Research Article

Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic *db/db* mice

Kwon-Il Seo[1], Myung-Sook Choi[2], Un Ju Jung[3], Hye-Jin Kim[2], Jiyoung Yeo[2], Seon-Min Jeon[4] and Mi-Kyung Lee[1]

[1] Department of Food and Nutrition, Sunchon National University, Jeonnam, Republic of Korea
[2] Department of Food Science and Nutrition, Kyungpook National University, Daegu, Republic of Korea
[3] Institute of Human Nutrition, College of Physicians and Surgeons, Columbia University, New York, USA
[4] Institute of Genetic Engineering, Kyungpook National University, Daegu, Republic of Korea

We investigated the effect of curcumin on insulin resistance and glucose homeostasis in male C57BL/KsJ-*db/db* mice and their age-matched lean non-diabetic *db/+* mice. Both *db/+* and *db/db* mice were fed with or without curcumin (0.02%, wt/wt) for 6 wks. Curcumin significantly lowered blood glucose and HbA_{1c} levels, and it suppressed body weight loss in *db/db* mice. Curcumin improved homeostasis model assessment of insulin resistance and glucose tolerance, and elevated the plasma insulin level in *db/db* mice. Hepatic glucokinase activity was significantly higher in the curcumin-supplemented *db/db* group than in the *db/db* group, whereas glucose-6-phosphatase and phosphoenolpyruvate carboxykinase activities were significantly lower. In *db/db* mice, curcumin significantly lowered the hepatic activities of fatty acid synthase, β-oxidation, 3-hydroxy-3-methylglutaryl coenzyme reductase, and acyl-CoA: cholesterol acyltransferase. Curcumin significantly lowered plasma free fatty acid, cholesterol, and triglyceride concentrations and increased the hepatic glycogen and skeletal muscle lipoprotein lipase in *db/db* mice. Curcumin normalized erythrocyte and hepatic antioxidant enzyme activities (superoxide dismutase, catalase, gluthathione peroxidase) in *db/db* mice that resulted in a significant reduction in lipid peroxidation. However, curcumin showed no effect on the blood glucose, plasma insulin, and glucose regulating enzyme activities in *db/+* mice. These results suggest that curcumin seemed to be a potential glucose-lowering agent and antioxidant in type 2 diabetic *db/db* mice, but had no affect in non-diabetic *db/+* mice.

Keywords: Antioxidant / Curcumin / Glucose homeostasis / Insulin resistance / Type 2 diabetes

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1 Introduction

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione] is the active component in Turmeric Rhizomes (*Curcuma Long Linn*), which are the major component of spices turmeric and curry. These spices have been widely used in traditional medicine in Southeast Asia, and their numerous biological effects have been associated with curcumin [1]. Practitioners of traditional Indian medicine believe that curcumin powder prevents many diseases including biliary disorders, anorexia, cough, diabetes, hepatic disorders, rheumatism, sinusitis, cancer, and Alzheimer's [2]. Several studies have indicated that curcumin plays a beneficial role in terms of being an antioxidant, anti-tumurogenic, and anti-inflammatory agent [3].

A recent study showed that curcumin-treated diabetic rats had lower blood glucose and glycated hemoglobin levels, in association with lower oxidative stress [4]. Fur-

Correspondence: Dr. Mi-Kyung Lee, Department of Food and Nutrition, Sunchon National University, 315 Maegok, Suncheon, Jeonnam, 540-742, Republic of Korea
E-mail: leemk@sunchon.ac.kr
Fax: +82-61-750-3608

Abbreviations: ACAT, acyl-CoA: cholesterol acyltransferase; **CAT,** catalase; **CPT**, carnitine palmitoyltransferase; **FAS**, fatty acid synthase; **FFA,** free fatty acid; **GK,** glucokinase; **G6Pase**, glucose-6-phosphatase; **GSH-Px**, glutathione peroxidase; **HbA_{1c},** glycosylated hemoglobin; **HOMA-IR,** homeostatic index of insulin resistance; **IPGTT,** intraperitoneal glucose tolerance test; **LPL,** lipoprotein lipase; **MDA,** malondialdehyde; **PEPCK**, phosphoenolpyruvate carboxykinase; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase

thermore, treatment with curcumin has been shown to reduce reactive oxygen species (ROS) levels in cells that are isolated from diabetic patients [5]. Experimental studies with diabetic animals demonstrated that curcumin supplementation can suppress cataract development [6] and the cross-linking of collagen [7], promotes wound healing [8], and lower blood lipid and glucose levels in a streptozotocin-treated diabetic animal model [9].

Glucose homeostasis is regulated primarily by the liver and skeletal muscle. Following a meal, most glucose disposal occurs in skeletal muscle, whereas fasting plasma glucose levels are determined primarily by a glucose output from the liver [10]. In particular, hepatic insulin resistance is associated with increased free fatty acid (FFA) that lowers the ability of insulin to suppress hepatic glucose production by activating gluconeogenesis yet inhibiting glycolysis [11]. Therefore, this study was to examine the effect of curcumin on insulin resistance, glucose homeostasis, and oxidative damage in C57BL/KsJ-*db/db* mice, a good model for type 2 diabetes that displays many of the characteristics of human disease including hyperphagia, hyperglycemia, insulin resistance, and progressive obesity [12].

