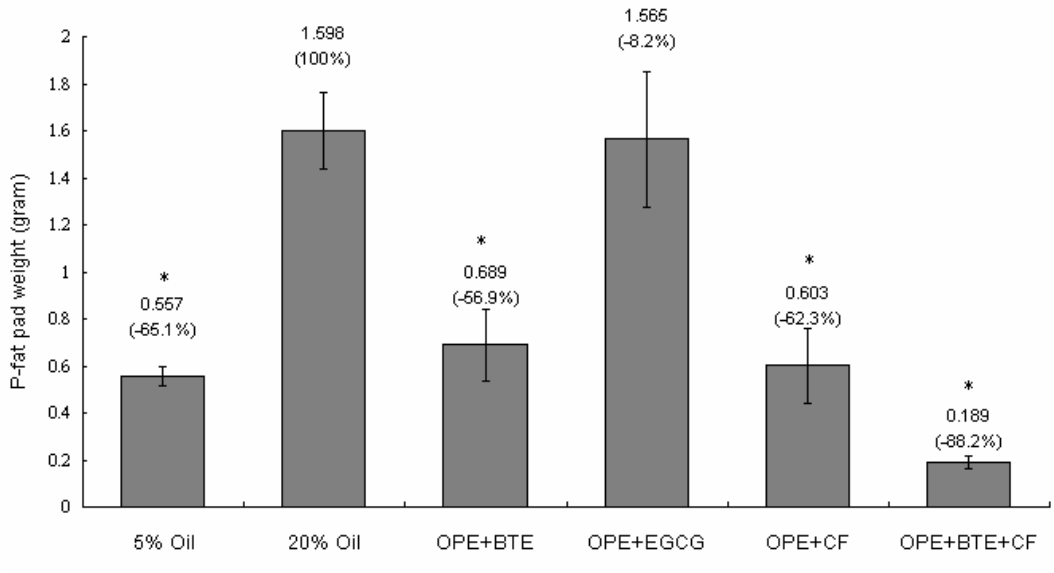


Figure 4.10. Different combinations of OPE, BTE, EGCG and CF decrease parametrial fat in CF-1 mice

Female CF-1 mice (6 weeks old; 10 mice per group) were fed with high-fat diet (AIN76A 20% corn oil); 0.2% OPE+0.2% BTE, 0.2% OPE+0.1% EGCG, 0.2% OPE+0.05% CF or 0.2% OPE+0.2% BTE+0.05% CF in high-fat diet for 10 weeks. The mice were sacrificed and abdominal fats (parametrial fats) were removed and weighed. Data are average weight of parametrial fat for 10 mice (mean±SE). "- %" represents inhibition of parametrial fat pad compared to high-fat diet = (1- P-fat weight of selected group/P-fat weight of 20% oil group) X100. *significant different ($p<0.05$).

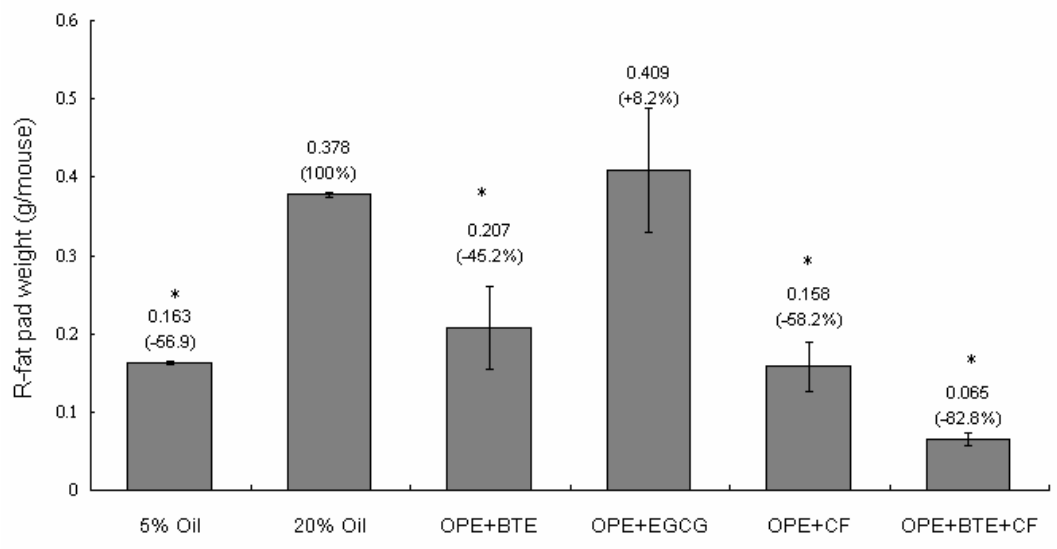


Figure 4.11. Different combinations of OPE, BTE, EGCG and CF decrease retroperitoneal fat in CF-1 mice

Female CF-1 mice (6 weeks old; 10 mice per group) were fed with high-fat diet (AIN76A 20% corn oil); 0.2% OPE+0.2% BTE, 0.2% OPE+0.1% EGCG, 0.2% OPE+0.05% CF or 0.2% OPE+0.2% BTE+0.05% CF in high-fat diet for 10 weeks. The mice were sacrificed and retroperitoneal fats (R-fat) were removed and weighed. Data are average weight of retroperitoneal fat for 10 mice (mean±SE). "- %" represents the inhibition of R-fat pad compared to high-fat diet = (1- R-fat weight of selected group/R-fat weight of 20% oil group) X100. *significant different ($p<0.05$).

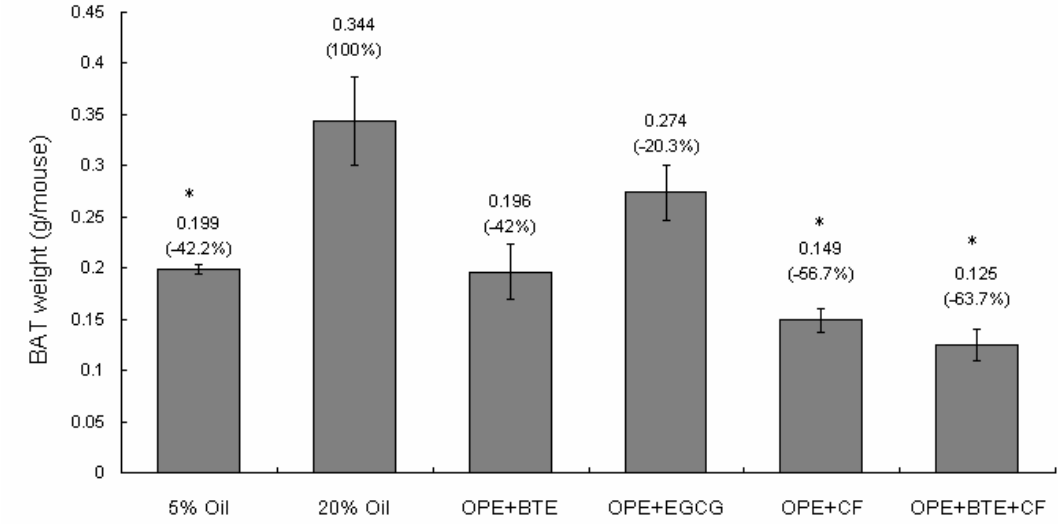


Figure 4.12. Different combinations of OPE, BTE, EGCG and CF decrease brown adipose tissue in CF-1 mice

Female CF-1 mice (6 weeks old; 10 mice per group) were fed with high-fat diet (AIN76A 20% corn oil); 0.2% OPE+0.2% BTE, 0.2% OPE+0.1% EGCG, 0.2% OPE+0.05% CF or 0.2% OPE+0.2% BTE+0.05% CF in high-fat diet for 10 weeks. The mice were sacrificed and brown adipose tissues (BAT) were removed and weighed. Data are average weight of brown adipose tissue for 10 mice (mean±SE). "-%" represents the inhibition of BAT compared to high-fat diet = (1- weight gained of selected group/weight gained of 20% oil group) X100.

4.1.13. Effect of various plant extracts on liver and spleen in mice fed with high-fat diet

In our study, liver weight and spleen weight were examined to determine if these modulated diets have undesirable side effects to the body. In general, enlargement of liver or spleen might be an indicator for pathologic development in body. Figure 4.13 shows the liver weight for these groups, there is no significant difference been observed among all groups. Besides, the spleen weight was also under normal condition for all groups (Figure 4.14).

In summary, we demonstrated that long-term feeding of orange peel extracts, black tea extracts and caffeine are beneficial for the suppression of high-fat diet-induced obesity, and that their effects may be attributed to the inhibition of adipose tissue formation and reduction of adipose tissue mass. Moreover, better results can be achieved by combination of different compounds. In this study, orange peel extracts acted synergistically with black tea extracts and caffeine to generate the most effective anti-obesity action. The results suggest that a sufficient supply of OPE, BTE and CF may prevent or improve obesity and possibly reduce the risk of associated diseases, such as coronary hear disease.

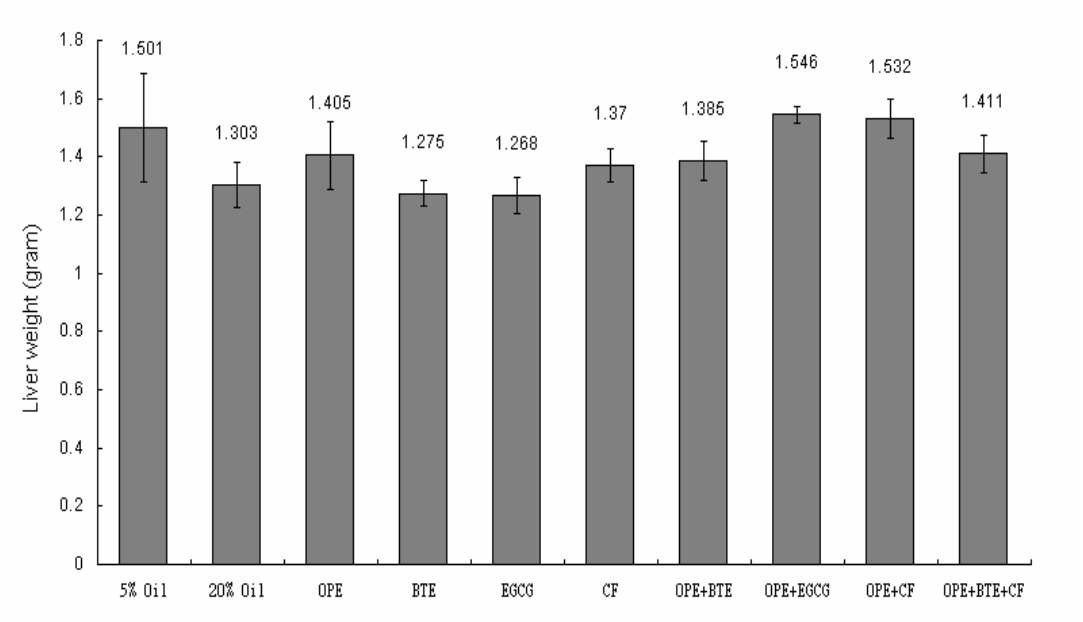


Figure 4.13. Effect of various plant extracts on liver in mice fed with high-fat diet

Female CF-1 mice (6 weeks old; 10 mice r per group) were given a low-fat diet (AIN76A 5% corn oil), a high-fat diet (AIN76A 20% corn oil), 0.2% OPE, 0.2% BTE, 0.05%CF in high fat diet or in combination of 0.2% OPE + 0.2% BTE, 0.2%OPE + 0.1%EGCG, 0.2%OPE +0.05%CF or 0.2%OPE + BTE + 0.05%CF in high-fat diet. Livers were weighed after 10 weeks of feeding the various diets. Data are mean±SE (n=10). *significant different ($p<0.05$).

