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Quercetin Curtails Obesity and Dyslipidemia, but Not Insulin Resistance in Long-Term Type 2 Diabetic Male Wistar Rats Fed the High-Fat, High-Sucrose Diet

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Abstract

It is unclear whether the persistence of Type 2 Diabetes (T2D)-associated insulin resistance in Wistar rats is entirely dependent on obesity and dyslipidemia or other factors are involved. We wanted to reveal whether alleviation of obesity and dyslipidemia by quercetin would sufficiently cure the insulin resistance in diabetic Wistar rats. For this purpose, ninety, male Wistar rats were randomized into three experimental groups (n=30): Normal Control (NC) fed chow diet, Diabetic Control (DC) fed High-Fat, High-Sucrose Diet (HFHSD) and diabetic, Quercetin-Treated (QT) fed the HFHSD and gavaged with quercetin at 50 mg.kg⁻¹ bw.day⁻¹. On Days 0, 60 and 120, Body Mass Index (BMI) and Abdominal Circumference:Thoracic Circumference (AC:TC) ratio were measured on ten rats from each group. Rats were then euthanized and fasting blood samples were withdrawn and used to quantify plasma glucose, Triacylglycerols (TAG), LDL-cholesterol, total cholesterol, C-Reactive Protein (CRP) and insulin concentrations. Insulin resistance score, Relative Pancreatic Weight (RPW, %) and number of islet of Langerhans were also determined.

We show that quercetin normalized BMI, AC:TC ratio, RPW (%) and dyslipidemia, and enhanced the islets number of Langerhans in the QT rats on Day 120 relative to the NC rats. In the diabetic DC rats, AC:TC ratio correlated positively with hyperglycemia and negatively with RPW (%). Quercetin lowered, but failed to normalize hyperinsulinemia, insulin resistance score, hyperglycemia and CRP in the QT rats relative to the NC rats suggesting that other factors are involved in the insulin resistance pathogenesis in T2D Wistar rats. Our data also suggest that AC:TC ratio is a predictor of the obesity-induced T2D in Wistar rats.

Keywords: Type 2 Diabetes; Obesity; Dyslipidemia; Insulin resistance; Hyperglycemia; Quercetin

Introduction

Obesity is defined as an excess adipose tissue accompanying BMI ≥ 30 kg/m² and large waist circumference [1]. Obesity is caused by energy intake that exceeds energy expenditure leading to development of metabolic syndrome [2] wide-spread inflammation and chronic health disorders.

In obese individuals, proinflammatory molecules, such as TNF- α , IL-1 and IL-6 are produced by the adipose tissue and thought to trigger β -cells injury and peripheral insulin resistance which culminate into Type 2 Diabetes (T2D) development [3]. Current interventions to lower obesity epidemic, such as consumption of nutrient-dense diet that provides less Calories, promotion of physical activity and use of anti-T2D medications and bariatric surgery have not been as efficient at down-regulating the upsurge of obesity [4]. Worldwide, more than 1 billion individuals are overweight, of whom 300 million are obese [1]. The huge number of afflicted individuals stimulated research that seeks an effective, safe dietary additive that cures the obesity-associated T2D and related chronic health disorders.

Quercetin is a phytochemical flavonoid that present in plants as hydrophilic glycosides. After hydrolysis of the glycosides, the average absorption of quercetin aglycone is estimated to be about 73% [5]. Several *in vivo* protective mechanisms limit the prooxidant and genotoxic potential of quercetin. Oral administration of quercetin to rats [6] and to mice [7] at 30,300 or 3,000 mg.kg⁻¹ bw.day⁻¹ did not trigger mutagenic or genotoxic effects in somatic cells of treated rats and mice in comparison to untreated control. Quercetin mitigates hyperglycemia [8] and promotes energy expenditure via up-regulating PPAR- α gene expression [9]. Quercetin decreases visceral and liver fat in mice [10] and body weight in rats [11], dyslipidemia in mice [9] and rats [12] and oxidative stress metabolic disorders and risk factors of

T2D. In particular, obesity and dyslipidemia have been implicated with induction of insulin resistance. However, whether alleviation of T2D-associated obesity and dyslipidemia would entirely cure the insulin resistance is poorly understood and yet to be conclusively determined.