2 Materials and methods

2.1 Animals and diets

Sixteen male C57BL/KsJ *db/db* mice and sixteen lean heterozygote non-diabetic *db/+* were purchased from Jackson Laboratory (Bar Harbor, ME). The animals were individually housed in stainless steel cages in a room at 22 ± 2°C on a 12 h light-dark cycle. The five week old *db/db* mice and *db/+* mice were fed a pelletized commercial chow diet for 2 wks after arrival, then the *db/+* and *db/db* mice were randomly divided into two groups (n=8), respectively. Thereafter, both *db/+* mice and *db/db* mice were fed a standard semisynthetic diet (AIN-76) [13, 14] with curcumin (0.2 g/kg diet, Sigma) or without for 6 wks. The mice had access to food and water *ad libitum*. At the end of the experimental period, the mice were anesthetized with Ketamine after withholding food for 12 h, and blood samples were taken from the inferior vena cava to determine the plasma biomarkers. Also, the liver was removed after collecting the blood, and rinsed with a physiological saline solution. All mice were treated in accordance with Sunchon National University Guidelines for the Care and Use of Laboratory Animals.

2.2 Blood glucose and glycosylated hemoglobin (HbA_{1c}) concentrations

The blood glucose concentration was monitored in venous blood drawn from the tail vein using a glucometer (Allmeicus, Korea) every week after a 6 h fast. The blood HbA_{1c} concentration was measured after hemolysis of the anticoagulated whole blood specimen. HbA_{1c} was determined immuno-turbidinetrically.

2.3 Intraperitoneal glucose tolerance test (IPGTT)

An IPGTT was performed at the fifth week. Following a 6 h fast, the mice were injected intraperitoneally with glucose at 1 g/kg body weight, and the blood glucose levels were determined in tail blood samples taken 0 (prior to glucose administration), 30, 60, and 120 min after the glucose administration.

2.4 Homeostatic index of insulin resistance (HOMA-IR)

HOMA-IR was calculated according to the homeostasis of the assessment as follows (Eq. 1) [15]:

$$\text{HOMA-IR} = [\text{fasting glucose (mmol/L)} \times \text{fasting insulin (}\mu\text{L U/mL)}]/22.51$$

2.5 Plasma biomarkers

Plasma insulin (Diagnostic System Laboratories, USA) and leptin (Linco, USA) levels were determined using radioimmunoassay kits. The plasma total cholesterol and triglyceride concentrations were determined using an enzymatic method (Sigma), while the plasma FFA concentration was determined using an enzymatic colorimetric method (Wako Chemicals, Richmond, VA).

2.6 Sample preparation

Blood samples were collected from the inferior vena cava using heparin-coated tubes. After centrifugation at $1000 \times g$ for 15 mim at 4°C, the plasma and buffy coat were carefully removed. The separated cells were then washed three times by resuspension in a 0.9% NaCl solution and the centrifugation was repeated. The washed cells were lyzed in an equal volume of water and mixed thoroughly. The hemoglobin concentration was estimated in an aliquot of the hemolysate, using a commercial assay kit (No. 525-A, Sigma). An appropriate dilution of the hemolysate was then prepared from the erythrocytes suspension by the addition of distilled water to estimate the catalase (CAT) and glutathione peroxidase (GSH-Px) activities.

In addition, to remove the hemoglobin by precipitation with chloroform:ethanol [16], 0.2 mL of an ethanol:choloroform (3:5, v/v) mixture was added to an aliquiot (0.5 mL) of the hemolysate cooled in ice. This mixture was stirred constantly for 15 min and then diluted with 0.1 mL of water. After centrifugation for 10 min at $1600 \times g$, the pale yellow supernatant was separated from the protein precipitate and used to assay superoxide dismutase (SOD).

The enzyme source fraction in the liver was prepared according to the method developed by Hulcher and Oleson

[17] with slight modifications. A 20% w/v homogenate was prepared in a buffer containing 0.1 mol/L triethanolamine, 0.02 mol/L EDTA and 2 mmol/L DTT (pH 7.0), then centrifuged at $600 \times g$ for 10 min to discard any cell debris, and the supernatant centrifuged at $10\,000 \times g$ followed by $12\,000 \times g$ for 20 min at 4°C to remove mitochondrial pellets. Thereafter, the supernatant was ultracentrifuged twice at $100\,000 \times g$ for 60 min at 4°C to obtain the cytosolic supernatant. The mitochondrial and microsomal pellets were then redissolved in 800 μL of a homogenization buffer and the protein content was determined by method of Bradford [18] using BSA as the standard.

2.7 Hepatic glucose regulating enzyme activities and glycogen content

Glucokinase (GK) activity was determined using a spectrophotometric continuous assay as described by Davidson and Arion [19] and Newgard *et al*. [20] with slight modifications, where the formation of glucose-6-phosphate was coupled to its oxidation by glucose-6-phosphate dehydrogenase and NAD^+ at 37°C. Glucose-6-phosphatase (G6Pase) activity was determined using the method of Alegre *et al*. [21] with slight modifications; the reaction mixture contained 40 mmol/L of sodium HEPES (pH 6.5), 14 mmol/L of glucose-6-phosphate, 18 mmol/L of EDTA, both previously adjusted to pH 6.5, 2 mmol/L of $NADP^+$, 0.6 IU/mL of mutarotase and 0.6 IU/mL of glucose dehydrogenase. Phosphoenolpyruvate carboxykinase (PEPCK) activity was monitored in the direction of oxaloacetate synthesis using the spectrophotometric assay developed by Bentle and Lardy [22] with slight modifications. The final volume of the purified enzyme (1 mL) was pipetted with a reaction mixture (pH 7.0) containing; 77 mmol/L sodium HEPES, 1 mmol/L inosine 5′-diphosphate, 1 mmol/L $MnCl_2$, 1 mmol/L DTT, 0.25 mmol/L NADH, 2 mmol/L phosphoenolpyruvate, 50 mmol/L of $NaHCO_3$ and 7.2 U malic dehydrogenase. The enzyme activity was then measured for 2 min at 25°C based on a decrease in the absorbance at 340 nm.