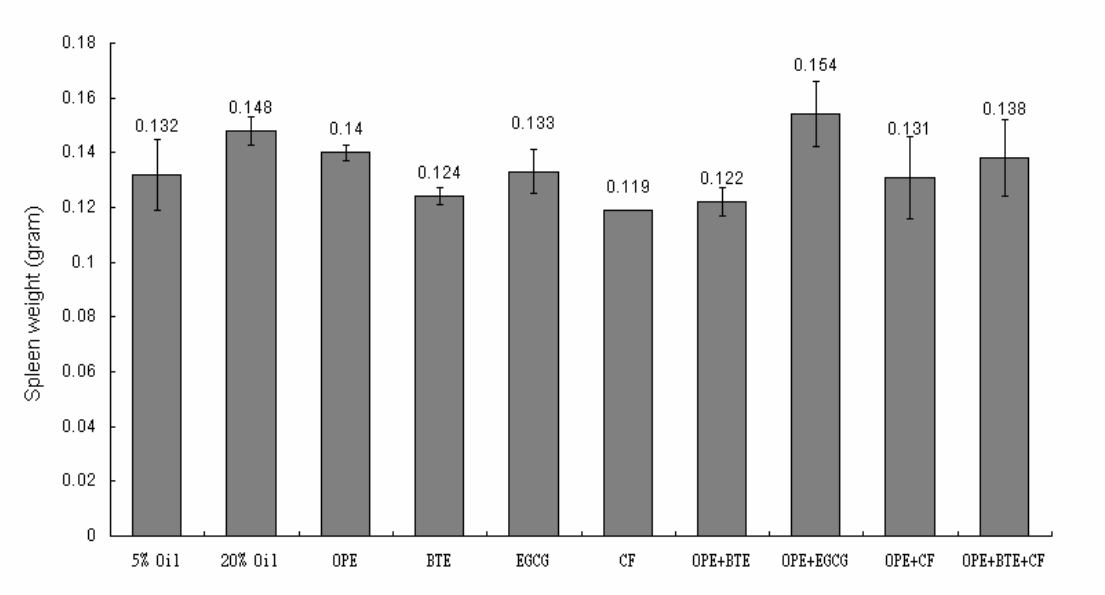


Figure 4.14. Effect of various plant extracts on spleen in mice fed with high-fat diet

Female CF-1 mice (6 weeks old; 10 mice r per group) were given a low-fat diet (AIN76A 5% corn oil), a high-fat diet (AIN76A 20% corn oil), 0.2% OPE, 0.2% BTE, 0.05%CF in high-fat diet or in combination of 0.2% OPE + 0.2% BTE, 0.2%OPE + 0.1%EGCG, 0.2%OPE +0.05%CF or 0.2%OPE + BTE + 0.05%CF in high-fat diet. Spleens were weighed after 10 weeks of feeding the various diets. Data are mean±SE (n=10). *significant different ($p<0.05$)

4.2. Experiment 2

4.2.1. Effects of high-fat diet and low-fat diet on body weight gain, abdominal fat size, lipid profile, glucose, and leptin levels

Although low-fat diet group (5% corn oil in AIN-76A diet) had higher food intake compared to the high-fat diet (20% corn oil in AIN-76A diet) group, the level of body weight gain, parametrial fat pad weight, retroperitoneal fat pad weight, and brown adipose tissue in mice fed on the high-fat diet were significantly increased compared to mice fed on the low-fat diet over 17 weeks (Table 4.4). Furthermore, the lipid profile, including High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), total cholesterol level, blood glucose and blood leptin level were significantly increased in mice fed on high-fat diet. The results clearly demonstrated that body weight gain, lipid profile and fat accumulation in mice were remarkably increased by the high-fat diet (Table 4.4).

4.2.2. Effects of various orange peel extracts on the body weight gain in mice fed on high-fat diet

Figure 4.15 shows the effect of orange peel extracts (WG 361) on body weight gain in mice fed on the high-fat diet. Addition of 0.2% OPE-WG 361, 0.01% OPE-WG 361, 0.05% OPE- WG 361 and 0.10% OPE-WG 361 in high-fat diets reduced the body weight gain by 14%, 9%, 11% and 23%, respectively (Figure 4.15), while addition of 0.03% OPE-WG 361 slightly promote the body weigh gain by 2% in mice fed on high-fat diet. Unlike 0.10% OPE-WG 362, high-fat diet that contained 0.01% OPE- WG 362 significantly suppressed body weight gain by 18% and 0.03% OPE-WG 362 also prevented body weight gain by 7% compared to mice fed on high-fat diet (Figure 4.16). In addition, different concentrations of orange peel extracts- WG

363 on body weight gain in mice fed on high-fat diet for 17 weeks were shown in Figure 4.17. The body weight gain were remarkably decreased by 0.03% OPE- WG 363 about 27%, dietary supplement of 0.01% OPE- WG 363 and 0.10% OPE- WG 363 also suppressed the body weight gain by 5% and 2%, respectively (Figure 4.17).

These results suggested that mice fed with high-fat diet supplemented daily with orange peel extracts had anti-obesity effect by suppressing body weight gain without a dose-dependent manner. Thus, we selected 0.2% OPE- WG 361, 0.01% OPE- WG 362 and 0.03% OPE- WG 363 groups which have the most effective anti-obesity activities to do further analysis.

	5% Corn oil AIN-76A diet	20% Corn oil AIN-76A diet
Initiated body weight (gram)	25.645 ± 1.50	26.09 ± 0.42
End body weight (gram)	40.17 ± 2.54	44.67 ± 2.31
Body weight gaining (gram)	14.525 ± 2.45	18.58 ± 2.01
Food intake (gram/mouse/day)	3.70 ± 0.07	3.36 ± 0.08
Parametrial fat pad weight (gram)	1.60 ± 0.34	2.48 ± 0.31
Retroperitoneal fat pad weight (gram)	0.65 ± 0.12	0.88 ± 0.16
Brown adipose tissue (gram)	0.56 ± 0.08	0.53 ± 0.06
HDL (mg/dL)	32.7 ± 5.49	51.4 ± 8.08
LDL (mg/dL)	32.96	62.26
Total cholesterol (mg/dL)	85.3 ± 1.96	132.5 ± 2.89
Triglyceride (mg/dL)	98.2 ± 16.65	94.2 ± 23.10
Glucose (mg/dL)	101.1 ± 7.22	124.9 ± 2.96
Leptin (ng/dL)	5.31 ± 0.68	8.70 ± 0.91
Liver weight (gram)	1.75 ±0.72	1.62 ± 0.06

Table 4.4. Effects of high-fat diet and low-fat diet on body weight gain, abdominal fat, blood lipid levels, blood glucose and leptin level in CF1 mice

Female CD-1 mice (5 weeks old; 10 mice per group) were given a low-fat diet (AIN-76A 5% corn oil) or a high-fat diet (AIN-76A 20% corn oil) and water *ad libitum* for 17 weeks. Food and water consumption, body weight were monitored weekly. After 17 weeks on the special diets, the mice were sacrificed. Parametrical fat, retroperitoneal fat and brown fat as well as liver were removed and weighed. Data are mean ±SE (n=10).

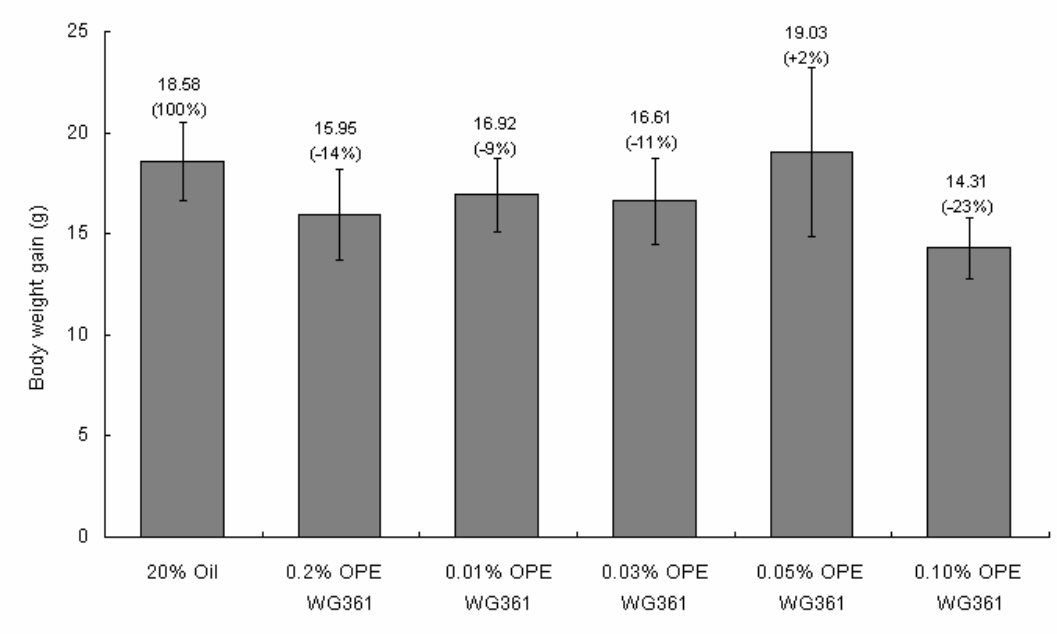


Figure 4.15. Effect of OPE - WG361 on body weight gain in mice fed on high-fat diet

Female CD-1 mice (5 weeks old; 10 mice per group) were fed with high-fat diet (AIN-76A 20% corn oil), and different concentration of orange peel extract – WG361 for 17 weeks. Body weights were monitored weekly. Data are mean±SE (n=10). Body weight gain= (Body weight at 17th week – body weight at 0 week). "- %" represents the percentage of inhibition on body weight gain by OPE – WG361 compared to high-fat diet = (1- weight gained of selected group/weight gained of 20% oil group) X100.

