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Phenolic Compounds Rutin and *o*-Coumaric Acid Ameliorate Obesity Induced by High-Fat Diet in Rats

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Dietary fat is one of the most important environmental factors associated with the incidence of cardiovascular diseases. In this study, the antiobesity effects of rutin (R) and *o*-coumaric acid (oCA) were investigated. Wistar rats were divided into normal and obese groups, and obese rats were prefed a high-fat diet (HFD) containing 40% beef tallow for 4 weeks. Then, R and *o*CA were given as a supplement to obese rats at doses of 50 and 100 mg/kg, respectively, for a period of 8 weeks. The results showed that body, liver organ, and adipose tissue weights of peritoneal and epididymal fat pads in the HFD+ R and HFD+*o*CA groups were significantly decreased as compared to those in the HFD group. Serum lipid profiles, insulin, and leptin were significantly decreased in the HFD+ R (high dose, HD) and HFD+*o*CA (HD) groups as compared to those in the HFD group. Hepatic triacylglycerol and cholesterol levels were significantly decreased in the HFD+ R (HD) and HFD+*o*CA (HD) groups as compared to those in the HFD group. Moreover, the consumption of R and *o*CA reduced oxidative stress and glutathione disulfide (GSSG) content, and enhanced the levels of glutathione (GSH), GSH peroxidase (GPx), GSH reductase (GRd), and GSH S-transferase (GST) in the hepatic tissue of rats with HFD-induced obesity. These results demonstrate that intake of R and *o*CA can be beneficial for the suppression of high-fat-diet-induced dyslipidemia, hepatosteatosis, and oxidative stress in rats.

KEYWORDS: Rutin; *o*-coumaric acid; dyslipidemia; hepatosteatosis; oxidative stress

INTRODUCTION

Obesity is closely associated with life-style-related diseases such as hyperlipidemia, hypertension, arteriosclerosis, type 2 diabetes mellitus, cancer, respiratory complications, and osteoarthritis (*1*). It is one of the main public health problems in developed and developing countries. The major health consequences of obesity are predictable given an understanding of the pathophysiology of increasing body fat. Muller et al. (*2*) indicated that the high intake of saturated fatty acids is associated with a high level of serum cholesterol and is strongly correlated with coronary death rates. Therefore, prevention and treatment of obesity are relevant to health promotion.

Epidemiological evidence has supported that dietary antioxidants play a role in the prevention of several chronic diseases such as cancer, cardiovascular disease, and diabetes (*3*). Phenolic compounds are constituents of fruits, vegetables, nuts, and plant-derived beverages such as tea, wine, and traditional Eastern medicines. They are reported to have antioxidative, antiviral, antiparasitic, and anticarcinogenic properties (*4*). Gorelik et al. (*5*) indicated that red wine polyphenols may prevent the adverse health effects of high-fat foods in humans. A number of studies have demonstrated that antioxidants may act as a regulator of obesity in mice or rats fed high-fat diets (HFDs) (*6*, *7*). Hsu and Yen (*6*) indicated that intake of gallic acid can be beneficial for the suppression of HFD-induced dyslipidemia, hepatosteatosis, and oxidative stress in rats.

Oxidative stress is one of the risk factors that links hyperlipidemia with the pathogenesis of atherosclerosis (*8*). Obesity seems to decrease antioxidant defense by lowering the levels of antioxidant enzymes (catalase, glutathione peroxidase, and glutathione reductase). Our *in vitro* study demonstrated that rutin and *o*-coumaric acid inhibit intracellular triglyceride and glycerol-3-phosphate dehydrogenase (GPDH) activity the best among the 15 phenolic acids and 6 flavonoids tested (*9*). Rutin and *o*-coumaric acid also inhibited the expression of peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding proteins (C/EBPα), and leptin, and up-regulated expression of adiponectin at the protein level. Nevertheless, data in the literature regarding the effect of rutin and *o*-coumaric acid on high-fat-diet-induced dyslipidemia, hepatosteatosis, and oxidative stress in rats remain unclear.

In this study, we investigated the antiobesity effect of rutin and *o*-coumaric acid in rats fed a high-fat diet. In addition, growth parameters, organ and adipose tissue weights, serum

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Table 1. Composition of Experimental High-Fat Diets[a]

ingredients (g/kg dietary weight)	ND	HFD control	HFD LD	HFD HD
casein	260	260	260	260
corn starch	500	150	150	150
sucrose	90	90	90	90
corn oil	50			
beef tallow		400	400	400
cellulose	50	50	49	48
mineral mixture[b]	40	40	40	40
vitamin mixture[b]	10	10	10	10
rutin (R)/*o*-coumaric acid (*o*CA)		0	1	2
metabolizable energy (kcal/g)	3.85	5.60	5.60	5.60

[a] For details of diets and procedures, see Materials and Methods. [b] Mineral and vitamin mixtures (AIN-76) were purchased from Oriental Yeast (Tokyo, Japan). ND, normal diet; HFD, high-fat diet; R, rutin; oCA, *o*-coumaric acid; LD, low dose; HD, high dose.

biochemical parameters, histology, and the antioxidant defense system were measured in rats fed a normal diet (ND) and a high-fat diet (HFD) with or without rutin (R) or *o*-coumaric acid (oCA).

MATERIALS AND METHODS

Materials. The *o*-coumaric acid and rutin were obtained from Sigma Chemical (St. Louis, MO). All other chemicals used were of the highest pure grade available.