Hepatic glycogen concentration was determined as described previously by Seifter *et al*. [23] with modifications. The liver tissue was homogenized in five volumes of a 30% w/v KOH solution and dissolved at 100°C for 30 min. The glycogen was determined by treatment with an anthrone reagent and measuring the absorbance at 620 nm.

2.8 Hepatic lipid regulating enzyme activities

Fatty acid synthase (FAS) activity was determined as described by Nepokroeff *et al*. [24] with slight modifications. The cytosolic enzyme (100 μL) was mixed with 125 mmol/L of potassium phosphate buffer (pH 7.0), 165 μmol/L of acetyl-CoA, 50 μmol/L of malonyl-CoA, 50 μmol/L of NADPH, 1 mmol/L of β-mercaptoethanol and 1 mmol/L EDTA. Absorbance was then measured for 2 min at 340 nm (30°C) on a spectrophotometer. β-Oxidation activity was determined as described by Lazarow [25] with slight modifications, where the reaction was initiated by adding 47 mmol/L of Tris-HCl (pH 8.0), 0.2 mmol/L of NAD, 990 μmol/L DTT, 5 μL albumin (1.5%), 5 μL Triton X-100 (2%), 0.1 mmol/L CoA, 0.01 mmol/L FAD, 1 mmol/L KCN and 5 μL of the mitochondrial fraction, then 10 μmol/L palmitoyl-CoA was added. The formation of NADH was measured for 5 min at 340 nm (37°C) on a spectrophotometer. The carnitine palmitoyltransferase (CPT) was assayed spectrophotometrically by following the release of CoA-SH from palmitoyl-CoA using the general thiol reagent 5,5′-dithiobis (2-nitrobenzoate), DTNB, as described by Bieber *et al*. [26] with slight modifications. The reaction mixture contained 0.1 mL aliquot of a premix containing 232 mmol/L Tris-HCl (pH 8.0), 1.1 mmol/L EDTA, 220 μmol/L L-carnitine, 24 μmol/L of DTNB, 7 μmol/L palmitoyl-CoA and 0.09% Triton X-100. The reaction was initiated by the addition of enzyme at 25°C. Absorbance was measured for 2 min at 412 nm on a spectrophotometer. The HMG-CoA reductase activities were determined in the microsomal fraction with [^{14}C]-HMG-CoA as the substrate based on a modification of the method of Shapiro *et al*. [27]. The activity was expressed as the synthesized mevalonate pmol/min/mg protein. The acyl-CoA:cholesterol acyltransferase (ACAT) activities were determined by the rate of the incorporation of [^{14}C]-Oleoyl CoA into cholesterol ester fractions, as described by Erickson *et al*. [28] and modified by Gillies *et al*. [29]. The activity was expressed as synthesized cholesteryloleate pmol/min/mg protein.

2.9 Lipoprotein lipase (LPL) activity in adipose tissue and skeletal muscle

A 10% w/v homogenate was prepared in a detergent-containing buffer (25 mmol/L ammonium chloride, 5 mmol/L EDTA, 10 mg/mL Triton X-100, 1 mg/mL SDS, 5 IU/mL heparin, 10 μg/mL leupeptin, 1 μg/mL pepstatin A, 3.5 μg/mL aprotinin, pH 8.5), then centrifuged at $20\,000 \times g$ (4°C) for 20 min to obtain the supernatant. Thereafter, LPL activity was determined according to the method developed by Nilsson-Ehle and Schotz [30] with slight modifications. To prepare the substrate, 600 mg of triolein, 36 mg of phosphatidyl cholin (egg yolk, Sigma) and ^{3}H-triolein (2.5×10^9 dpm, specific activity; 22 Ci/mmol) were added and dried in N_2 gas. The dried substrate was then added to 10 mL of glycerol and sonicated. The prepared substrate, heat-inactivated serum (at 56°C for 30 min), and a 0.3 mol/L of Tris-HCl (pH 8.5) buffer containing 0.2 mol/L NaCl, 0.02% w/v heparin and 12% w/v BSA were then used for the assay mixture and 120 μL preincubated at 37°C for 5 min. Next, the reaction was initiated by adding 5 μL of the tissue preparations and 75 μL of distilled water to make a final volume of

0.2 mL, which was then heated at 37°C for 1 h. The FFA that formed was isolated using a liquid-liquid partition system [31]. Thereafter, the reaction was stopped by the addition of 3.25 mL of methanol:chloroform:heptane (1.41:1.25:1, v:v:v) and 1.05 mL of a 0.1 mol/L sodium carbonate buffer (pH 10), then the reaction mixture was centrifuged for 15 min at 1500 × *g*. Finally, 0.4 mL of the supernatant was subjected to scintillation counting (Packard Tricarb 1600TR, Packard, Australia).