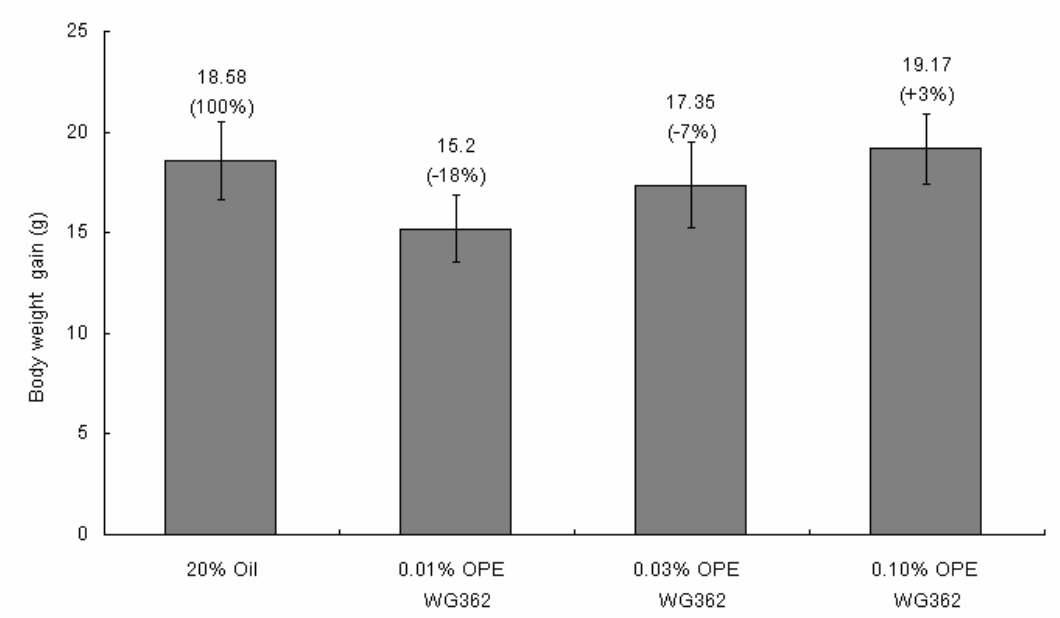


Figure 4.16. Effect of OPE - WG362 on body weight gain in mice fed on high-fat diet

Female CD-1 mice (5 weeks old; 10 mice per group) were fed with high-fat diet (AIN-76A 20% corn oil), and different concentration of orange peel extract – WG362 for 17 weeks. Body weights were monitored weekly. Data are mean±SE (n=10). Body weight gain= (Body weight at 17th week – body weight at 0 week). "- %" represents the percentage of inhibition on body weight gain by OPE – WG362 compared to high-fat diet = (1- weight gained of selected group/weight gained of 20% oil group) X100.

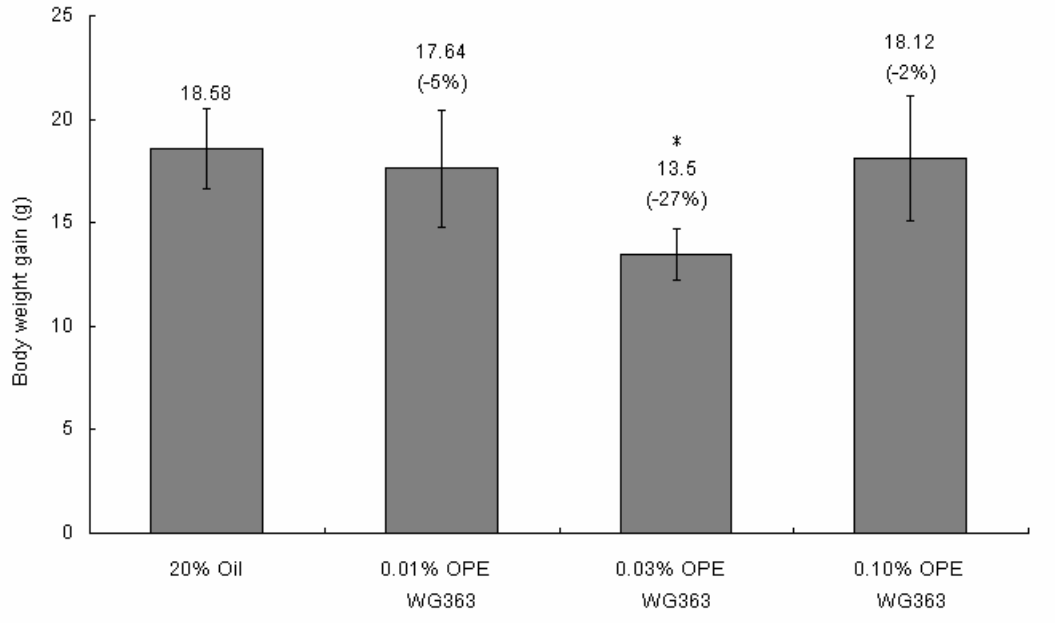


Figure 4.17. Effect of OPE - WG363 on body weight gain in mice fed on high-fat diet

Female CD-1 mice (5 weeks old; 10 mice per group) were fed with high-fat diet (AIN-76A 20% corn oil), and different concentration of orange peel extract – WG363 for 17 weeks. Body weights were monitored weekly. Data are mean±SE (n=10). Body weight gain= (Body weight at 17th week – body weight at 0 week). "- %" represents the percentage of inhibition on body weight gain by OPE – WG363 compared to high-fat diet = (1- weight gained of selected group/weight gained of 20% oil group) X100.

4.2.3. Effect of feeding various orange peel extracts on food intake in CD-1 mice

Figure 4.18 shows the effect of selected orange peel extracts on food intake in mice fed on the high-fat diet and low-fat diet, there is no significant difference among all groups, including 0.2% OPE- WG 361 and 0.03% OPE- WG 363 in high-fat diet. Only administration of 0.01% OPE- WG 362 lowed the average food intake in mice fed on high-fat diet (Figure 4.18). In other word, addition of orange peel extracts in the high-fat diets did not influence the mice appetite as well as energy intake remains debated.

4.2.4. The anti-obesity effects of various orange peel extracts on the abdominal fat and brown adipose tissue

High-fat diet that contained 0.2% OPE- WG 361, 0.01% OPE- WG 362 and 0.03% OPE- WG 363 significantly suppressed body weight gain by 14%, 18% and 27%, respectively, compared to mice fed on high-fat diet (Figure 4.15, Figure 4.16, Figure 4.17, respectively). The effect of orange peel extracts on parametrial fat pad were shown in Figure 4.19: 0.2% OPE- WG 361 lowered the P-fat pad weight by 29%, 0.01% OPE- WG 362 lowered the P-fat pad weight by 16%, and 0.03% OPE-WG 363 lowered the P-fat pad weight by 38% (Figure 4.19). Although the 0.2% OPE- WG 361 diet remarkably reduced the P-fat pad, the diet did not have influence on retroperitoneal fat pad in mice. Unlike 0.2% OPE- WG 361 group, 0.01% OPE- WG 362 and 0.03% OPE- WG 363 significantly suppressed the retroperitoneal fat pad weight by 22% and 23%, respectively (Figure 4.20). The effect of orange peel extracts on adipose tissue can also been observed in brown adipose tissue. At the 17 weeks, feeding 0.2% OPE- WG 361, 0.01% OPE- WG 362 and 0.03% OPE- WG 363 had inhibited the brown adipose tissue about 9%, 13%, and 19%, respectively, as

compared to the high-fat diet group (Figure 4.21). These results suggested that mice fed with high-fat diet supplemented daily with orange peel extracts had anti-obesity effect, which suppresses the accumulation of adipose tissue and body weight gain in CD-1 mice.

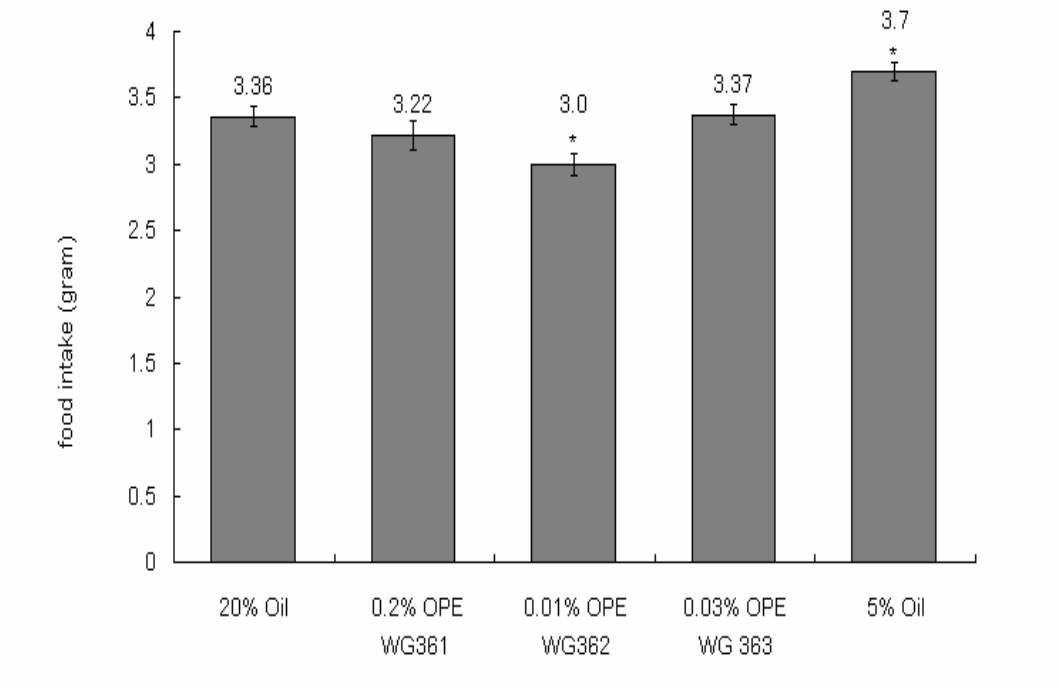


Figure 4.18. Monitoring daily food intakes in mice fed on orange peel extract in the high-fat diets

Female CD-1 mice (5 weeks old; 10 mice per group) were given a low-fat diet (AIN-76A 5% corn oil), a high fat-diet (AIN-76A 20% corn oil) or high-fat diet contained 0.2% OPE-WG361, 0.01%OPE-WG362, or 0.03%OPE-WG363 and water *ad lititum* for 17 weeks. Food intake was monitored weekly. Data are mean±SE (n=10). *significant different ($p<0.05$).