Animals, Diets, and Experimental Design. Four-week-old male Wistar rats were purchased from the National Science Council Animal Center, Taipei, Taiwan. Animals were housed individually in stainless steel cages in an air-conditioned room at 23 ± 2 °C, 55−60% relative humidity, and a 12 h light/dark cycle, and were given a laboratory rodent chow diet for 1 week. The rats were divided into normal and obese groups and then fed normal diets (ND) and high-fat diets (HFD), respectively. The obese groups (n = 6/group) were prefed a high-fat diet containing 40% beef tallow for 4 weeks. The HFD group was then divided into three groups according to whether they received supplemental rutin and *o*-coumaric acid for 8 weeks: the HFD, HFD+R (high dose, HD), or HFD+oCA (HD) group and the HFD+R (low dose, LD) or HFD+oCA (LD) group received the HFD supplemented with rutin or *o*-coumaric acid at levels of 0, 50, and 100 mg/kg, respectively. The rats were provided with semisynthetic diets (**Table 1**) and water *ad libitum* throughout the experimental period. These animals should normally be able to consume 5% of their body weight daily. The diets were stored in a 4 °C cold chamber. Body weights, food intakes, and food efficiency were measured every day for 8 weeks. The food efficiency (g/kcal) was calculated by dividing body weight gain (g/day) by energy intake (kcal/day) over the diet period. After an overnight fasting, blood was withdrawn from the abdominal aorta under diethyl ether anesthesia, and serum was harvested. The visceral tissues were immediately excised, rinsed, weighed, and frozen in liquid nitrogen. All experimental procedures involving animals were conducted in accordance with the National Institutes of Health (NIH). This experiment was approved of by the Institutional Animal Care and Use Committee (IACUC) of National Chung Hsing University, Taichung, Taiwan.

Measurement of Serum Parameters. Blood was placed into a sterile Vacutainer plastic tube (BD Vacutainer, Plymouth, U.K.). Serum was separated by centrifugation (4000 rpm, 10 min) and transferred to Eppendorf tubes. All serum samples were stored at −80 °C until analysis. The serum concentrations of triacylglycerol (TAG), glucose, phospholipid, total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, creatinine, Na^+, K^+, and Cl^- were measured with commercial kits (Bayer Corporation, Tarrytown, NY). The concentrations of insulin and leptin were measured with rat insulin ELISA kits (Mercodia AB, Uppsala, Sweden) and leptin ELISA kits (BioVendor, Brno, Czech Republic).

Hematoxylin/Eosin (H&E) and Oil-Red O Staining. Liver samples were collected following euthanasia, fixed in 10% formalin buffered solution, cut into 5-μm sections, and stained with hematoxylin/eosin (H&E). Moreover, livers from the animals were frozen in liquid nitrogen, embedded in an optimal temperature cutting compound, cut into 5 μm sections, and stained with Oil-Red O. H&E and Oil-Red O staining were performed using standard techniques.

Hepatic Lipid Analysis. Liver lipids were extracted according to the methods of Folch et al. (*10*), and the concentrations of TAG and cholesterol were measured by the methods of Fletcher (*11*) and Sperry and Webb (*12*).

Lipid Peroxidation Assay. Determination of malondialdehyde (MDA) by thiobarbituric acid (TBA) was used as an index of the extent of lipid peroxidation according to the methods of Buege and Aust (*13*). Briefly, 0.5 mL of homogenate was added to 2 mL of TBA reagent containing 0.375% TBA, 15% trichloroacetic acid, and 0.25 mol/L HCl. Samples were boiled for 15 min, cooled, and centrifuged at 1700*g* for 15 min at 4 °C. The absorbance of the supernatants was measured spectrophotometrically at 532 nm. The concentrations of TBA-reactive substances were calculated using 1,1,3,3-tetraethoxypropane (TEP) as a standard. The protein concentration was determined using a standard commercial kit (Bio-Rad Laboratories, Hercules, CA). The results are expressed as MDA formation per milligram of protein.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. Determination of TEAC was carried out using the method of Arnao et al (*14*). $ABTS^{\bullet+}$ is generated by the interaction of ABTS (100 μmol/L), H_2O_2 (50 μmol/L), and peroxidase (4.4 U/mL). To measure antioxidant activity, 0.25 mL of serum was mixed well with an equal volume of ABTS, H_2O_2, peroxidase, and 1.5 mL of deionized water. The absorbance was measured at 734 nm after interacting with sample solution for 10 min. The decrease in absorption at 734 nm after addition of the reactant was used to calculate the TEAC value. A dose−response curve was plotted for trolox, and antioxidant ability was expressed as the TEAC. The higher the TEAC value of a sample, the stronger the antioxidant activity.

Determination of GSH and GSSG in the Liver. The GSH/GSSG ratio was determined after hepatic tissue was homogenized with phosphate-buffered saline at a pH of 7.4. The GSH/GSSG ratio was determined using a glutathione assay kit (Cayman Chemical Company, Ann Arbor, MI). The amounts of total glutathione (GSH) and glutathione disulfide (GSSG) were photometrically determined using a microplate reader (Awareness Technology, Palm City, FL) at 405 nm, and the amounts of GSH and GSSG and the GSH/GSSG ratio were calculated.