We hypothesized that long-term state of obesity and dyslipidemia although initially induce insulin resistance in T2D rats, but cure of obesity and dyslipidemia might not normalize the insulin resistance as other aberrant homeostatic alterations may have developed. Our objective was to elucidate whether cure of obesity and dyslipidemia by quercetin would lead to complete normalization of the insulin resistance in long-term T2D Wistar rats or other factors would block quercetin effect.

Materials and Methods

Kits and reagents

Quercetin was purchased from the Sigma Aldrich USA. Biochemical kits for lipids profiles were purchased from Biosystems S.A. Costa Brava 30, 08030 Barcelona, Spain. Insulin kits were purchased from BSOH Company, Japan.

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Received October 21, 2016; **Accepted** December 26, 2016; **Published** December 30, 2016

Citation: Abuzaid AA, Osman MO, Elkhawad AO (2016) Quercetin Curtails Obesity and Dyslipidemia, but Not Insulin Resistance in Long-Term Type 2 Diabetic Male Wistar Rats Fed the High-Fat, High-Sucrose Diet. Adv Pharmacoeconom Drug Saf 5: 213. doi: [10.4172/2167-1052.1000213](https://doi.org/10.4172/2167-1052.1000213)

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Animals

Healthy male Wistar rats (90 rats), six-week old, weighting 220-240 g, were used in the experiment. Each three rats were housed in a separate cage in the Restricted Animal Facility of the Faculty of Pharmacy, University of Khartoum. All rats were adapted for two weeks on stranded chow diet (Table 1) then randomized into three treatment groups (n=30): Normal Control (NC) fed the standard chow diet, Diabetic Control (DC) Fed High-Fat, High-Sucrose Diet (HFHSD; Table 2) and diabetic, Quercetin-Treated Group (QT) fed the HFHSD and gavaged with quercetin at 50 mg/kg bw⁻¹.day⁻¹ until Day 120 of the study. All diets were fed *ad libitum* and rats were given free access to drinking water. The environment within the room was maintained to provide a temperature of 22-25°C, a relative humidity of 35-45%, and 12/12 h light/dark cycle.

Induction of obesity, type 2 diabetes and insulin resistance

To induce long-term obesity, T2D and insulin resistance, the rats in the DC and QT Groups were fed the HFHSD *ad libitum* for 12 months. By the end of the 12th month (Day 0), rats in the different groups were housed individually and the rats in the QT Group were gavaged quercetin at 50 mg.kg⁻¹ bw.day⁻¹ until the end of the study. The HFHSD constitutes 15% protein, 30% fat (20% of which was beef lard), 47% carbohydrates (35% of which was refined sugar), 5% fiber and 3% multivitamin multimineral complex (adopted from Srinivasan et al. [13] with modifications). The rats in the NC Group continued to consume the standard chow diet *ad libitum* until the end of the study.

Samples collection and analyses

Determination of the anthropometrical parameters: Following an overnight fast on Days 0, 60, and 120, the body weight was determined in grams, body length (from nose to anus) was measured in centimeters and BMI was calculated by dividing the body weight by squared body length. To determine the AC:TC ratio, AC was measured

directly anterior to the forefeet in centimeters and TC was measured immediately behind the foreleg in centimeters [14].

Blood sample collection and dissection of pancreas: On days 0, 60 and 120 the rats were euthanized by decapitation under light halothane[®] anesthesia. Fasting blood samples were withdrawn by cardiac puncture and plasma was separated by centrifugation at 5,000 × g for 15 min in a refrigerating centrifuge and stored at -80°C until used for quantification of plasma glucose, triacylglycerols (TAG), LDL-cholesterol, total cholesterol, CRP and insulin concentrations. The pancreas was dissected aseptically in a biosafety cabinet and processed for morphometric and histomorphometric analysis.