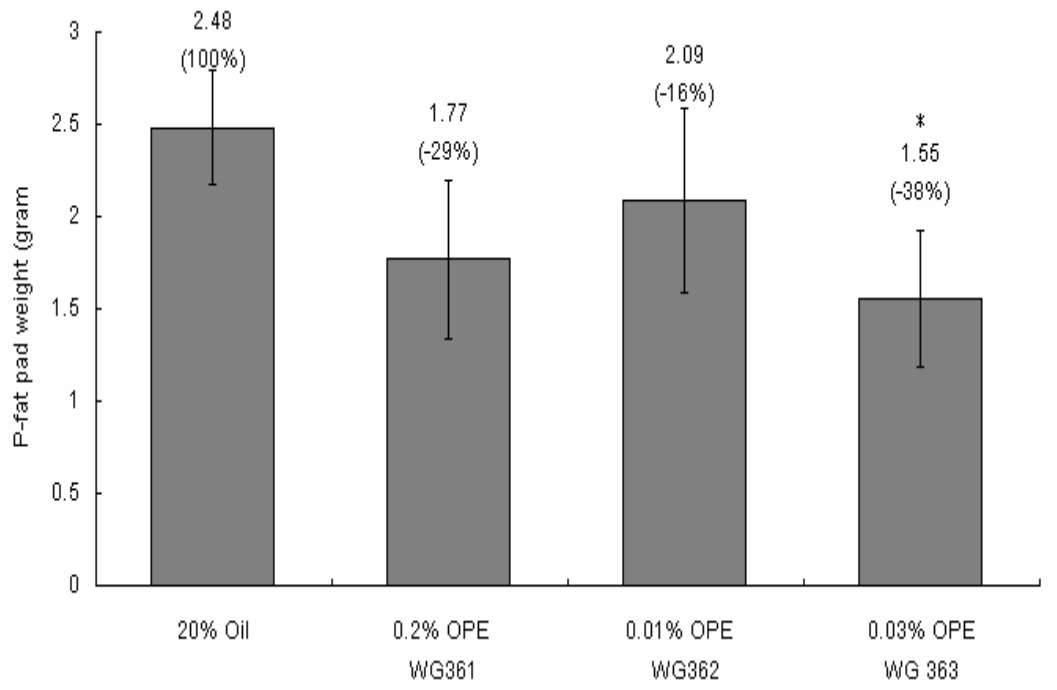


Figure 4.19. Dietary OPE decreases parametrial fat (P-fat) in CD-1 mice

Female CD-1 mice (5 weeks old; 10 mice per group) were fed with high-fat diet (AIN76A 20% corn oil), or 0.2% OPE-WG361, 0.01%OPE-WG362, or 0.03%OPE-WG363 in high-fat diet for 17 weeks. The mice were sacrificed and abdominal fats (parametrial fats) were removed and weighed. Data are average weight of parametrial fat for 10 mice (mean±SE). "- % " represents the percentage of inhibition on P-fat mass by OPE compared to high-fat diet = (1- weight gained of selected group/weight gained of 20% oil group) X100. *significant different ($p<0.05$).

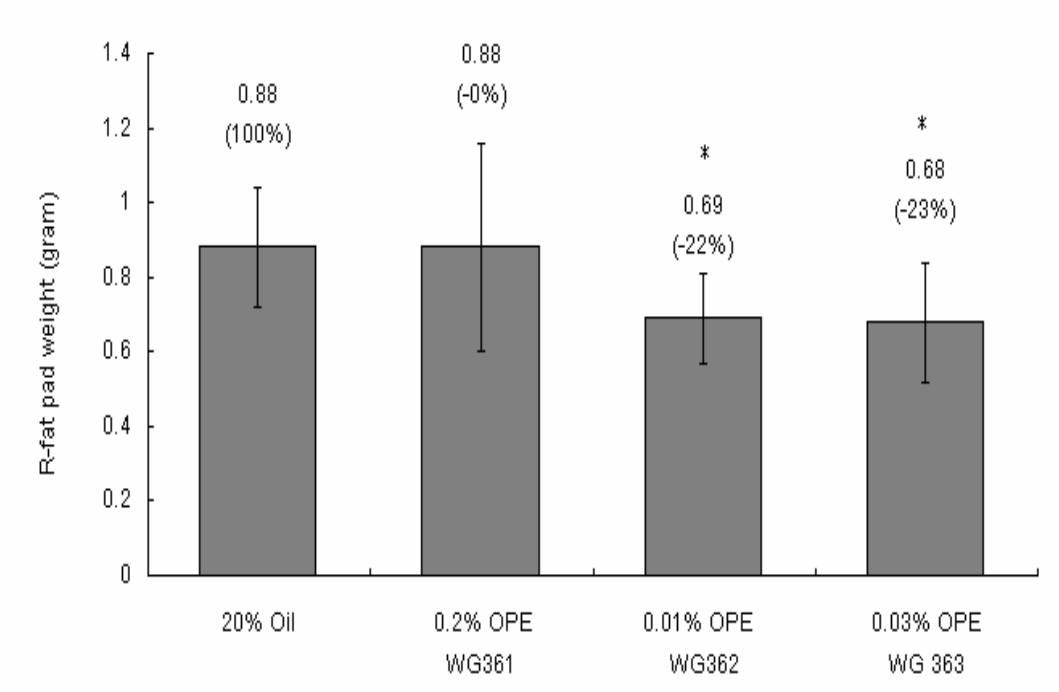


Figure 4.20. Dietary OPE decreases retroperitoneal fat (R-fat) in CD-1 mice

Female CD-1 mice (5 weeks old; 10 mice per group) were fed with high-fat diet (AIN76A 20% corn oil), or 0.2% OPE-WG361, 0.01% OPE-WG362, or 0.03% OPE-WG363 in high-fat diet for 17 weeks. The mice were sacrificed and retroperitoneal fat were removed and weighed. Data are average weight of retroperitoneal fat for 10 mice (mean±SE). "- % " represents the percentage of inhibition on R- fat pad mass by OPE compared to high-fat diet = (1- weight gained of selected group/weight gained of 20% oil group) X100. *significant different ($p<0.05$).

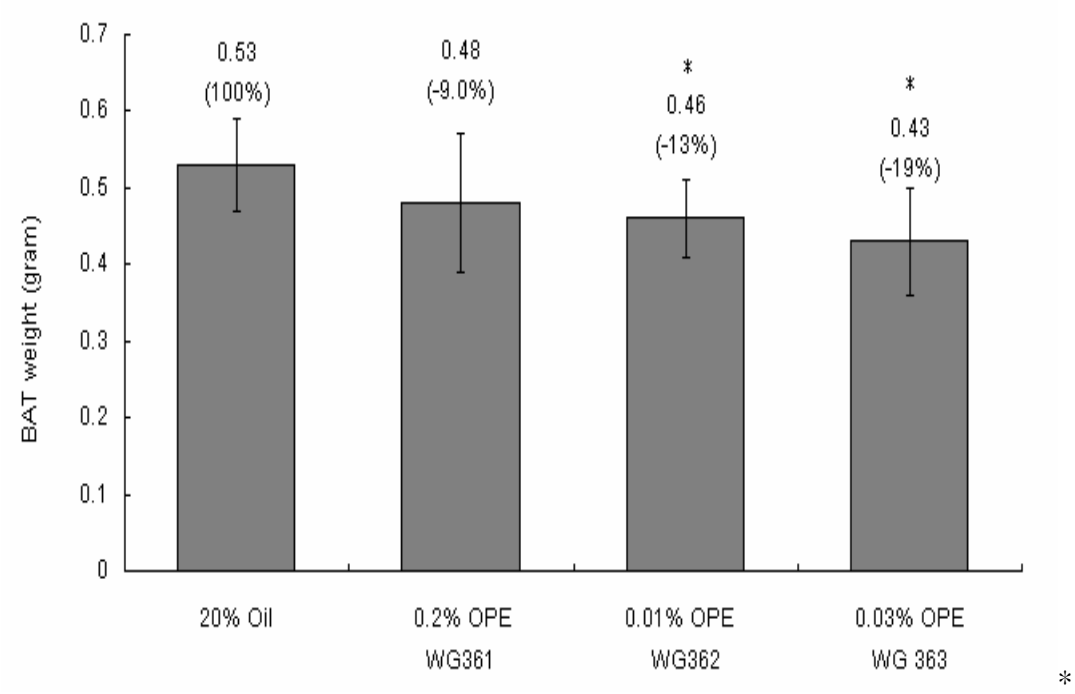


Figure 4.21. Dietary OPE decreases brown adipose tissue mass (BAT) in CD-1 mice

Female CD-1 mice (5 weeks old; 10 mice per group) were fed with high-fat diet (AIN76A 20% corn oil), or 0.2% OPE-WG361, 0.01% OPE-WG362, or 0.03% OPE-WG363 in high-fat diet for 17 weeks. The mice were sacrificed and brown adipose tissue were removed and weighed. Data are average weight of BAT for 10 mice (mean±SE). "- % " represents the percentage of inhibition on BAT mass by OPE compared to high-fat diet = (1- weight gained of selected group/weight gained of 20% oil group) X100.
*significant different ($p<0.05$).

4.2.5. The anti-obesity effects of various orange peel extracts on blood lipid levels

Figure 4.22 illustrates blood triglycerides levels of mice fed on different orange peel extracts for a 17 weeks period. In the 0.2% OPE-WG 361, 0.01% OPE-WG 362 and 0.03% OPE-WG 363 feeding, about 18%, 15%, and 13%, respectively, decreased in blood triglycerides levels were observed after 17 weeks (Figure 4.22). The results in Figure 4.23 showed the effects of 0.2% OPE-WG 361, 0.01% OPE-WG 362 and 0.03% OPE-WG 363 on total cholesterol levels in mice fed with high-fat diet. Addition of 0.2% OPE-WG 361, 0.01% OPE-WG 362 and 0.03% OPE-WG 363 in high-fat diets reduced the total cholesterol levels by 7%, 31% and 30%, respectively (Figure 4.23). Moreover, the levels of HDL-Cholesterol were significantly decreased by 0.2% OPE-WG 361, 0.01% OPE-WG 362 and 0.03% OPE-WG 363 about 29%, 25%, and 48% lower than the high-fat diet, respectively (Figure 4.24). Figure 4.25 shows the effect on LDL-Cholesterol after feeding different kinds of orange peel extracts in mice after 17 weeks. The level of LDL in 0.2% OPE-WG 361 group was increased about 14% at the 17th week but remarkably decreased by 0.01% OPE-WG 362 and 0.03% OPE-WG 363 diets about 40% and 21%, respectively (Figure 2.25). It was observed that with an increase of the body weight and the abdominal fat, there was also an increase of the triglycerides, total cholesterol, HDL and LDL levels in blood circulation. The important role of the cholesterol and lipoprotein concentrations in coronary heart disease has been reported by many scientists. Excessive adipose tissue and high levels of cholesterol, LDL and triglyceride lead to a high risk for the development of coronary heat disease and metabolic syndrome (Kuo *et al*., 2005). In conclusion, dietary supplement of orange peel extracts can lower the level of triglyceride, total cholesterol as well as lower the level of LDL and HDL in mice. These results strongly suggested orange peel

extracts exerted a hypolipidemic effect and might acted as a chemopreventive agent for obesity.