Determination of Antioxidant Enzymes in the Liver. All antioxidant enzyme activities were determined after hepatic tissue was homogenized with phosphate-buffered saline at a pH of 7.0. The GSH peroxidase (GPx) activity was determined according to the method of Lawerence & Burk (*15*). Liver homogenate solution (100 μL) was mixed with 800 μL of 100 mmol/L potassium phosphate buffer (pH 7.4) containing 1

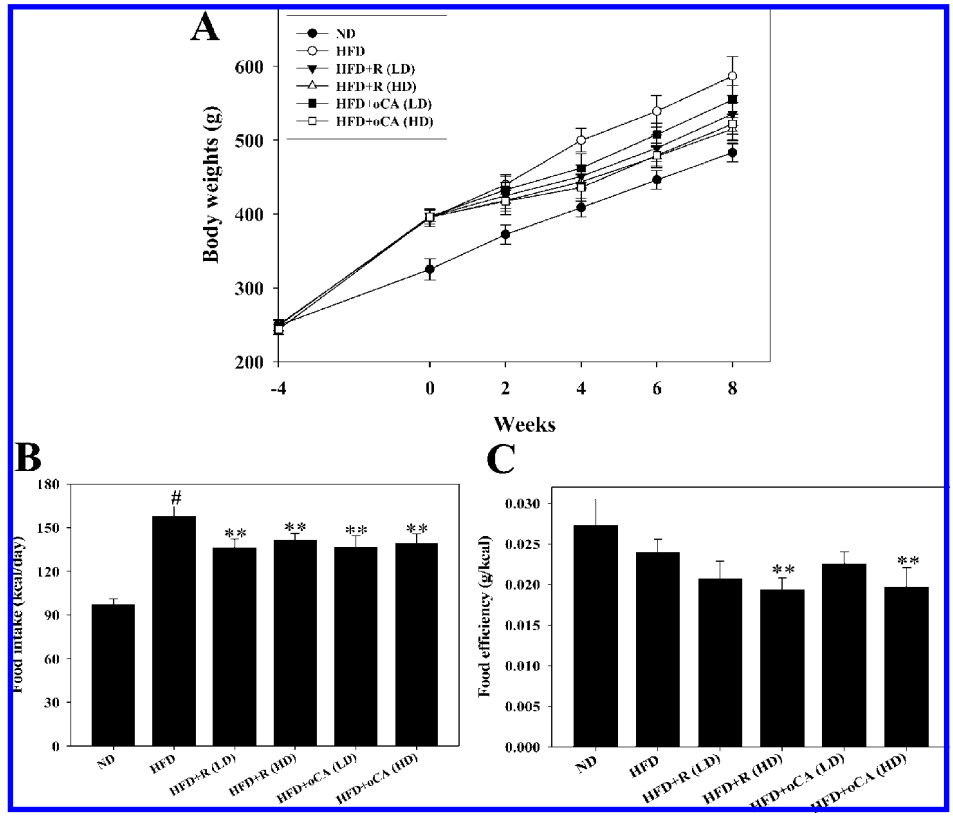

Figure 1. Effects of rutin and *o*-coumaric acid on (A) body weights, (B) food intake, and (C) the food efficiency of rats with obesity induced by a high-fat diet. Data are presented as mean ± SD (n = 6/group). Results were statistically analyzed with Student's t-test (#p < 0.01 compared with the ND group; *p < 0.05 compared with the HFD group; **p < 0.01 compared with the HFD group).

mmol/L EDTA, 1 mmol/L NaN_3, 0.2 mmol/L NADPH, 1 U/mL GSH reductase, and 1 mmol/L GSH. After 5 min, 2.5 mmol/L H_2O_2 (100 μL) was added to start the reaction. The absorbance change at 340 nm was recorded over the course of 3 min. Enzyme activity was calculated by E_{340} = 6220/M per cm (extinction coefficient), and the result is expressed as units of nmol NADPH/min per mg protein.

The GSH reductase (GRd) activity was determined according to the method of Bellomo et al. (*16*). Liver homogenate solution (100 μL) was mixed with 900 μL of 100 mmol/L potassium phosphate buffer (pH 7.0) containing 1 mmol/L $MgCl_2 \cdot 6H_2O$, 50 mmol/L GSSG, and 0.1 mmol/L NADPH, and was incubated for 3 min at room temperature. The absorbance change at 340 nm was recorded over the course of 3 min. The enzyme activity was calculated by E_{340} = 6220/M per cm (extinction coefficient), and the result is expressed as units of nmol NADPH/min per mg protein.

The GSH *S*-transferase (GST) activity was determined according to the method of Habig et al. (*17*). Liver homogenate solution (100 μL) was mixed well with 880 μL of 100 mmol/L potassium phosphate buffer (pH 6.5) containing 100 mmol/L GSH and 50 mmol/L CDNB (1-chloro-2,4-dinitrobenzene). The absorbance change at 340 nm was recorded over the course of 3 min. The enzyme activity was calculated by E_{340} = 9.6/mM per cm (extinction coefficient), and the result is expressed as units of nmol CDNB-GSH conjugate formed/min per mg protein.

Superoxide dismutase (SOD) activity was determined by a SOD assay kit-WST (Dojindo Molecular Technologies Inc., Maryland) as specified by the manufacturer. Absorbance was measured spectrophotometrically in a FLUOstar galaxy spectrophotometer (BMG Labtechnologies Ltd., Germany) at 450 nm. The SOD activity was calculated using the following equation: SOD activity (inhibition rate %) = $\{[(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2})]/(A_{blank1} - A_{blank3})\} \times 100$. The value (%) is expressed as SOD activity of the ND group at 100%.