Determination of plasma metabolites, CRP and insulin

Plasma glucose concentration was determined by using the One Touch[®] Glucometer and confirmed spectrophotometrically. Plasma TAG, total cholesterol, LDL-cholesterol and CRP were determined spectrophotometrically by using commercial kits based on the manufacturers' instructions. Additionally, plasma insulin was analyzed by using immune-enzymatic assay TOSOH AIA-360 Chemistry Analyzer. Insulin resistance was calculated by using the homeostasis model assessment of insulin resistance (HOMA-IR=Insulin, μIU/mL × glucose, mg/dL)/405 as previously described [15].

Morphometric and histomorphometric analyses

The RPW (%) was determined after adipose tissue was precisely removed from the euthanized rats on Days 0, 60 and 120 using a sensitive digital scale. Pancreases tissues intended for histopathological works were rapidly fixed in 10% formalin in PBS, dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin wax. Pancreatic sections (5 μm thick) were stained with Hematoxylin and Eosin (H&E) and used for detection of islet of Langerhans [16]. Four sections were examined from each animal in the different experimental groups. The number of pancreatic islets of Langerhans per section was determined under 10 high power fields. The number of islets was assessed by counting all islets section of different non-overlapping fields for the same slide of each animal. The histopathological images were captured by using the SPOT idea[™] CMOS|5.0 Mp digital camera mounted on Olympus CH20i (Olympus BX51) microscope equipped with grid and micrometer and hooked a SPOT idea[™] CMOS software.

Statistical analysis

The data were analyzed by using the Two-way ANOVA procedure of the statistical software GraphPad Prism 5[®] (GraphPad Software Inc., La Jolla, CA, USA). Bonferroni test was used for the pairwise comparison of the means with significance set at α=0.05 and 95% confidence intervals. The Pearson Correlation Coefficient (*r*) was computed to determine the association between each two variables. Data were expressed as the means ± SEM. Statistical significance was declared when *P*<0.05. Means with superscript differ significantly at *P*<0.05.

Results

Anthropometrical analyses

Feeding the HFHSD to the DC and QT rats significantly (all *P*<0.001; Figur 1A) increased the BMI of the diabetic DC rats on Days 0, 60 and 120 and the QT rats on Day 0 only relative to the normal control NC rats. Interestingly, by Days 60 and 120, quercetin administration

	Weight %	Kcal %
Fat	11.4	13.103
Carbohydrates	51.4	59.877
Corns starch	28.6	-
Protein	23.2	27.020
Mineral-vitamin mix	10.1	-
Fiber	3.9	-
Total	100	100

Adopted from rat diet 5012 (LabDiet, St. Louis, MO)

Table 1: The macro- and micronutrients constituents and energy of the chow diet fed to the NC rats.

	Weight %	Kcal %
Fat	30	52.53
Carbohydrates	46	35.8
Sucrose	34%	-
Corn starch	13%	-
Protein	15	11.67
Mineral mix	3.0	-
Vitamin mix	1	-
Cellulose	5	-
Total	100	100

The fat constituent contained 20% beef lard. Values are calculated from ingredients mix

Table 2: The macro- and micronutrients constituents and energy (Calories) of the obesogenic, diabetogenic HFHSD fed to the DC and QT rats.