2.10 Antioxidant enzyme activities

SOD activity was spectrophotometrically measured using a modified version of the method developed by Marklund and Marklund [32]. Briefly, SOD was detected on the basis of its ability to inhibit superoxide-mediated reduction. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. CAT activity was measured using Aebi's [33] method with slight modifications, in which the disappearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$ was used to determine CAT activity. The GSH-Px activity was measured using Paglia and Valentine's [34] method with slight modifications. The reaction mixture contained 1 mmol/L glutathione, 0.2 mmol/L NADPH, and 0.24 units of glutathione reductase in a 0.1 mol/L Tris-HCl (pH 7.2) buffer. The reaction was initiated by adding 0.25 mmol/L H_2O_2 and the absorbance was measured at 340 nm for 5 min. A molar extinction coefficient of 6.22 $mM^{-1}cm^{-1}$ was used to determine the activity.

2.11 Lipid peroxidation

As a marker of lipid peroxidation production, the erythrocyte or hepatic malondialdehyde (MDA) concentrations were measured using the method of Ohkawa *et al*. [35]. Two hundred microliters of the erythrocyte and hepatic homogenate (20%, w/v) were mixed with 200 μL of 8.1% w/v SDS, 1.5 mL of 20% w/v acetic acid (pH 3.5), and 1.5 mL of 0.8% w/v thiobarbituric acid. The reaction mixture was then heated at 95°C for 60 min. After cooling, the hepatic mixture was added to 1.0 mL of distilled H_2O and 5.0 mL of a butanol:pyridine (15:1) solution. The reaction mixture was then centrifuged at 800 × *g* for 15 min and the resulting colored layer was measured at 532 nm using 1,1,3,3-tetraethoxypropane (Sigma) as the standard.

2.12 Statistical analysis

All data are presented as the mean ± SE. Statistical analyses were performed using the SPSS program (SPSS, Chicago, IL). Student's *t*-test was used to assess the differences between the groups. The *db*/+ group was compared with the *db*/+ curcumin, *db/db,* and *db/db* curcmin groups. The effect of the curcumin supplement was also compared within the type 2 diabetic mice groups. Values of $p < 0.05$ were considered to be statistically significant.

3 Results

3.1 Body weight and food intake

As shown in Table 1, body weights in the *db/db* groups were significantly higher than in the non-diabetic *db/+* groups. We observed that the body weight reduction of *db/db* mice was significant at 6 wks (30.80 ± 1.24 g *vs*. 33.03 ± 1.08 g, $p < 0.05$). Curcumin supplement did not affect body weight of *db/+* mice, however it suppressed a reduction in body weight within the *db/db* groups. The final body weight of the curcumin-supplemented mice was significantly higher compared to the *db/db* group by 1.5-fold (Table 1). During the experimental period, the food intake in the *db/db* groups were also higher than in the *db/+* groups, however curcumin did not affect the food intake in both *db/+* and *db/db* groups (data not shown).

3.2 Fasting blood glucose level and IPGTT

Baseline (0 wk) fasting glucose levels did not differ between the groups, however the blood glucose values of the *db/db* groups were significantly higher than those of *db/+* groups during the six week testing period (Fig. 1). Curcumin supplement did not change blood glucose level in non-diabetic group, however it significantly lowered blood glucose level in diabetic *db/db* group starting from the first week. This resulted in lowering the glucose level by 22% in the *db/db* mice at the sixth week (Fig. 1). Although we also did not observe change of IPGTT in *db/+* mice, curcumin significantly improved the glucose tolerance in *db/db* groups over the entire IPGTT (Fig. 2). As such, the curcu-

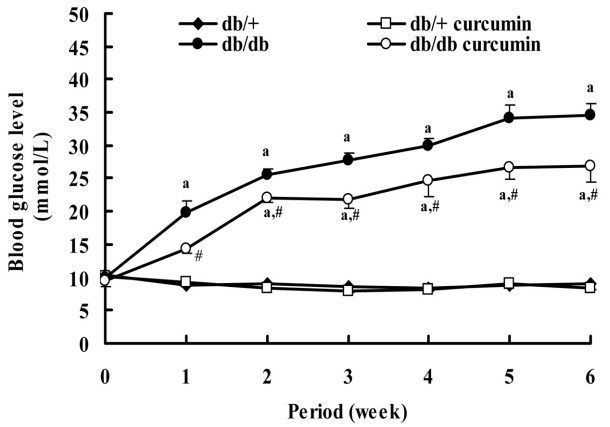

Figure 1. Blood glucose level in *db/db* mice and *db/+* mice fed diet supplemented with curcumin. Values are expressed as mean ± SE, [a] $p < 0.05$ *vs.* to *db/+* group based on Student's *t*-test, [#] $p < 0.05$ *vs.* to *db/db* group based on Student's *t*-test.

Table 1. Body weight in *db/db* mice and *db/+* mice fed diet supplemented with curcumin[a)]

	db/+	*db/+* curcumin	*db/db*[b)]	*db/db* curcumin[b)]
Body weight (g)				
0 wk	20.33 ± 1.21	20.17 ± 0.51	32.07 ± 0.99	32.04 ± 0.93
1 wk	21.96 ± 1.53	21.47 ± 0.45	34.22 ± 0.88	34.88 ± 0.79
2 wks	22.80 ± 1.75	22.40 ± 0.44	35.28 ± 087	36.78 ± 1.07
3 wks	23.56 ± 1.67	23.00 ± 0.66	35.16 ± 0.86	37.17 ± 1.40
4 wks	24.16 ± 1.47	23.95 ± 0.81	34.00 ± 1.03	36.77 ± 1.79
5 wks	25.06 ± 1.40	24.72 ± 0.72	33.03 ± 1.08	36.21 ± 1.24
6 wks	24.70 ± 1.55	25.05 ± 0.68	30.80 ± 1.24	35.37 ± 1.42[c)]

a) Values are expressed as mean ± SE.
b) $p < 0.05$ *vs. db/+* group based on Student's *t*-test.
c) $p < 0.05$ *vs. db/db* group based on Student's *t*-test.

min improved fasting blood glucose and postprandial glucose level in only type 2 diabetic mice.