Glycerol-3-phosphate Dehydrogenase (GPDH) Inhibition. Fat and liver tissues were homogenized according to the method of Shillabeer et al. (*18*). The liver tissue was homogenized with phosphate−carbonate buffer (70 mmol/L $NaHCO_3$, 85 mmol/L K_2HPO_4, 9 mmol/L KH_2PO_4, 1 mmol/L EDTA, pH 8.0, containing 1 mmol/L dithiothreitol), and aliquots of the supernatant were frozen at −80 °C until they were used for the GPDH assay. The fat tissue was homogenized with 10 mmol/L HEPES buffer containing 0.25 mol/L sucrose, 1 mmol/L EDTA, and 1 mmol/L dithiothreitol. The floating fat layer was removed, and the aliquots were determined for GPDH inhibition. An inhibitory test of GPDH activity was determined according to the procedure of Wise and Green (*19*). Protein concentration was determined using the BioRad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA). The absorbance change at 340 nm was recorded over the course of 3 min. The value (%) is expressed as the GPDH inhibition of the HFD group at 100%.

Statistical Analysis. All data are presented as mean ± SD of three different determinations. Differences between variants were analyzed by the Student's t-test for unpaired data. Values of $p < 0.05$ were regarded as statistically significant.

RESULTS

Effects of Rutin and *o*-Coumaric Acid on Body Weight and Tissue Weights. In our animal model, HFD groups were fed a high fat-diet containing 40% beef tallow for 4 weeks. The HFD groups were then divided into three groups (n = 6/group) according to whether they received supplemental rutin (R) or *o*-coumaric acid (oCA) for 8 weeks. As shown in **Figure 1**A,

Table 2. Effects of Rutin and *o*-Coumaric Acid on the Weights of Organs and Adipose Tissue of Rats with Obesity Induced by a High Fat-Diet[a]

tissue weights (mg/g rat)	ND	HFD	HFD+R (LD)	HFD+R (HD)	HFD+oCA (LD)	HFD+oCA (HD)
heart	2.6 ± 0.3	2.5 ± 0.2	2.5 ± 0.3	2.5 ± 0.2	2.6 ± 0.3	2.7 ± 0.2
liver	25.1 ± 1.0	29.1 ± 1.8[b]	26.7 ± 1.9[c]	24.8 ± 1.4[d]	28.1 ± 2.4	26.5 ± 2.0[d]
spleen	1.7 ± 0.1	1.5 ± 0.1	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2
lung	0.5 ± 0.4	3.4 ± 0.3	3.2 ± 0.4	3.3 ± 0.4	3.6 ± 0.2	3.6 ± 0.2
kidney	5.1 ± 0.5	4.9 ± 0.9	4.9 ± 0.6	4.8 ± 0.3	4.8 ± 0.2	4.9 ± 0.3
peritoneal fat	20.3 ± 2.1	43.7 ± 5.8[b]	36.4 ± 5.0[d]	32.9 ± 3.0[d]	39.0 ± 5.5[d]	34.0 ± 4.1[d]
epididymalfat	20.1 ± 2.5	43.2 ± 3.7[b]	32.1 ± 6.1[d]	28.3 ± 3.9[d]	36.4 ± 7.7[d]	30.8 ± 3.4[d]

[a] For details of diets and procedures, see the Materials and Methods. Each value is expressed as the mean ± SD (n = 6/group). Results were statistically analyzed with Student's t-test. [b] $p < 0.01$ compared with the ND group. [c] $p < 0.05$ compared with the HFD group. [d] $p < 0.01$ compared with the HFD group.

Table 3. Effects of Rutin and *o*-Coumaric Acid on the Serum Biochemical Parameters of Rats with Obesity Induced by a High Fat-Diet[a]

biochemical parameters	ND	HFD	HFD+R (LD)	HFD+R (HD)	HFD+oCA (LD)	HFD+oCA (HD)
TAG (mg/dL)	69.3 ± 11.7	94.7 ± 10.7[b]	80.5 ± 6.7[c]	77.0 ± 10.8[d]	92.5 ± 11	86.2 ± 13
glucose (mg/dL)	100 ± 14	102 ± 22	117 ± 27	120 ± 19	117 ± 20	122 ± 20
phospholipid (mg/dL)	87.2 ± 10.4	116 ± 10[b]	101 ± 10.1	93.2 ± 6.3[d]	103 ± 10[c]	98 ± 8.0[d]
total cholesterol (mg/dL)	53.0 ± 5.5	76.2 ± 13.0[b]	62.7 ± 11.9[c]	59.2 ± 7.7[c]	67.3 ± 11	65.2 ± 7.0
LDL-cholesterol (mg/dL)	16.5 ± 2.3	23.4 ± 4.6[b]	19.9 ± 6.3	17.7 ± 2.8[d]	21.3 ± 1.8[d]	18.4 ± 4.8[d]
HDL-cholesterol (mg/dL)	35.8 ± 5.1	15.0 ± 2.2[b]	33.6 ± 6.8[d]	30.1 ± 3.9[d]	29.9 ± 6.2[c]	31.3 ± 2.5[c]
AST (U/L)	107 ± 10	107 ± 15	88.5 ± 21	94.4 ± 18	91.5 ± 14	95.3 ± 15
ALT (U/L)	38.7 ± 3.5	38.6 ± 2.4	34.1 ± 5.5	35.5 ± 7.7	46.6 ± 10	40.1 ± 8.1
uric acid (mg/dL)	2.4 ± 0.3	2.6 ± 0.04	2.4 ± 0.4	2.3 ± 0.4	2.6 ± 0.3	2.5 ± 0.2
creatinine (mg/dL)	0.6 ± 0.2	0.7 ± 0.02	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
Na^+ (mmol/L)	144 ± 4	149 ± 9	150 ± 8	152 ± 7	153 ± 6	151 ± 7
K^+ (mmol/L)	4.5 ± 0.4	4.7 ± 0.3	4.7 ± 0.5	4.9 ± 0.3	4.7 ± 0.1	5.0 ± 0.9
Cl^- (mmol/L)	109 ± 6	111 ± 6	110 ± 5	112 ± 5	117 ± 5	112 ± 5
leptin (ng/L)	2.5 ± 0.3	7.1 ± 0.9[b]	4.5 ± 0.8[d]	3.2 ± 0.6[d]	3.8 ± 0.7[d]	2.9 ± 0.6[d]
insulin (ng/L)	4.3 ± 0.7	8.8 ± 1.1[b]	8.6 ± 0.8	6.4 ± 0.8[d]	7.7 ± 1.2	5.7 ± 1.0[d]