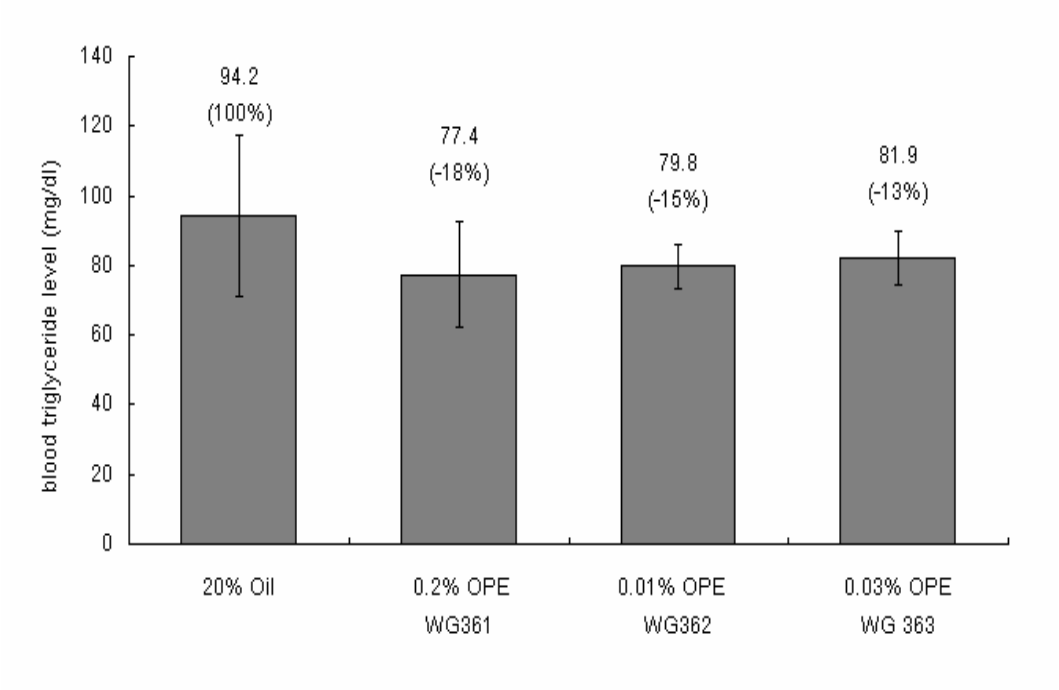


Figure 4.22. Effect of orange peel extracts on blood triglyceride level in mice fed on a high-fat diet over 17 weeks

Groups include 0.2% OPE-WG361, 0.01% OPE-WG362, and 0.03% OPE-WG363 in high-fat diet. Values are mean±SE (n=10 per group). "- %" represents the percentage of inhibition on blood triglyceride level by OPE compared to high-fat diet = (1-triglyceride level of selected group/triglyceride level of 20% oil group) X100. *significant different ($p<0.05$).

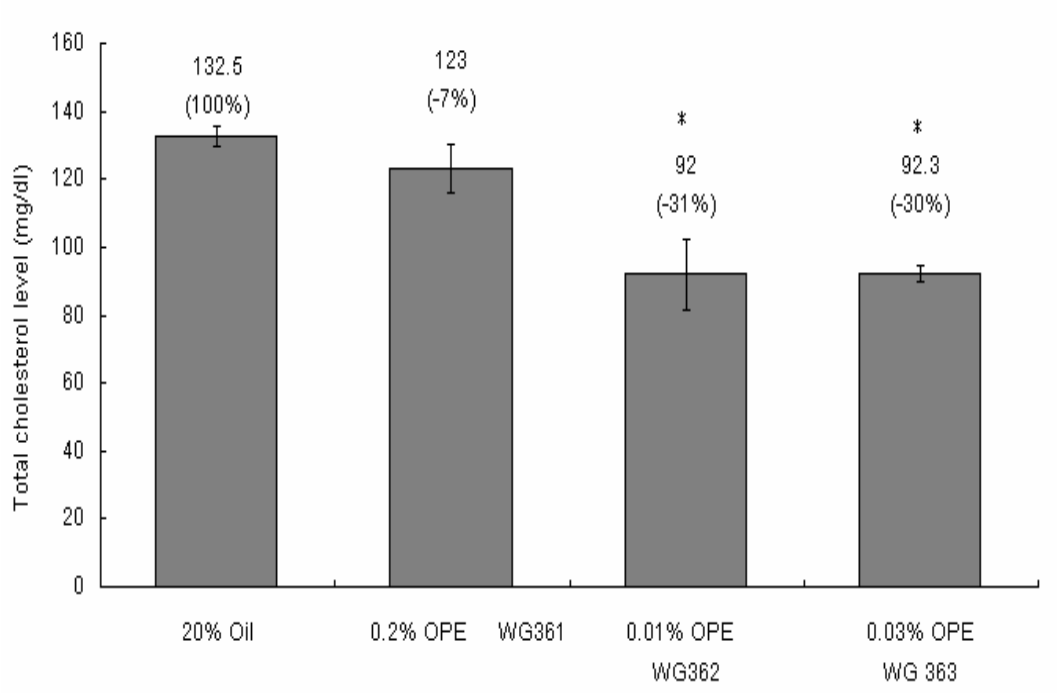


Figure 4.23. Effect of orange peel extracts on blood total cholesterol level in mice fed on a high-fat diet over 17 weeks

Groups include 0.2% OPE-WG361, 0.01% OPE-WG362, and 0.03% OPE-WG363 in high-fat diet. Values are mean±SE (n=10 per group). "- %" represents inhibition of blood total cholesterol compared to high-fat diet = (1- cholesterol level of selected group/cholesterol level of 20% oil group) X100. *significant different ($p<0.05$).

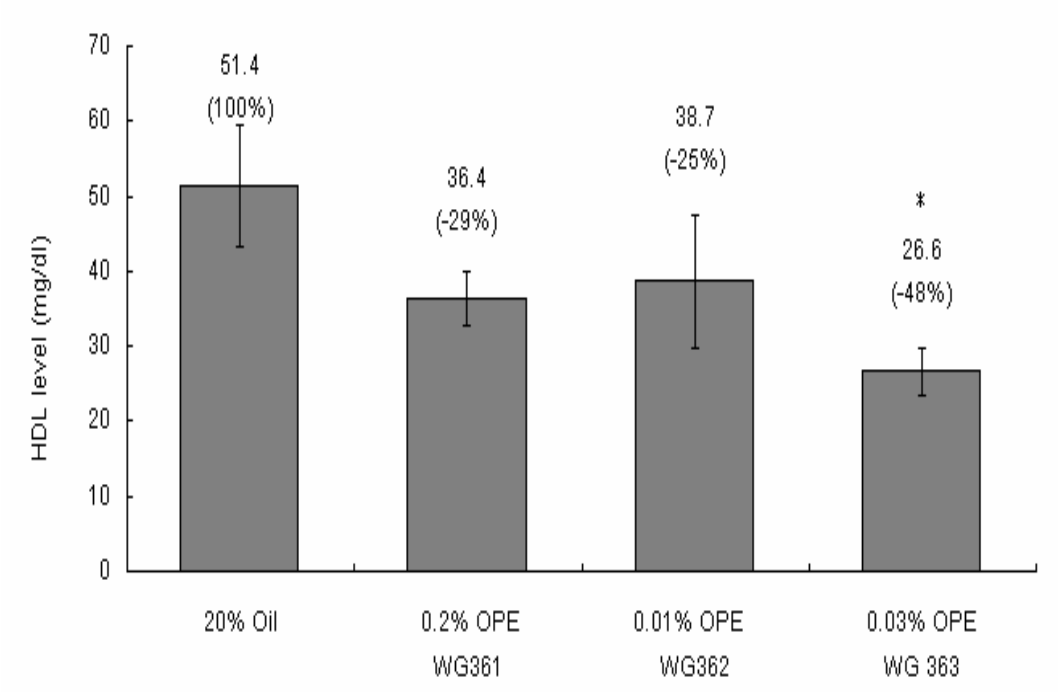


Figure 4.24. Effect of orange peel extracts on blood HDL level in mice fed on a high-fat diet over 17 weeks

Groups include 0.2% OPE-WG361, 0.01% OPE-WG362, and 0.03% OPE-WG363 in high-fat diet. Values are mean±SE (n=10 per group). "- %" represents the percentage of inhibition on blood HDL level by OPE compared to high-fat diet = (1- HDL level of selected group/HDL level of 20% oil group) X100. *significant different ($p<0.05$).

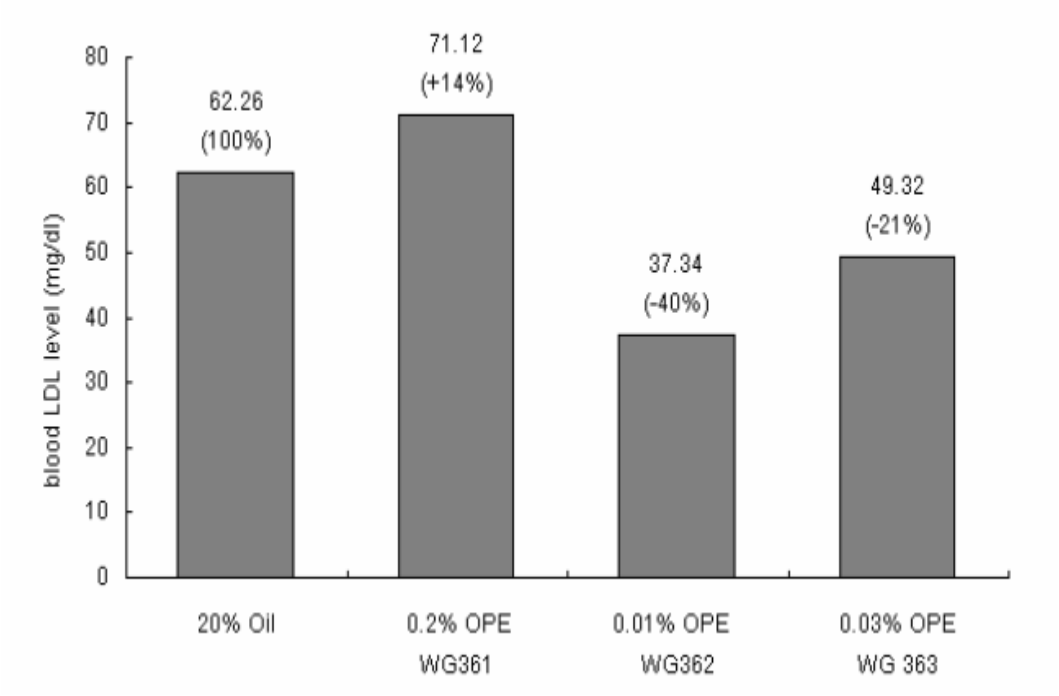


Figure 4.25. Effect of orange peel extracts on blood LDL level in mice fed on a high-fat diet over 17 weeks

Groups include 0.2% OPE-WG361, 0.01% OPE-WG362, and 0.03 % OPE-WG363 in high-fat diet. Values are mean±SE (n=10 per group). "- %" represents the percentage of inhibition on blood LDL level by OPE compared to high-fat diet = (1- LDL level of selected group/LDL level of 20% oil group) X100.

4.2.6. The effects of various orange peel extracts on blood glucose level in CD-1 mice

Figure 4.26 shows the effect on blood glucose after feeding different kinds of orange peel extracts after 17 weeks. The level of blood glucose in 0.2% OPE-WG 361 group was slightly decrease about 2% at the 17th week but significantly decreased by 0.01% OPE-WG 362 and 0.03% OPE-WG 363 diet about 20% and 19%, respectively (Figure 2.26). In conclusion, administration of dietary orange peel extracts intake substantially decreases diet-induced obesity, hyperglycemia, and hypercholesterolemia in mice without a dose-dependent manner.