3.3 Blood HbA_{1c} concentration, plasma biomarkers, and HOMA-IR

Blood HbA_{1c} concentrations in the *db/db* groups were approximately 3-fold higher than in the non-diabetic *db/+* groups, however the curcumin supplement significantly lowered the blood HbA_{1c} concentration within the *db/db* mice groups by 7.8% (Table 2). The plasma leptin concentrations were significantly higher in the *db/db* groups than in the non-diabetic *db/+* groups. The curcumin supplementation within the *db/db* groups elevated the leptin concentration compared to the diabetic control *db/db* group by 1.6-fold (Table 2). The plasma insulin level of the curcumin-supplemented *db/db* mice was significantly higher than in the control *db/db* mice by 17% (Table 2). HOMA-IR in the *db/db* groups was significantly higher than in the *db/+* groups by 7-fold, however curcumin significantly improved HOMA-IR in *db/db* mice (Table 2). No differences were observed within the non-diabetic *db/+* groups.

3.4 Plasma lipid levels

Plasma fatty acid, total cholesterol, and triglyceride concentrations were significantly higher in the *db/db* group than in the *db/+* group, however the curcumin supplement significantly lowered these lipid profiles by 17, 19, and 10%, respectively, within the type 2 diabetic mice groups (Table 2). No differences were observed within the non-diabetic *db/+* groups.

3.5 Hepatic glucose regulating enzyme activities and glycogen level

Hepatic GK activity was significantly lower in the *db/db* group than in the *db/+* group, yet the G6Pase and PEPCK activities were significantly higher in the *db/db* groups compared to the *db/+* groups (Table 3). Curcumin supplement significantly elevated hepatic GK activity within the *db/db* groups by 42%, while it significantly suppressed the elevation of hepatic gluconeogenic enzyme activities, G6Pase and PEPCK, in *db/db* mice (Table 3). The hepatic glycogen concentration in the *db/db* group was about 2.5 times higher than in the *db/+* group. The curcumin supplement significantly elevated glycogen storage in the liver within the *db/db* groups (Table 3). In non-diabetic *db/+* mice, curcumin did not alter hepatic GK, P6Pase, and PEPCK activities and glycogen content.

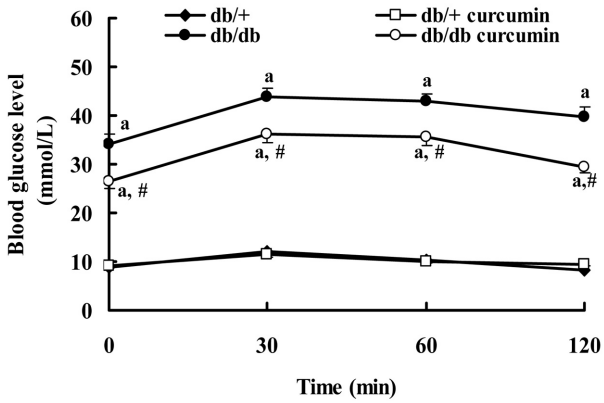

Figure 2. Glucose tolerance test in *db/db* mice and *db/+* mice fed diet supplemented with curcumin. After a 6 h fast, male mice (12 wks old) were intraperitoneally injected with glucose (1 g/kg body weight). The blood glucose concentration was measured at the indicated times. Values are expressed as mean ± SE, [a] $p < 0.05$ *vs.* to *db/+* group based on Student's *t*-test, [#] $p < 0.05$ *vs.* to *db/db* group based on Student's *t*-test.

3.6 Hepatic lipid regulating enzyme activities

The activities of hepatic FAS, β-oxidation, CPT, HMG-CoA reductase, and ACAT were significantly higher in the *db/db* group than in the non-diabetic *db/+* group (Table 4). The elevated hepatic lipid regulating enzymes activities were significantly lower in the curcumin-supplemented group compared to the *db/db* group (Table 4). No differences were observed within the non-diabetic *db/+* groups.

Table 2. Blood HbA1c, plasma leptin, insulin and lipids levels, and HOMA-IR in *db/db* mice and in *db/+* mice fed diet supplemented with curcumin[a)]

	db/+	*db/+* curcumin	*db/db*	*db/db* curcumin[b)]
HbA_{1c} (%)	4.77 ± 0.02	4.60 ± 0.06	14.29 ± 0.02[c)]	13.18 ± 0.02[c)]
Leptin (μg/L)	7.50 ± 0.71	7.10 ± 0.49	21.84 ± 12.22[c)]	34.90 ± 2.92[c)]
Insulin (μL U/mL)	47.33 ± 1.33	47.50 ± 2.50	48.16 ± 1.22	56.40 ± 2.20
HOMA-IR[d)]	18.71 ± 1.14	17.50 ± 1.74	74.92 ± 1.16[c)]	67.15 ± 2.77[c)]
FFA (mmol/L)	0.39 ± 0.02	0.38 ± 0.01	0.85 ± 0.01[c)]	0.71 ± 0.01[c)]
TC (mmol/L)	3.36 ± 0.12	3.46 ± 0.18	4.76 ± 0.27[c)]	3.85 ± 0.22
TG (mmol/L)	1.22 ± 0.14	1.05 ± 0.10	1.77 ± 0.08[c)]	1.59 ± 0.12[c)]

FFA: free fatty acid, TC: total cholesterol, TG: triglyceride.
a) Values are expressed as mean ± SE.
b) $p < 0.05$ *vs.* to *db/db* group based on Student's *t*-test.
c) $p < 0.05$ *vs.* to *db/+* group based on Student's *t*-test.
d) HOMA-IR: calculated from glucose (mmol/L) × fasting insulin (μL U/mL)/22.5.