[a] For details of diets and procedures, seeMethods. Each value is expressed as the mean ± SD (n = 6/group). Results were statistically analyzed with Student's t-test [b] $p < 0.01$ compared with the ND group; [c] $p < 0.05$ compared with the HFD group; [d] $p < 0.01$ compared with the HFD group. TAG, triacylglycerol; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

body weight was significantly increased (21%) in the HFD group as compared to that in the ND group. After 8 weeks of feeding, body weights in the HFD+ R and HFD+oCA (LD and HD) groups were significantly decreased as compared to those in the HFD group. Food intake was significantly higher in the HFD group compared to the ND group (**Figure 1**B). Food efficiencies in the HFD+R (HD) and HFD+oCA (HD) groups were significantly decreased as compared to those in the HFD group ($p < 0.01$). The organ and adipose tissue weights of the four groups are depicted in **Table 2**. There were no significant differences in the organ weights of the heart, spleen, lung, and kidney among the four groups, but the weights of the liver and adipose tissue (peritoneal and epididymal fat) in the HFD+ R and HFD+oCA groups were significantly decreased as compared to those of the HFD group ($p < 0.01$).

Effects of Rutin and *o*-Coumaric Acid on Dyslipidemia in Obese Rats. The effects of rutin and *o*-coumaric acid on dyslipidemia of rats with obesity induced by a high-fat diet are depicted in **Table 3**. Serum levels of TAG, phospholipids, total cholesterol, and LDL-cholesterol in the HFD+R (HD) group were significantly decreased as compared to those in the HFD group ($p < 0.05$). Serum levels of phospholipid and LDL-cholesterol in the HFD+oCA groups were significantly decreased as compared to those in the HFD group ($p < 0.05$), excluding the HFD+oCA (LD) group. Serum levels of glucose were not significantly different among the four groups. Serum levels of HDL-cholesterol in the HFD+R and HFD+oCA groups were significantly increased as compared to those in the HFD group ($p < 0.05$). There were no significant differences in the serum levels of AST, ALT, uric acid, creatinine, Na^+, K^+, and Cl^- among the four groups. Serum levels of insulin and leptin were significantly increased in the HFD group as compared to those of the ND group ($p < 0.01$), whereas the levels of serum insulin in the HFD+R (HD) and HFD+oCA (HD) groups and leptin in the HFD+R and HFD+oCA groups were significantly decreased as compared to those in the HFD group ($p < 0.01$).

Effects of Rutin and *o*-Coumaric Acid on Hepatosteatosis in Obese Rats. As demonstrated in **Figure 2**A, hematoxylin and eosin staining showed normal liver architecture in the ND group. The HFD group had more profound steatosis with macrovesicular fat accumulation. The HFD+R and HFD+oCA (LD and HD) groups showed microvesicular fat accumulation. These changes were confirmed with Oil-Red O staining. Staining with Oil-Red O confirmed the presence of lipid droplets within the hepatocytes of the rats fed a HFD (**Figure 2**B). The number of lipid droplets in the HFD+R and HFD+oCA (LD and HD) groups was smaller than that of the HFD group. As shown in **Figure 2**C and D, hepatic TAG and cholesterol levels in the HFD group were significantly increased as compared to those of the ND group ($p < 0.01$), whereas those in the HFD+R (HD) and HFD+oCA (HD) groups were significantly decreased as compared with the HFD group ($p < 0.01$).

Effects of Rutin and *o*-Coumaric Acid on Oxidative Stress in Obese Rats. Table 4 shows the effects of rutin and *o*-coumaric acid on the oxidative stress of rats with obesity induced by a high fat-diet. Lipid peroxidation levels in the liver are expressed as MDA content. Liver MDA contents were significantly increased to 13.1 nmol/mg protein ($p < 0.01$) in the HFD group when compared with the ND group (2.8 nmol/mg protein). The HFD+R (HD) and HFD+oCA (HD) groups demonstrated significantly ($p < 0.01$) decreased MDA contents of 7.7 and 9.7 nmol/mg protein, respectively. TEAC values are expressed as trolox equivalents (mmol/mg protein) in rat livers. The HFD group had significantly reduced TEAC values ($p < 0.05$) in the liver. The HFD+R and HFD+oCA (HD) groups