4.2.7. The effects of various orange peel extracts on blood leptin level in CD-1 mice

Research has shown that serum leptin concentrations were higher in obese individuals and leptin concentrations were increased with increasing body fat (Campfield, 1999; Campfield *et al*., 1998; Maffei *et al*., 1995). In obese humans, blood leptin levels generally correlate with the amount of fat stored in the body (Bays, 2004). The effect of orange peel extracts on serum leptin can also been observed in Figure 4.27. At the 17 weeks, feeding 0.2% OPE-WG 361 had slightly lowered the serum leptin level by 10% and 0.01% OPE-WG 362 and 0.03% OPE-WG 363 had remarkably lowered the serum leptin level by 70% and 42% as compared to the high-fat diet group, respectively (Figure 4.27).

4.2.8. The effects of various orange peel extracts on liver in CD-1 mice

Liver weight was examined to determine if these modulated diets have undesirable side effects to the body. In general, enlargement of liver or spleen might

be an indicator for pathologic development in body. Figure 4.28 shows the liver weight for these groups, there is no significant difference/enlargement been observed among all groups.

Finally, our results showed that feeding of different degrees of orange peel extracts to mice fed high-fat diet results in the reduction of body weight, white adipose tissue, brown adipose tissue as well as lipid profile includes total cholesterol, triglyceride, LDL and HDL in blood. Furthermore, long term consumption of dietary orange peel extracts intake for 17 weeks considerably decreases diet-induced obesity, hyperglycemia, and hypercholesterolemia in mice. These results strongly suggested that orange peel extracts exerted a hypolipidemic effect and therefore might have a protective effect against the obesity risks and its complications.

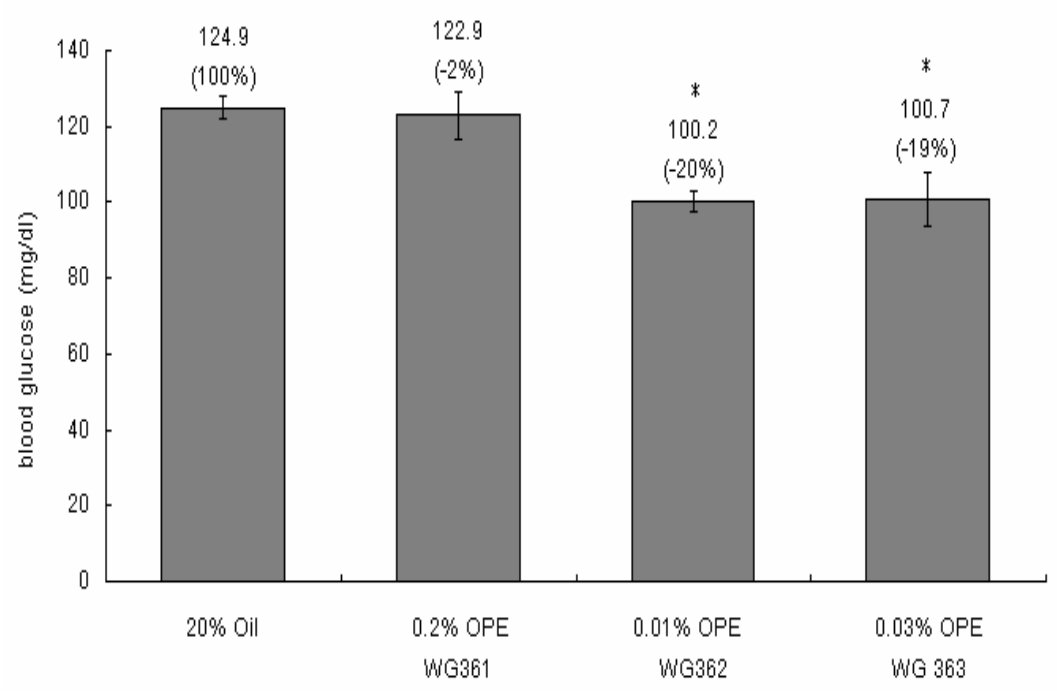


Figure 4.26. Effect of orange peel extracts on blood glucose level in mice fed on a high-fat diet over 17 weeks

Groups include 0.2% OPE-WG361, 0.01% OPE-WG362, and 0.03% OPE-WG363 in high-fat diet. Values are mean±SE (n=10 per group). "- %" represents the percentage of inhibition on blood glucose level by OPE compared to high-fat diet = (1- glucose level of selected group/glucose level of 20% oil group) X100. *significant different ($p<0.05$).

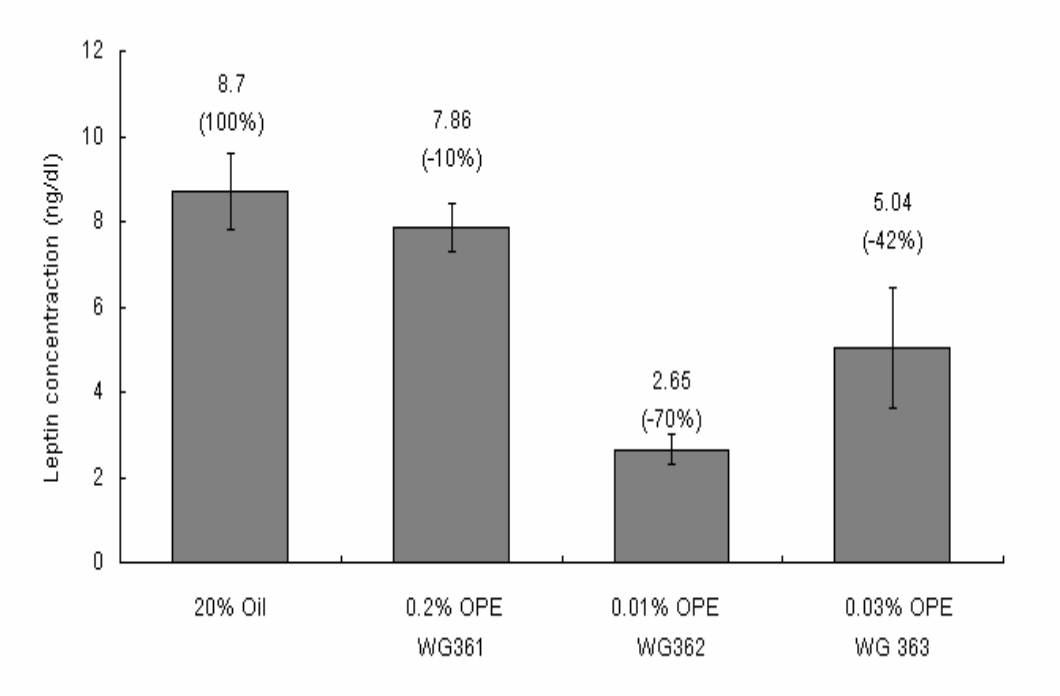


Figure 4.27. Effect of orange peel extracts on blood leptin level in mice fed on a high-fat diet over 17 weeks

Groups include 0.2% OPE-WG361, 0.01% OPE-WG362, and 0.03% OPE-WG363 in high-fat diet. Values are mean±SE (n=10 per group). "- %" represents the percentage of inhibition on blood leptin level by OPE compared to high-fat diet = (1- leptin level of selected group/leptin level of 20% oil group) X100.

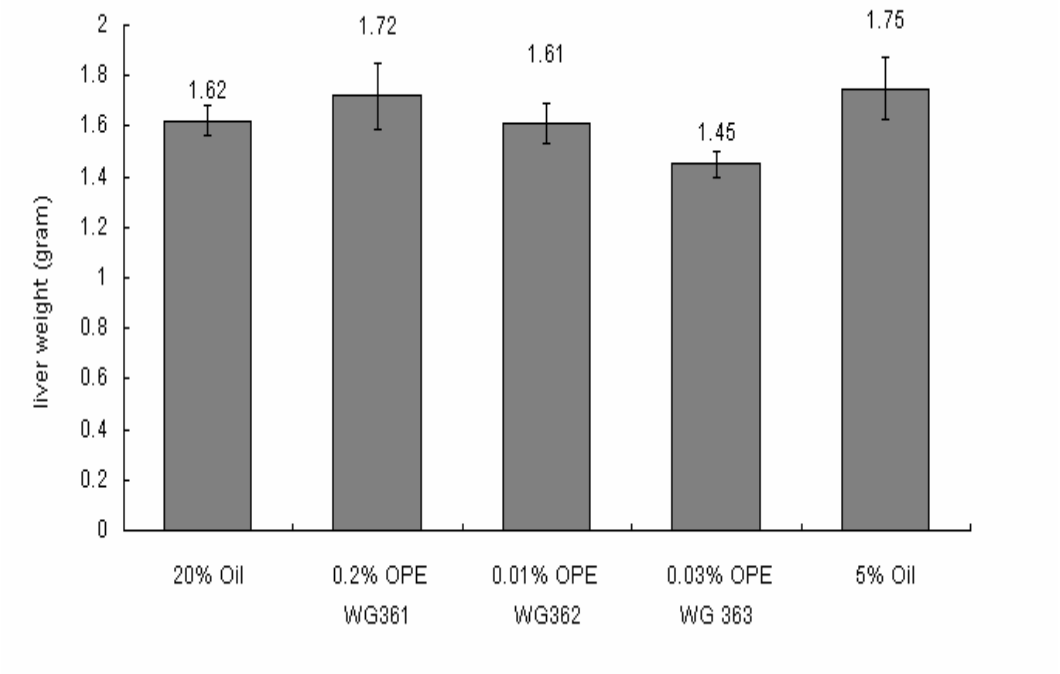


Figure 4.28. Effect of orange peel extracts on liver in mice fed with high-fat diet

Female CD-1 mice (5 weeks old; 10 mice per group) were given a low-fat diet (AIN-76A 5% corn oil), a high-fat diet (AIN-76A 20% corn oil), or high-fat diet contain 0.2% OPE-WG361, 0.01% OPE-WG362, or 0.03% OPE-WG363. Livers were weighed after 17 weeks of feeding the various diets. Data are mean±SE (n=10). *significant different ($p<0.05$).

5. REFERENCES

http://www.cdc.gov/nccdphp/dnpa/obesity/index.htm CDC

Arciero, P. J.; Gradner, A. W.; Calles-Escandon, J.; Benowitz, N. L.; Poehlman, E. T. Effects of caffeine ingestion on NE kinetics, fat oxidation, and energy expenditure in younger and older men. *Am. J. Physiol.* **1995**, *268*, E1192-1198.

Astrup, A.; Toubro, S. Thermogenic, metabolic, and cardiovascular responses to ephedrine and caffeine in man. *Int. J. Obes. Relat. Metab. Disord.* **1993**, *17*(Suppl1), S41 – S43.

Astrup, A.; Toubro, S.; Cannon, S.; Hein, P.; Breum, L.; Madsen, J. Caffeine: a double-blind, placebo-controlled study of its thermogenic, metabolic, and cardiovascular effects in healthy volunteers. *Am. J. Clin. Nutr.* **1990**, *51*, 759 – 767.