Table 3. Hepatic GK, G6Pase and PEPCK activities and glycogen level in *db/db* mice and *db/+* mice fed diet supplemented with curcumin[a)]

	db/+	*db/+* curcumin	*db/db*[b)]	*db/db* curcumin[c)]
GK (nmol/min/mg protein)	21.85 ± 3.71	21.00 ± 1.03	11.38 ± 1.57	27.39 ± 1.91
G6Pase (nmol/min/mg protein)	149.45 ± 3.67	147.38 ± 1.49	280.43 ± 6.71	210.56 ± 8.33[b)]
PEPCK (nmol/min/mg protein)	9.12 ± 0.09	8.41 ± 0.12	25.63 ± 0.78	21.93 ± 0.54[b)]
Glycogen (mg/g of liver)	41.63 ± 2.91	38.86 ± 3.90	86.10 ± 4.84	108.08 ± 5.41[b)]

a) Values are expressed as mean ± SE.
b) $p < 0.05$ *vs.* to *db/+* group based on Student's *t*-test.
c) $p < 0.05$ *vs.* to *db/db* group based on Student's *t*-test.

3.7 LPL activities in adipose tissue and skeletal muscle

The LPL activities of adipose tissue were significantly lower in the *db/db* groups than in the non-diabetic *db/+* groups, whereas, those of the skeletal muscle were significantly higher in the *db/db* groups (Fig. 3). Although curcumin did not affect adipose tissue LPL activity, skeletal muscle LPL activity was significantly higher in the curcumin-supplemented *db/db* mice than in the *db/db* groups (Fig. 3). No differences were observed within the non-diabetic *db*/+ groups.

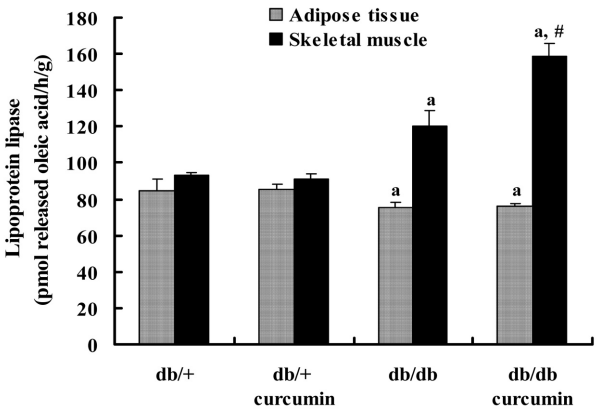

Figure 3. LPL activities of adipose tissue and skeletal muscle in *db/db* mice and *db/+* mice fed diet supplemented with curcumin. Values are expressed as mean ± SE, [a] $p < 0.05$ *vs.* to *db/+* group based on Student's *t*-test, [#] $p < 0.05$ *vs.* to *db/db* group based on Student's *t*-test.

3.8 Antioxidant defense enzyme activities and lipid peroxide in the erythrocytes and liver

Erythrocyte SOD and CAT activities were significantly higher in the *db/db* group than in the *db/+* group, but the GSH-Px activity was lower (Table 5). Supplementation with curcumin lowered the erythrocyte SOD and CAT activities and elevated GSH-Px activity in *db/db* groups. Hepatic SOD activity did not differ between the groups, however CAT and GSH-Px activities were higher in the *db/db* group than in the *db/+* group. These increased CAT and GSH-Px activities were significantly lowered by the curcumin supplement when compared within the *db/db* groups (Table 5).

As such curcumin seemed to normalize altered erythrocyte and hepatic antioxidant enzyme activities within the *db/db* mice. Erythrocyte and hepatic MAD levels, index of lipid peroxide, were significantly higher in *db/db* group than in the *db/+* group by 1.5-fold and 1.8-fold, respectively (Table 5). However, curcumin effectively lowered MDA levels in

Table 4. Hepatic FAS, β-oxidation, CPT, HMG-CoA reductase and ACAT activities in *db/db* mice and *db/+* mice fed diet supplemented with curcumin[a)]

	db/+	*db/+* curcumin	*db/db*[b)]	*db/db* curcumin[c)]
FAS (nmol/min/mg protein)	15.55 ± 0.88	11.57 ± 1.77	28.92 ± 3.45	14.01 ± 1.73
β-oxidation (nmol/min/mg protein)	2.86 ± 0.27	2.60 ± 0.31	19.60 ± 1.57	3.07 ± 0.31
CPT (nmol/min/mg protein)	1.98 ± 0.08	2.03 ± 0.12	14.28 ± 1.08	3.85 ± 0.18
HMG-CoA reductase (pmol/min/mg protein)	191.03 ± 5.49	179.75 ± 5.49	243.86 ± 7.89	204.83 ± 9.91
ACAT (pmol/min/mg protein)	171.84 ± 2.30	184.56 ± 4.92	305.63 ± 13.13	207.66 ± 12.68

a) Values are expressed as mean ± SE.
b) $p < 0.05$ *vs.* to *db/+* group based on Student's *t*-test.
c) $p < 0.05$ *vs.* to *db/db* group based on Student's *t*-test.