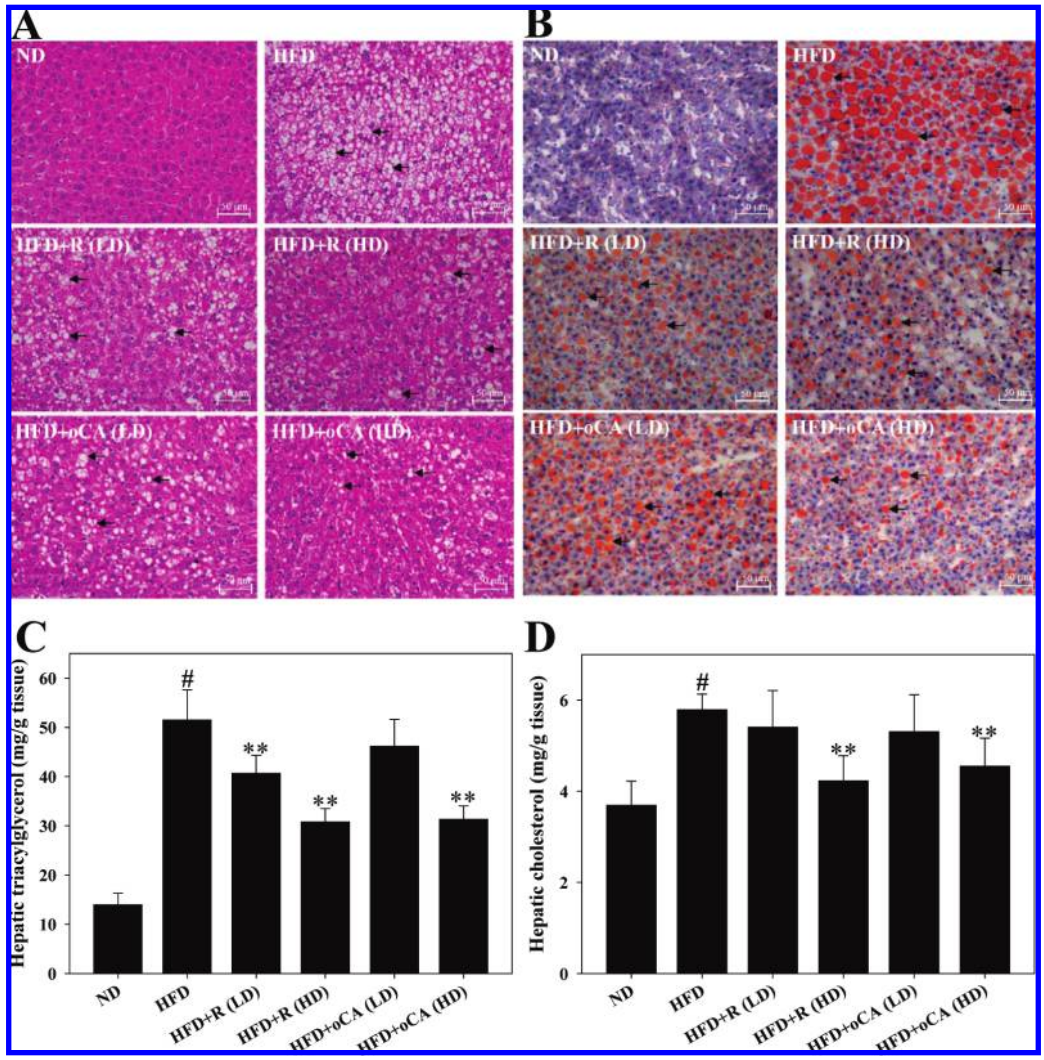

Figure 2. Effects of rutin and *o*-coumaric acid on hepatosteatosis of rats with obesity induced by a high-fat diet. Livers were stained with (A) hematoxylin and eosin or (B) Oil-Red O. Original magnification: 200×. (C) Hepatic triglyceride and (D) Hepatic cholesterol. Data are presented as mean ± SD (n = 6/group). Results were statistically analyzed with Student's t-test ($^{\#}p < 0.01$ compared with the ND group; $^{**}p < 0.01$ compared with the HFD group).

Table 4. Effects of Rutin and *o*-Coumaric Acid on the Oxidative Stress of Rats with Obesity Induced by a High-Fat Diet[a]

	ND	HFD	HFD+R (LD)	HFD+R (HD)	HFD+oCA (LD)	HFD+oCA (HD)
MDA (nmol/mg protein)	2.8 ± 0.9	13.1 ± 1.7[b]	12.0 ± 1.2	7.7 ± 0.9[d]	11.3 ± 1.1	9.7 ± 0.9[d]
TEAC (mmol trolox/mg protein)	0.75 ± 0.06	0.53 ± 0.09[b]	0.63 ± 0.03[c]	0.70 ± 0.05[d]	0.61 ± 0.1	0.64 ± 0.1[d]
GSH (μmol/mg protein)	3.38 ± 0.20	0.77 ± 0.07[b]	1.54 ± 0.15[d]	3.03 ± 0.35[d]	1.4 ± 0.1[d]	2.6 ± 0.3[d]
GSSG (μmol/mg protein)	0.29 ± 0.04	0.45 ± 0.02[b]	0.37 ± 0.08[c]	0.29 ± 0.07[d]	0.4 ± 0.1	0.3 ± 0.1[d]
GSH/GSSG ratio	11.8 ± 1.5	1.72 ± 0.16[b]	4.40 ± 1.39[d]	10.6 ± 1.9[d]	3.8 ± 1.0[c]	8.3 ± 1.8[d]
GPx (nmol/mg protein)	190 ± 7.4	123 ±15[b]	149 ± 16[d]	179 ± 11[d]	154 ± 9[d]	177 ± 15[d]
GRd (nmol/mg protein)	38.0 ± 5.6	16.3 ± 2.2[b]	23.1 ± 3.4[c]	33.1 ± 4.2[d]	25.7 ± 2.4[d]	33.3 ± 8.8[d]
GST (nmol/mg protein)	611 ± 41	366 ±48[b]	394 ± 38	462 ± 49[d]	458 ± 47[d]	494 ± 53[d]
SOD (%)	100 ± 7.8	18.8 ± 6.9[b]	69.8 ± 8.2[d]	82.3 ± 11[d]	54.8 ± 8.6[d]	78.3 ± 4.8[d]