Bays, H.; Blonde, L.; Rosenson, R. Adiposopathy: how do diet, exercise and weight loss drug therapies improve metabolic disease in overweight patients? *Expert. Rev. Cardiovasc. Ther.* **2006**, *4(6)*, 871-895.

Bays, Harold E. Current and investigational antiobesity agents and obesity therapeutic treatment targets. *Obesity research* **2004**, *12*, 1197-1211.

Borradaile, N. M.; Carroll, K. K.; Kurowska, E. M. Regulation of HepG2 cell apolipoprotein B metabolism by the citrus flavanones hesperetin and naringenin. *Lipids* **1999**, *34(6)*, 591–598.

Bukowiecki, L. J.; Lupien, J.; Follea, N.; Jahjah, L. Effects of sucrose, caffeine, and cola beverages on obesity, cold resistance, and adipose tissue cellularity. *Am. J. Physiol.* **1983**, *244*, R500-R507.

Campfield, L. A. Multiple facets of OB protein (leptin) physiology: integration of central and peripheral mechanisms in the regulation of energy balance, in progress in obesity research. **1999**, *8* (Eds.) Ailhaud, G. and Guy-Grand, B. John Libbey & Company, London, pp. 327-335.

Campfield, L. A.; Smith, F. J. Oberview: neurobiology of OB protein (leptin). *Proc. Nutr. Soc.* **1998**, *57*, 429-440.

Chen, M. D.; Lin, W. H.; Song, Y. M.; Lin, P. Y.; Ho, L. T. Effect of caffeine on the level of brain serotonin and catecholamine in the genetically obese mice. *Chin. Med. J.* **1994**, *53*, 257-261.

Cheng, T. O. Is green tea better than black tea in reducing atherosclerosis? *Circulation* **2004**, 110-332.

Cheng, T. O. All teas are not created equal, the Chinese green tea and cardiovascular

health. *Int. J. Cardiology.* **2006**, 301-308.

Cinti, S. The role of brown adipose tissue in human obesity. *Nutr. Metab. Cardiovasc. Dis.* **2006**, *16(8)*, 569-574.

Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adult. NIH publication No. 98-4083 1998

Cryer, A.; Van, **R.L.R.** New perspectives in adipose tissue: structure, function and development. **1985**.

Davies, M. J.; Judd, J. T.; Baer, D. J.; Clevidence, B. A.; Paul, D. R.; Edwards, A. J.; Wiseman, S. A.; Muesing, R. A.; Chen. S. C. Black Tea Consumption Reduces Total and LDL Cholesterol in Mildly Hypercholesterolemic Adults. *J. Nutr.* **2003**, *133(10)*, 3298S - 3302.

Diepvens, K.; Westerterp, K. R.; Westerterp-Plantenga, Margriet S. Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea. *Am. J. Physiol. Regul. Intergr. Comp. Physiol.* **2007**, *292*, R77-R85.

Dulloo, A. G. Ephedrine, xanthines and prostaglandin-inhibitors: actions and interactions in the stimulation of thermogenesis. *Int. J. Obes. Relat. Metab. Disord.* **1993**, *17 Suppl 1*, S35-S40.

Dulloo, A. G.; Duret C.; Rohrer, D.; Girardier, L.; Mensi, N.; Fathi, M.; Chantre, P.; Vandermander, J. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-hour energy expenditure and fat oxidation in humans. *Am. J. Clin. Nutr.* **1999**, *70*, 1040-1045.

Dulloo, A. G.; Seydoux, J.; Girardier, L.; Chantre, P.; Vandermander, J. Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. *Int. J. Obes. Relat. Metab. Disord.* **2000**, *24*, 252-258.

Grundy, S. M. Metabolic syndrome: a multiplex cardiovascular risk factor. *J. Clin. Endocrinol. Metab.* **2007**, *92(2)*, 399-404.

Hausman, Gary J.; Martin, Roy. Biology of the adipocyte: Research approaches. **1987**.

Hasegawa, N.; Mori, M. Effect of powdered green tea and its caffeine content on lipogenesis and lipolysis in 3T3-L1 cell. *J. Health Sci.* **2000**, *46*, 153-155.

Hara, Y. in: Ho, C. T.; Osawa, Y.; Huang, M. T.; Rosen, R. T. (Eds.) Food Phytochemicals for Cancer Prevention II, American Chemical Society, Washington, DC, **1994**, pp. 35–50.

Higdon, V.; Frei, Balz. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Critical reviews in food science and nutrition* **2003**, *43(1)*, 89-143.

Ho, C. T. Novel hydroxylated polymehtoxyflavones from orange peel as nutraceutical ingredients for food and beverages. *International conference on Nutraceuticals and functional foods.*

Hongu, N.; Sachan, D. S. Caffeine, carnitine and choline supplementation of rats decreases body fat and sereum leptin concentration as does exercise. *J. Nutr.* **2000**, *130*, 152-157.

Ito, Y.; Sasaki, R.; Shinohara, R.; Yagyu, S.; Suzuki S.; Aoki, K. *Med. Biol.* **1994**, *129*, 21–24.

Jequier, E. Leptin signaling, adiposity, and energy balance. Ann N Y Acad Sci. 2002, *967*, 379-388.

Jung, R. T.; Shetty, P. S.; Jame, W. P. T.; Barrand, M. A.; Callingham, B. A. Caffeine: its effect on catecholamine and metabolis in lean and obese humans. *Clin. Sci.* **1981,** *60*, 527-535.

Kao, Y. H.; Chang, H. H.; Lee, M. J.; Chen, C. L. Tea, obesity and diabetes. *Mol. Nutr. Food Res.* **2006**, *50*, 188-210.

Kao, Y. H.; Hiipakka, R. A.; Liao, S. Modulating of endocrine systems and food intake by green tea epigallocatechin gallate. *Endocrinology* **2000**, *141*, 980-987.

Kao, Y. H.; Hiipakka, R. A.; Liao, S. Modulation of obesity by a green tea catechin. *Am. J. Clin. Nutr.* **2000**, *72*, 1232-1241.

Kazuo Kobayashi-Hattori; Akie Mogi; Yoshinobu Matsumoto; Toshichika Takita. Effects of caffeine on the body fat and lipid metabolism of rats fed on a high-fat diet. *Biosci. Biotechnol. Biochim.* **2005**, *69*, 2219-2223.

Klaus, S.; Pultz, S.; Thone-Reineke, C.; Wolfram, S. Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing ebergy obsorption and increasing fat oxidation. *Int. J. Obes. Relat. Metab. Disord.* **2005**, *29*, 615-623.

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

Kuo, K-L; Weng, M-S; Chiang, C-T; Ysai, Y-J; Lin-Shiau, S-Y; Lin, J-K. Comparative studies on the hypolipidemic and growth suppressive effects of oolong, black, pu-erh, and green tea leaves in rats. *J. Agric. Food Chem.* **2005**, *53*, 480-489.

Kurowska, E. M.; Manthey, J. A. Regulation of lipoprotein metabolism in HepG2 cells by citrus flavonoids. *Adv. Exp. Med. Biol.* **2002**, *505*, 173–179.

Kurowska, E. M.; Manthey, J. A. Hypolipidemic effects and absorption of citrus polymethoxylated flavones in hamsters with diet-induced hypercholesterolemia. *J. Agric. Food. Chem.* **2004**, *52(10)*, 2879–86.

Lawrence, V. J.; Kopelman, P. G. Medical consequences of obesity. *Clin. Dermatol.* **2004**, *22(4)*, 296-302.

Leblanc, J.; Richard, D.; Racotta, I. S. Metabolic and hormone-related responses to caffeine in rats. *Pharmacol. Res.* **1995**, *32*, 129-133.

Lee, Ki Won; Lee, Hyong Joo; Lee, C. Y. Antioxidant activity of black tea vs. green tea. *J. Nutr.* **2002**, p. 785.

Leung, L. K.; Su, Y.; Chen, R.; Zhang, Z.; Huang, Y.; Chen, Z. Y. Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *J. Nutr.* **2001**, *131*, 2248-2251.

Li, S.; Pan, M. H.; Ho, C.-T. unpublished results, 2007.

Li, S.; Yu, H.; Ho, C.-T. Nobiletin: efficient and large quantity isolation from orange peel extract. *Biomedical Chromatography* **2006**, *20*, 133-138.

Liao, S.; Kao, Y. H.; Hiipakka, R. A. Green tea: biochemical and biological basis for health benefit. *Vitam. Horm.* **2001**, *62*, 1-94.

Lin, J. K.; Lin-Shiau, S. Y. Mechanisms of hypolipidemic and anti-obesity effects of tea and tea polyphenols. *Mol. Nutr. Food Res.* **2006**, *50(2)*, 211-217.

Low, A. K.; Bouldin, M. J.; Sumrall, C. D.; Loustalot, F. V.; Land, K. K. A clinician's approach to medical management of obesity. *Am. J. Med. Sci.* **2006**, *331(4)*, 175-182.

Maffei, M.; Halaas, J.; Ravussin, E.; Pratley, R. E.; Lee, G. H.; Zhang, Y.; Fei, H.; Kim, S.; Lallone, R.; Ranganathan, S.; Kern, P. A.; Friedmen, J. M. Leptin levels in human and rodent: measurement of plasma leptin and ob mRNA in obese and weight-reduced subjects. *Nat. Med.* **1995**, *1*, 1155-1161.

Mazzucotelli A.; Lanqin D. Fatty acid mobilization and their use in adipose tissue. *J. Soc. Biol.* **2006**, *200(1*), 83-91.

Michna, L.; Lu, Y. P.; Lou, Y. R.; Wanger, G. C.; Conney, A. H. Stimulatory effect of oral administration of green tea and caffeine on locomotor activity in SKH-1 mice. *Life Sci.* **2003**, *73*, 1383-1392.

Middleton, E. Jr.; Kandaswami, C.; Theoharides, T. C. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.* **2000**, *52(4)*, 673-751.

Muramatsu, K.; Fuduyo, M.; Hara, Y. Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *J. Nutr. Sci. Vitaminol*. **1986**, 32, 613-622.

Murase, T.; Nagasawa, A.; Suzuki, J.; Hase, T.; Tokimitsu I. Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. *Int. J. Obes. Relat. Metab. Disord.* **2002**, *26(11)*, 1459-1464.

Nagao, T.; Meguro, S.; Soga, S.; Otsuka, A.; Tomonobu, K; Fumoto, S.; Chikama,

A.; Mori, K.; Yuzawa, M.; Watanabe, H.; Hase, T.; Tanaka, Y.; Tokimitsu. I.; Shimasaki, H.; Itakura, H. Tea catechins suppress accumulation of body fat in humans. *J. Oleo. Sci.* **2001**, *50*, 717-728.

Naslund, I.; Halltren, P.; Sjostrom, L. Fat cell weight and number before and after gastric surgery for morbid obesity in women. *Int. J. Obes.* **1998**, *12*, 191-197.

Pasman, W. J.; Westerterp-Plantenga, M. S.; Saris, W. H. M. The effectiveness of long-term supplementation of carbohydrate, chromium, fibre and caffeine on weight maintenance. *Int. J. Obes.* **1997**, *21*, 1143–1151.