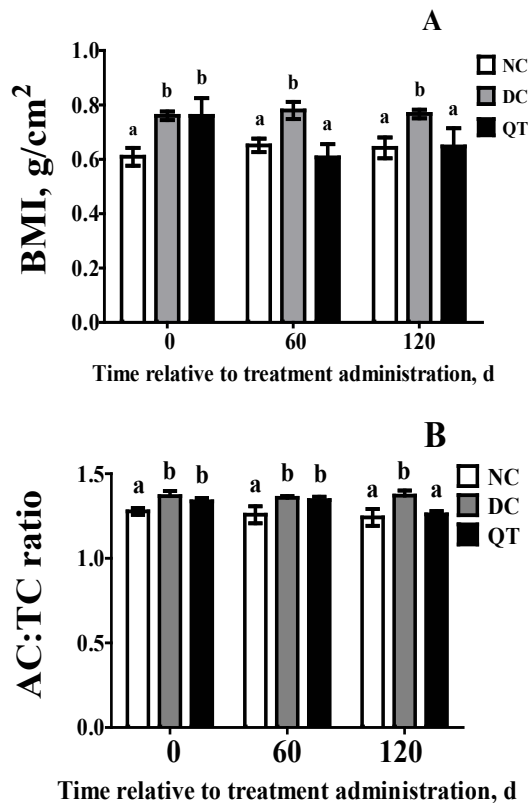


Figure 1: The BMI (g/cm²) and AC:TC ratio values on Days 0, 60 and 120 of male Wistar rats fed the standard chow diet (NC), fed the HFHSD (DC) or fed the HFHSD and gavaged with quercetin (QT) at 50 mg.kg⁻¹ bw.day⁻¹ until Day 120 of the study. Values are the means ± SEM. Means with different superscripts differ significantly. Statistical significance declared at $P \leq 0.05$. Panel A: The BMI (g/cm²) values. Feeding the HFHSD to the DC and QT rats significantly (all $P < 0.001$; Figure 1A) increased the BMI of the diabetic DC rats on Days 0, 60 and 120 (0.7597 ± 0.02 ; 0.7795 ± 0.03 ; 0.7667 ± 0.02 , respectively) and the QT rats on Day 0 only (0.7597 ± 0.02) relative to the normal control NC rats (0.6093 ± 0.01 ; 0.6511 ± 0.009 ; 0.6419 ± 0.01 , respectively). On Days 60 and 120, quercetin administration normalized (both $P > 0.05$) the BMI of the QT rats relative to the NC rats (0.6072 ± 0.01 ; 0.6472 ± 0.01 vs. 0.6511 ± 0.01 ; 0.6419 ± 0.02 , respectively). Panel B: The AC:TC ratio of the DC rats became significantly greater ($P < 0.0001$) on Days 0, 60 and 120 relative to NC rats (1.3683 ± 0.03 ; 1.3577 ± 0.01 ; 1.3708 ± 0.03 vs. 1.2774 ± 0.02 ; 1.2576 ± 0.05 ; 1.2418 ± 0.05 units, respectively). In contrast, the AC:TC ratio of the QT rats was greater ($P \leq 0.001$) only on Days 0 and 60 (relative to the NC rats (1.3370 ± 0.02 and 1.3446 ± 0.02 vs. 1.2774 ± 0.02 ; 1.2576 ± 0.05 units, respectively). By Shown in Figure 1B, quercetin lowered ($P < 0.0001$) AC:TC ratio of the QT rats compared with the DC rats and not different ($P > 0.05$) relative to the NC rats (1.2599 ± 0.02 vs. 1.3708 ± 0.03 and 1.2418 ± 0.05 units, respectively) on Day 120.

normalized (both $P > 0.05$) the BMI of the QT rats (Figure 1A).

The AC:TC ratio of the DC rats became significantly greater ($P < 0.0001$) on Days 0, 60 and 120 relative to NC rats. In contrast, the AC:TC ratio of the QT rats was greater ($P \leq 0.001$) on Days 0 and 60. Quercetin, however, lowered ($P < 0.0001$) AC:TC ratio of the QT rats compared with the DC rats, but not different ($P > 0.05$) relative to the NC rats (Figure 1B). Illustrated in Table 3, BMI did not correlate with the AC:TC ratio in all the experimental rats.

Morphometric and histomorphometric analyses

The RPW (%) significantly (all $P < 0.0001$) decreased in the DC rats on Days 0, 60 and 120 and in the QT rats on Day 0 compared to NC rats.