Table 5. Antioxidant enzyme activities and MDA level in *db/db* mice and *db/+* mice fed diet supplemented with curcumin[a)]

	db/+	*db/+* curcumin	*db/db*	*db/db* curcumin
Erythrocytes				
SOD (unit/g Hb)	0.78 ± 0.09	0.68 ± 0.10	1.61 ± 0.05[b)]	0.66 ± 0.03[c)]
CAT (μmol/min/g Hb)	50.56 ± 4.64	53.11 ± 4.95	73.72 ± 3.12[b)]	62.20 ± 3.35[c)]
GSH-Px (μmol/min/g Hb)	8.84 ± 0.44	10.99 ± 0.63[b)]	6.41 ± 0.22[b)]	7.54 ± 0.15[b, c)]
MDA (nmol/g Hb)	310.26 ± 16.65	317.76 ± 11.32	479.72 ± 19.16[b)]	393.36 ± 23.39[b, c)]
Liver				
SOD (unit/mg protein)	33.16 ± 1.80	36.23 ± 0.81	36.79 ± 1.18	33.65 ± 1.85
CAT (μmol/min/mg protein)	0.80 ± 0.05	0.59 ± 0.07	2.14 ± 0.18[b)]	1.21 ± 0.10[c)]
GSH-Px (nmol/min/mg protein)	8.83 ± 0.27	8.64 ± 0.08	11.15 ± 0.11[b)]	8.54 ± 0.52[c)]
MDA (nmol/g of liver)	14.31 ± 1.02	14.91 ± 0.89	25.22 ± 0.62[b)]	15.78 ± 1.33[c)]

a) Values are expressed as mean ± SE.
b) $p < 0.05$ *vs.* to *db/+* group based on Student's *t*-test.
c) $p < 0.05$ *vs.* to *db/db* group based on Student's *t*-test.

the erythrocytes and liver within the *db/db* groups (Table 5), but no differences were observed within the non-diabetic *db*/+ groups.

4 Discussion

This study demonstrates that the dietary curcumin supplement improved insulin resistance and hyperglycemia in *db/db* mice, obese-diabetic animals with insulin resistance, but had no effects on non-diabetic *db/+* mice. In the present study, curcumin significantly lowered blood glucose levels and HOMA-IR when compared to those in the diabetic control *db/db* mice by 22 and 10%, respectively. HOMA-IR has been suggested as a biomarker to assess insulin resistance and secretion and is a useful clinical index for insulin sensitivity and pancreatic β-cell functions in epidemiological studies [15]. Although HOMA-IR has several limitations in terms of accuracy and reliability [36], it expresses essentially hepatic insulin resistance [37]. We also observed curcumin significantly improved the glucose tolerance within *db/db* groups without changing of IPGTT in *db/+* mice.

The *db/db* mouse, which has a mutation in the leptin receptor gene that caused abnormal splicing, acquires obesity and develops type 2 diabetes as a consequence of loss of leptin function [38]. This type of genetic control over the pathogenetic mechanisms can lead to insufficient insulin secretion [39]. These results show that curcumin is able to improve insulin resistance with a simultaneous increase in plasma leptin and insulin levels. The plasma insulin levels of *db/db* mice increase rapidly during the first weeks of life, but they dramatically decrease at an age 10 – 12 wks to normal or less than normal levels [40], resulting in drastic body weight loss at the time of death [12]. However, the curcumin supplement suppressed body weight loss in *db/db* mice by sustaining plasma leptin and insulin levels. Curcumin increased plasma leptin and insulin levels by 60 and 17% in *db/db* groups, respectively, however it did not affect those of *db/+* mice. Although, we can not provide direct evidence for the effect of curcumin on insulin release in *db/db* mice, recently curcumin was reported to enhance insulin release by induction of β-cell electrical activity [41]. In our previous studies, plasma insulin levels showed a positive correlation with leptin levels in *db/db* mice [42, 43]. Insulin induces leptin synthesis and secretion [44] through the regulation of glucose metabolism in adipocytes [45]. The exact relationship between increased leptin levels and decreased blood glucose level could not be clarified, however we

observed a negative correlation between plasma leptin and blood glucose concentration ($r = -0.622$, $p < 0.01$) within *db/db* mice. Harris *et al.* [46] reported that chronic intraperitonal leptin infusion (7 days) significantly reduced fasting glucose in male C57BL/Ks-*db/db* mice, by suggesting that it is mediated by leptin receptors other than the hypothalamic long-form receptors. However, no clear explanation is available at this moment.

Insulin resistance profoundly contributes to the pathophysiology of type 2 diabetes and it reduces glucose utilization and increases glucose production from the liver, thus leading to hyperglycemia [47]. The liver plays a unique role in controlling carbohydrate metabolism by maintaining a normal range of glucose concentration. Knowledge of the processes involved in maintaining glucose homeostasis as well as insulin resistance is a prerequisite for developing new therapeutic approach in diabetes as well as in liver disease [48]. Our results show that curcumin influences the glucose and lipid metabolism in the liver as well as in muscle. We observed hepatic GK activity was significantly lower in the *db/db* mice than in the *db/+* mice, whereas glycogen content of *db/db* mice was higher compared to the *db/+* mice. These findings contrast with those of previous reports by Yen and Stamm [49] who showed that GK activity was higher in the *db/db* mice than in the control mice, and by Roesler and Khandelwal [50] who reported little difference in hepatic glycogen levels between *db/db* and *db/+* mice. However, Coleman and Hummel [51] reported glycogen level was 2–3-fold higher in diabetic mice relative to control. In *db/db* mice, curcumin increased hepatic GK activity and glycogen content, while it inhibited G6Pase and PEPCK activities. In our previous studies, we also found that similar amounts of various flavonoid compounds increased GK activity and its mRNA expression and lowered G6Pase and PEPCK activities and their mRNA expression in the liver of *db/db* mice [42, 52]. These results suggest that down-regulation of gluconeogenic enzymes and the up-regulation of glycolytic enzymes by curcumin have contributed towards reduced blood glucose concentration in curcumin-supplemented *db/db* mice.