Racotta, I. S.; Leblanc, J.; Richard, D. The effect of caffeine on food intake in rats: involvement of corticotropin-releasing factor and the sympatho-adrenal system. *Pharmacol. Biochem. Behav.* **1994**, *48*, 887–892.

Raskin, I.; Ribnicky, D. M; Komarnytsky, S.; Ilic, N.; Poulev, A.; Borisjuk, N.; Brinker, A.; Moreno, D. A.; Ripoll, C.; Yakoby, N.; O' Neal J. M.; Cornwell, T.; Pastor, I.; Fridlender, B. Plants and human health in the twenty-first century. *Trends Biotechnol.* **2002**, *20*, 522-531.

Reeves, A. F.; Rees, J. M.; Schiff, M.; Hujoel, P. Total body weight and waist circumference associated with chronic periodontitis among adolescents in the United States. *Arch. Pediatr. Adolesc. Med.* **2006**, *160(9)*, 894-899.

Rezende, F. A.; Rosado, L. E.; Ribeiro, Rde C.; Vidigal, Fde C.; Vasques, A. C.; Bonard, I. S.; de Carvalho C. R. Body mass index and waist circumference : association with cardiovascular risk factors. *Arq. Bras. Cardiol.* **2006**, *87(6)*, 728-734.

Robertson, A. The chemistry and biochemistry of black tea production – the non-volatiles. Tea: cultivation to consumption. **1992**, M. Clifford. London, Chapman and Hall: 555-601.

Saelens, B. E.; Seeley, R. J.; van Schaick, K.; Donnelly, L. F.; O'Brien, K. J. Visceral abdominal fat is correlated with whole-body fat and physical activity among 9-y-old children at risk of obesity. *Am. J. Clin. Nutr.* **2007**, *85(1)*, 46-53.

Siddique, I. A.; Afaq, F.; Adhami, V. M.; Ahmad, N.; Mukhtar, H. Antioxidants of the beverage tea in promotion of human health. *Antioxid. Redox. Signal*. **2004**, *6*, 571-82.

Tremblay, A.; Masson, E.; Leduc, S.; Houde, A.; Despres, J. P. Caffeine reduces spontaneous energy intake in men but not in women. *Nutr. Res.* **1988**, *8*, 553–558.

Vaidya, V. Health and treatment strategies in obesity. *Adv Psychosom Med. Basel, Karger,* **2006**.

Vinson, J. A.; Dabbagh, Y. A. Effect of green and black tea supplementation on lipids, lipid oxidation and fibrinogen in hamsters: mechanisms for the epidemiological benefits of tea drinking. *FEBS Lett.* **1998**, *433*, 44-46.

Westerterp-Plantenga, M. S.; Lejeune, M. P.; Kovacs, E. M. Body weight loss and weight maintenance in relation to habitual caffeine intake and green tea supplementation. *Obes. Res*. **2005**, *13*, 1195–1204.

Westerterp-Plantenga, M.; Diepvens, K.; Joosen, A. M.C.P.; Berube-Parent, S.; Tremblay, A. Metabolic effects of spices, teas, and caffeine. *Physiology & Behavior* **2006**, *89*, 85-91.

Whitman, S. C.; Kurowska, E. M.; Manthey, J. A.; Daugherty, A. Nobiletin, a citrus flavonoid isolated from tangerines, selectively inhibits class a scavenger receptor-mediated metabolism of acetylated LDL by mouse macrophages. *Atherosclerosis* **2005**, *178*:25.

Wolfram, S.; Raederstorff, D.; Wang, Y.; Teixeira, S. R.; Elste V.; Weber, P. TEAVIGO (epigallocatechin gallate) supplementation prevents obesity in rodents by reducing adipose tissue mass. *Ann. Nutr. Metab.* **2005**, *49*, 54-63.

Wolfram, S.; Wang, Y.; Thielecke, F. Anti-obesity effects of green tea: from bedside to bench. *Mol. Nutr. Food Res.* **2006**, *50*, 176-187.

Yang C. S.; Wang, Z. Y. J. Effect of tea polyphenols and EGCG on nasopharyngeal carcinoma cell proliferation and the mechanisms involved. *Natl. Cancer Inst.* **1993**, *85*, 1038-1049

Yang C. S. Tea and health. *Nutrition* **1999**, *15*, 946-949.

Yang, M.; Wang, C.; Chen, H. Green, oolong and black tea extracts modulate lipid metabolism in hyperlipidemia rats fed high-sucrose diet. *J. Nutr. Biochem.* **2001**, *12*, 14-20.

Yang, T. T. C.; Koo, M. W. L. Hypocholesterolemic effects of Chinese tea. *Pharmocol. Res.* **1997**, *35*, 505-512.

Yokozawa, T.; Dong, E.; Nakagawa, T.; Kim, D. W.; Hattori, M.; Nakagawa, H. Effects of Japanese black tea on atherosclerotic disorders. *Biosci. Biotechnol. Biochem*. **1998**, *62*, 44-48.

Yoshida, T.; Sakane, N.; Umekawa, T.; Kondo, M. Relationship between basal metabolic rate, thermogenic response to caffeine, and body weight loss following combined low calorie and exercise treatment in obese women. *Int. J. Obes.* **1994**, *18*, 345 – 50.

Zheng, G.; Sayama, K.; Okubo, T.; Juneja, L. R.; Oguni, I. Anti-obesity effects of three major components of green tea, catechins, caffeine and theanine, in mice. *In Vivo* **2004**, *18*, 55-62.

Treatment	Food Intake (g)	Water Intake (ml)	Initial Body Weight (g)	End Body Weight (g)	Body Weight Gain (g)	P Fat (g)	R Fat (g)	BAT (g)
1. 20% oil, H	3.36±0.08	4.81±0.12	26.09±0.42	44.67±2.31	18.580	2.48±0.31	0.88±0.16	0.53±0.06
2. 0.2% WG361, N	3.76±0.12	5.80±0.12	26.47±0.62	38.99±1.89	12.520	1.33±0.28	0.56±0.10	0.38±0.07
3. 0.2% WG361, H	3.22±0.11	4.67±0.10	26.08±0.59	42.03±2.82	15.950	1.77±0.43	0.88±0.28	0.48±0.09
4. 0.01% WG361, H	3.18±0.09	4.57±0.12	25.70±0.41	42.62±1.98	16.920	2.06±0.29	0.95±0.13	0.69±0.15
5. 0.03%WG361, H	2.98±0.08	4.45±0.16	25.92±0.40	42.53±4.83	16.610	2.53±0.54	0.97±0.17	0.69±0.13
6. 0.05%WG361, H	3.25±0.11	4.52±0.11	25.65±0.80	44.68±5.06	19.030	2.84±1.15	0.97±0.28	0.72±0.20
7. 0.10%WG361, H	3.20±0.10	5.70±0.29	25.77±0.60	40.08±1.93	14.310	1.91±0.38	0.76±0.14	0.49±0.07
8. 0.01%WG362, H	3.00±0.08	5.05±0.23	25.87±0.77	41.07±2.17	15.200	2.09±0.50	0.69±0.12	0.46±0.05
9. 0.03%WG362, H	3.26±0.07	4.74±0.12	26.04±0.65	43.39±2.50	17.350	2.62±0.50	0.97±0.13	0.60±0.10
10. 0.1%WG362, H	3.34±0.10	4.83±0.10	25.95±0.56	45.12±1.95	19.170	2.50±0.48	1.03±0.18	0.75±0.13
11. 0.01%WG363, H	3.36±0.09	4.54±0.07	25.83±0.57	43.57±2.44	17.640	2.89±0.70	0.93±0.15	0.53±0.08
12. 0.03% WG363, H	3.37±0.07	4.71±0.11	25.83±0.57	39.33±1.74	13.500	1.55±0.37	0.68±0.16	0.43±0.07
13. 0.1%WG363, H	3.25±0.10	4.52±0.12	25.775±0.90	43.89±3.04	18.115	2.81±0.56	1.09±0.24	0.70±0.12
14. 5% oil, N	3.70±0.07	4.91±0.14	25.645±1.50	40.17±2.54	14.525	1.60±0.34	0.65±0.12	0.56±0.08

Appendix 1. Effects of OPE on body weight gain, parametrial fat (P-fat), retroperitoneal fat (R-fat) and brown adipose tissue in mice. Female CD-1 mice were given a low-fat diet or a high-fat diet and water for 17 weeks. Food and water consumption, body weight were monitored weekly. Parametrical fat, retroperitoneal fat and brown fat as well as spleen and liver were removed and weighed.

Treatment	HDL mg/dl	LDL mg/dl	TC mg/dl	TG mg/dl	Glucose mg/dl	Leptin ng/dl
1. 20% oil, H	51.4±8.08	62.26	132.5±2.89	94.2±23.10	124.9±2.96	8.70±0.91
2. 0.2% WG361, N	40.4±5.36	36.96	97.8±2.46	102.2±9.87	123.2±8.51	3.52±0.70
3. 0.2% WG361, H	36.4±3.51	71.12	123.0±6.99	77.4±15.31	122.9±6.17	7.86±0.56
4. 0.01% WG361, H	44.0±5.70	40.06	104.3±11.99	101.2±20.60	102.0±7.26	4.21±0.31
5. 0.03%WG361, H	36.7±3.18	147.60	202.5±44.12	91.0±12.88	114.7±8.88	5.51±0.99
6. 0.05%WG361, H	39.8±5.24	91.00	149.7±19.47	94.5±7.33	105.5±7.17	6.57±1.60
7. 0.10%WG361, H	46.2±7.00	93.32	153.6±19.65	70.4±2,19	121.4±1.76	7.80±6.91
8. 0.01%WG362, H	38.7±8.89	37.34	92.0±10.55	79.8±6.43	100.2±2.96	2.65±3.62
9. 0.03%WG362, H	44.4±9.82	74.34	138.3±21.55	97.8±20.1	119.1±5.17	6.46±9.38
10. 0.1%WG362, H	43.6±8.72	63.64	125.1±23.33	89.3±7.31	111.9±6.69	25.87±8.70
11. 0.01%WG363, H	45.8±7.69	58.66	121.3±10.1	84.2±13.65	119.1±5.36	6.15±0.46
12. 0.03% WG363, H	26.6±3.21	49.32	92.3±2.28	81.9±7.75	100.7±6.89	5.04±1.42
13. 0.1%WG363, H	52.3±13.5	83.40	156.6±27.3	104.5±21.87	117.6±4.51	9.80±0.39
15. 5% oil, N	32.7±5.49	32.96	85.3±1.96	98.2±16.65	101.1±7.22	5.31±0.68

Appendix 2. Effects of OPE on blood HDL, LDL, total cholesterol (TC), triglycerides (TG), glucose and leptin levels in CD-1 mice. Female CD-1 mice (5 weeks old; 10 mice per group) were given a low-fat diet or a high-fat diet and water for 17 weeks. After 17 weeks on the special diets, the mice were sacrificed and blood samples were collected. Data are mean±SE (n=10).