[a] For details of diets and procedures, see Materials and Methods. Each value is expressed as the mean ± SD (n = 6/group). Results were statistically analyzed with Student's t-test. [b] $p < 0.01$ compared with the ND group. [c] $p < 0.05$ compared with the HFD group. [d] $p < 0.01$ compared with the HFD group. MDA, malondialdehyde; TEAC, trolox equivalent antioxidant capacity; GSH, glutathione; GSSG, glutathione disulfide; GPx, glutathione peroxidase; GRd, glutathione reductase; GST, glutathione *S*-transferase; SOD, superoxide dismutase.

had significantly increased ($p < 0.01$) TEAC values in the liver. The GSH contents and GSH/GSSG ratio in the HFD+R and HFD+oCA groups were significantly increased as compared to those of the HFD group ($p < 0.05$). The GSSG contents in the HFD+R and HFD+oCA (HD) groups were significantly decreased as compared to that of the HFD group ($p < 0.01$). The HFD group had a significant reduction in antioxidant enzyme activities ($p < 0.01$) in the livers of rats with obesity induced by a high-fat diet. The GSH-dependent antioxidant enzyme activities of GPx, GRd, and GST showed 35, 57, and 40% reductions, respectively, in the HFD group when compared to those of the ND group. Antioxidant enzymes (GPx, GRd, and GST) in the HFD+R and HFD+oCA groups were significantly increased as compared to those in the HFD group

Table 5. Effects of Rutin and *o*-Coumaric Acid on GPDH Inhibition in the Fat and Liver Tissues of Rats with Obesity Induced by a High-Fat Diet[a]

	GPDH inhibition (%)[b]		
	peritoneal fat	epididymal fat	liver
ND	21.6 ± 6.1	35.1 ± 7.8	55.0 ± 12.6
HFD	100 ±9.5[c]	100 ± 10[c]	100 ± 11[c]
HFD+R (LD)	67.0 ± 11.3[e]	76.6 ± 11.1[e]	85.4 ± 9.6[d]
HFD+R (HD)	49.6± 7.0[e]	61.7 ± 10.7[e]	69.6 ± 8.4[e]
HFD+oCA (LD)	67.0 ± 11.8[e]	80.1 ± 10.2[e]	89.5 ± 13.1
HFD+oCA (HD)	52.3 ± 10.2[e]	73.8 ± 9.6[e]	66.5 ± 7.6[e]

[a] For details of diets and procedures, see Materials and Methods. [b] The value (%) was expressed as the GPDH inhibition of the HFD group at 100%. Each value is expressed as the mean ± SD ($n = 6$/group). Results were statistically analyzed with Student's *t*-test. [c] $p < 0.01$ compared with the ND group. [d] $p < 0.05$ compared with the HFD group. [e] $p < 0.01$ compared with the HFD group. GPDH, glycerol-3-phosphate dehydrogenase.

($p < 0.05$), excluding the HFD+R (LD) group. The SOD activity in the HFD+R and HFD+oCA groups was significantly increased as compared to that in the HFD group ($p < 0.01$).

Effects of Rutin and *o*-Coumaric Acid on GPDH Activity in Obese Rats. Table 5 shows the effects of rutin and *o*-coumaric acid on GPDH activity in the fat and liver tissues of rats with obesity induced by a high-fat diet. GPDH activity in the HFD group was significantly increased in the adipose tissue (peritoneal and epididymal fat) as compared to values obtained for the ND group ($p < 0.01$). The HFD+R and HFD+oCA groups demonstrated significantly decreased ($p < 0.01$) GPDH activity in adipose tissue when compared with the HFD group. The GPDH activity in the livers of the HFD+R and HFD+oCA (HD) groups was significantly decreased as compared to that of the HFD group ($p < 0.05$).

DISCUSSION

In this study, the obese groups ($n = 6$/group) were prefed a high-fat diet containing 40% beef tallow for 4 weeks. Rutin was then given as a supplement at levels of 50 and 100 mg/kg for a period of 8 weeks. The range of doses used in the present study was consistent with those used in other studies examining the effect of rutin in mice fed a high-fat diet (*20*). The present study was designed to establish if short periods of alternations between the various diets (ND, HFD, and HFD+R or HFD+oCA) would result in different levels of dyslipidemia, hepatosteatosis, and oxidative stress in rats. We found that feeding the rats rutin and *o*-coumaric acid for 8 weeks suppressed the increases in body weight, organ weight of the liver, and adipose tissue weights of peritoneal and epididymal fat induced by a HFD (**Figure 1**A and **Table 2**).