On Days 60 and 120, however, quercetin administration significantly (both $P < 0.0001$) increased the RPW (%) in the QT rats to be greater compared with the DC rats, but not different ($P > 0.05$) compared with the NC rats. Also, quercetin increased ($P < 0.01$) the islets of Langerhans number in the QT rats relative to the DC rats, slightly ($P = 0.08$; Table 4) compared with the NC rats on Day 120.

Correlation between the anthropometrical and morphometrical parameters

BMI did not correlate with AC:TC ratio (all $r \leq 0.2994$, $P > 0.05$; Table 3), RPW (all $r \leq -0.4180$, $P > 0.05$; Table 3) and hyperglycemia ($r \leq 0.3831$, $P > 0.05$; Table 3), in all of our experimental groups (Table 3). We observed a strong inverse correlation between the AC:TC ratio and RPW in the QT and NC rats ($r = -0.7085$; $P \leq 0.05$ and $r = -0.8428$, $P \leq 0.01$, respectively). Also, plasma glucose significantly ($r \geq 0.665$; $P \leq 0.05$) correlated positively with AC:TC ratio in QT rats (Table 3).

Development of dyslipidemia

Under effect of the HFHSD, the dyslipidemia parameters plasma TAG, LDL-cholesterol and total cholesterol significantly (all $P < 0.0001$) increased in the diabetic DC rats on Days 0, 60 and 120, and in the QT

Treatment groups				
Parameters	Statistics	DC	QT	NC
BMI vs. AC:TC	<i>r</i>	0.1659	0.1400	0.2994
	<i>P</i>	0.6468	0.7187	0.4712
BMI vs. RPW	<i>r</i>	-0.4180	-0.3257	-0.4172
	<i>P</i>	0.1923	0.3312	0.2038
BMI vs. PG	<i>r</i>	0.2160	0.3831	0.3676
	<i>P</i>	0.5489	0.2745	0.3703
AC:TC vs. RPW	<i>r</i>	-0.4417	-0.7085	-0.8428
	<i>P</i>	0.0523	0.0049	0.0086
AC:TC vs. PG	<i>r</i>	0.7937	0.6650	0.5286
	<i>P</i>	0.0140	0.0361	0.0780