Prolonged exposure to high fatty acid concentrations (lipotoxicity) influences both insulin action and insulin secretion, and it can contribute directly to the deterioration of pancreatic β-cell functions that accompanies the development of diabetes [53]. In our study, the plasma FFA and triglyceride concentrations were significantly higher in the *db/db* group than in the *db/+* group by 2.2-fold and 1.5-fold, however the curcumin supplement lowered those of *db/db* mice. LPL is a rate-limiting enzyme that hydrolyzes circulating triglyceride, leading to the generation of FFA, which store triglyceride in adipose tissue and serve energy source in skeletal muscle and heart [54]. Impaired insulin action not only stimulates lipolysis, increasing delivery of FFA to the liver and consequently increasing production of hepatic triglyceride but also reduces LPL activity [55]. We also observed similar results that the LPL activity in adipose tissue was significantly lower in the diabetic *db/db* group than in the non-diabetic *db/+* group, but hepatic triglyceride concentration was significantly higher in the diabetic *db/db* group (13.69 ± 0.40 mg/g *vs.* 10.86 ± 0.12 mg/g, $p < 0.05$). Interestingly, curcumin did not affect the LPL activity in adipose tissue and hepatic triglyceride concentration in *db/db* mice (data not shown). The LPL activity in skeletal muscle was significantly higher in the diabetic *db/db* group than in the non-diabetic *db/+* group. This muscle LPL activity was even further elevated by curcumin supplement in *db/db* group. Our results are consistent with other's report that the activation of LPL in adipose tissue is delayed, whereas LPL activity in skeletal muscle is increased by hyperinsulinemia in obesity and type 2 diabetes [56]. Somehow, curcumin supplement only elevated LPL activity in skeletal muscle of *db/db* mice. This appeared to contribute to lower plasma triglyceride concentration in curcumin-supplemented *db/db* mice, although further study is required to support the action of curcumin on altering the LPL activity in skeletal muscle. Furthermore, insulin resistance is commonly associated with several abnormalities in lipid metabolism, including increased plasma fatty acid levels, hypertriglycemia, hypercholesterolemia and enhanced hepatic lipogenesis. In the current study, we observed that the hepatic FAS, β-oxidation, CPT, HMG-CoA reductase and ACAT activities were significantly higher in the *db/db* mice than in the *db/+* mice, however the supplementation of curcumin significant lowered these lipid regulating enzyme activities in *db/db* mice. Curcumin significantly lowered plasma FFA and cholesterol concentrations by altering their metabolic enzyme activities.

Hyperglycemia increases oxidative stress through the overproduction of ROS [57]. These ROS contribute to organ injury in systems such as the heart and liver [58], and oxidative damage is generally increased in diabetes [59]. In particular, erythrocytes are susceptible to oxidative damage resulting from a high concentration of oxygen and hemoglobin [60]. In the present study, erythrocyte SOD and CAT activities were significantly higher in the *db/db* control group than in the *db/+* control group, whereas GSH-Px activity was lower in the *db/db* group. Ahmed *et al*. [61] also reported that serum SOD and CAT activities were significantly higher in type 2 diabetic patients as compared to the control. These antioxidant enzymes were also suggested as being markers for vascular injury in type 2 diabetes [61]. The hepatic SOD activity did not differ between *db/db* group and *db/+* group, whereas CAT and GSH-Px activities were significantly increased in *db/db* mice. In the current study, curcumin normalized the antioxidant enzymes activities of erythrocyte and liver in *db/db* mice. It is plausible that curcumin supplementation seemed to alter these enzymes activities in erythrocyte and liver toward maintaining antioxidant homeostasis in *db/db* mice. Elevated glucose levels induce oxidative stress that is ultimately

reflected by the increased MDA levels in the erythrocytes and liver of *db/db* mice. Lipid peroxidation is also considered responsible for the impairment of endothelial cells, capillary permeability, and fibroblast and collagen metabolism [62]. In this study, curcumin significantly lowered MDA levels in the erythrocytes and liver of *db/db* mice, thus indicating a decreased rate of lipid peroxidation. As such curcumin may decrease the production of free radicals which could lead to normalizing antioxidant activity in the erythrocytes and liver.

In conclusion, these results suggest that curcumin was beneficial in improving insulin resistance and glucose homeostasis in *db/db* mice, which seems to be medicated through elevation of plasma insulin level that caused activation of glycolysis and inhibition of gluconeogenic and lipid metabolic enzymes in liver, and increasing LPL activity in skeletal muscle, although further detailed mechanism needs to be elucidated. In addition, curcumin seemingly contributed to a reduction in oxidative stress that was induced by hyperglycemia in the erythrocytes and liver, thereby being beneficial in preventing diabetic complication.

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