Adipose tissue is vital for maintaining whole body energy homeostasis and consists of adipocytes, which store TAG as a fuel for the body. Lavie and Milani (*21*) indicated that obesity adversely affects plasma lipids, especially by increasing TAG and decreasing HDL-cholesterol level. Jayakumar et al. (*22*) indicated that the HFD might lead to an increase in the synthesis of phospholipids and cholesterol esters in rats. We found that the HFD+R (HD) group had significantly decreased levels of TAG, phospholipids, total cholesterol, and LDL-cholesterol (**Table 3**). Serum levels of phospholipids and LDL-cholesterol in the HFD+oCA groups were significantly decreased as compared to those in the HFD group. Fried et al. (*23*) indicated that basal levels of leptin are known to be strongly positively correlated with body fat in rats fed a high-fat diet. Serum levels of insulin and leptin in the HFD+R (HD) and HFD+oCA (HD) groups were significantly decreased as compared to those in the HFD group. Our data indicated that intake of rutin and *o*-coumaric acid (50 and 100 mg/kg rat) for 8 weeks in Wistar rats did not affect serum biochemical parameters (AST, ALT, uric acid, creatinine, Na^+, K^+, and Cl^-). Hasumura et al. (*24*) reported that intake of rutin for 13 weeks was determined to be a no-observed-adverse-effect level (NOAEL) and a no-observed effect level (NOEL) in male (539 mg/kg/day) and female (3227 mg/kg/day) Wistar rats. The data obtained for serum biochemical parameters (AST, ALT, uric acid, creatinine, Na^+, K^+, and Cl^-) might also support the dosage used as a no-observed-adverse-effect level (NOAEL) in male rats. Therefore, we assume that rutin and *o*-coumaric acid have an antiobesity effect via suppression of dyslipidemia, hepatosteatosis, and oxidative stress in obese rats.

The liver is the central organ for cholesterol, phospholipid, TAG, and lipoprotein metabolism. Human and animal studies indicate that hepatic steatosis results in accumulation of excess fatty acid, the end-product of *de novo* fatty acid synthesis (*25*). In the histology study (hematoxylin-eosin staining and oil-red O staining), the number of lipid droplets in the HFD+R and HFD+oCA groups was significantly reduced as compared to that of the HFD group (**Figure 2**A). We also found that intake of rutin or *o*-coumaric acid for 8 weeks suppressed the increases in the levels of hepatic TAG and cholesterol induced by a HFD (**Figure 2**B). Our previous study indicates that intake of gallic acid for 10 weeks suppressed the increases in the levels of hepatic TAG and cholesterol induced by a HFD (*6*).

Hyperlipidemia is known to enhance the risk of coronary heart disease, fatty liver disease, and carcinogenesis. ROSs play major roles in the initiation and progression of cardiovascular dysfunction associated with diseases such as hyperlipidemia (*26*). MDA, a stable metabolite of the free radical-mediated lipid peroxidation cascade, is widely used as a marker of lipid peroxidation (*27*). Lipid peroxide levels in the tissue were found to be significantly elevated in the HFD group. We found that the HFD+R (HD) and HFD+oCA (HD) groups showed significantly decreased MDA production in the liver (**Table 4**). The TEAC assay was originally utilized for analyses of plasma and other solutions (*28*). It is also useful for determination of overall antioxidant levels in tissue homogenates. Our data indicated that intake of rutin or *o*-coumaric acid for 8 weeks enhances the decreases in hepatic TEAC values induced by a HFD (**Table 4**). Enzymatic antioxidants such as SOD, catalase, or GPx can scavenge ROSs and free radicals or prevent their formation (*29*). We found that GSH contents were depleted in rats with obesity induced by a high-fat diet and were restored after treatment with rutin (**Table 4**). We also found that antioxidant enzyme activities (GPx, GRd, GST, SOD) in the HFD group were significantly decreased, and the HFD+R and HFD+oCA groups had significantly increased activities of antioxidant enzymes in liver.

The cytosolic enzyme GPDH appears to play an important role in the conversion process. GPDH occupies a central position in the triglyceride synthesis pathway, at the point where it branches from the glycolytic pathway (*19*). Our data indicate that the HFD+R and HFD+oCA groups had significantly decreased GPDH activity in the fat and liver tissues of rats with obesity induced by a high-fat diet (**Table 5**). Our previous study also indicates that antioxidants (capsaicin, rutin, and *o*-coumaric acid) can inhibit GPDH activity in 3T3-L1 adipocytes (*9, 30*).

In conclusion, the present results demonstrate for the first time that the addition of rutin or *o*-coumaric acid to the diet decreases body weight gain, the weights of liver and adipose tissue, serum parameters (TAG, phospholipid, total cholesterol, LDL-cholesterol, insulin, and leptin), and hepatic steatosis. Rutin

or *o*-coumaric acid reduced oxidative stress (reduced lipid peroxidation and GSSG, and enhanced TEAC, GSH, GSH/GSSG ratio, GPx, GRd, GST, and SOD) and GPDH activity in rats with obesity induced by a high-fat diet. These results provide initial evidence that rutin and *o*-coumaric acid may be useful for the treatment of obesity.

ABBREVIATIONS USED

AST, aspartate aminotransferase; ALT, alanine aminotransferase; CDNB, 1-chloro-2,4-dinitrobenzene; C/EBP, CCAAT/enhancer-binding proteins; FER, food efficiency ratio; GPDH, glycerol-3-phosphate dehydrogenase; GRd, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione S-transferase; GPx, glutathione peroxidase; HD, high dose; HDL, high-density lipoprotein; HFD, high-fat diet; H&E, hematoxylin/eosin; LD, low dose; LDL, low-density lipoprotein; MDA, malondialdehyde; ND, normal diet; oCA, *o*-coumaric acid; PPARγ, peroxisome proliferator-activated receptor γ; R, rutin; SOD, superoxide dismutase; TAG, triacylglycerol; TBA, thiobarbituric acid; TEAC, trolox equivalent antioxidant capacity.

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