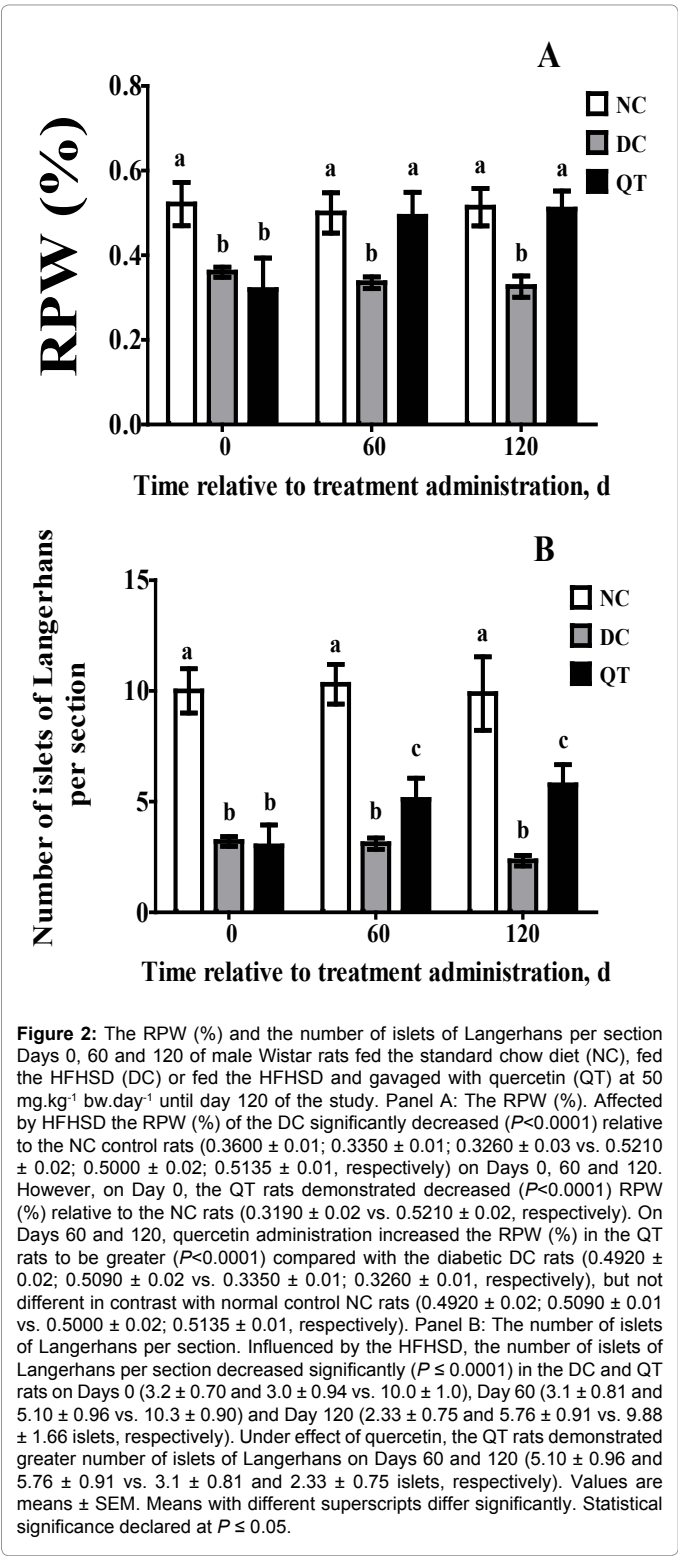
r =Pearson Correlation Coefficients (Prob>| r | under H_0 : $Rho=0$). BMI stands for body mass index, g/cm², AC:TC stands for abdominal circumference to thoracic circumference ratio, RPW stands for relative pancreatic weight and PG stands for plasma glucose concentration in mg/dL. DC stands for diabetic control group, QT for diabetic quercetin-treated group and NC for non-diabetic control group. Quercetin was gavaged at 50 mg.kg⁻¹ bw.day⁻¹ until Day 120 of the study. Values are the means ± SEM. Statistical significance declared at $P \leq 0.05$.

Table 3: The correlation among anthropometrical parameters, RPW, and plasma glucose concentration on Day 120 of male Wistar rats fed the standard chow diet (NC), fed the HFHSD (DC) or fed the HFHSD and gavaged with quercetin (QT).

Parameter	Statistics	Treatment groups		
		DC	QT	NC
PG vs. IRS	<i>r</i>	0.7000	0.8490	0.9473
	<i>P</i>	0.0500	0.007	0.0001
PG vs. IsL	<i>r</i>	-0.5519	-0.5553	-0.6354
	<i>P</i>	0.0150	0.0790	0.0660
IRS vs. IsL	<i>r</i>	-0.6283	0.0129	0.0483
	<i>P</i>	0.0699	0.9738	0.6700

r =Pearson Correlation Coefficients (Prob>| r | under H_0 : $Rho=0$). DC stands for diabetic control group, QT stands for diabetic quercetin-treated group, NC stands for non-diabetic control group, PG stands for plasma glucose, IRS stands for insulin resistance score, and IsL stands for islets of Langerhans number. Quercetin was gavaged at 50 mg.kg⁻¹ bw.day⁻¹ until Day 120 of the study. Statistical significance was declared at $P \leq 0.05$.

Table 4: The correlation among plasma glucose concentration, insulin resistance score and the number of islets of Langerhans on Day 120 of male Wistar rats fed the standard chow diet (NC), fed the HFHSD (DC) or fed the HFHSD and gavaged with quercetin (QT).



rats on Day 0 compared with the normal control NC rats (Figures 2A-2C). Administration of quercetin significantly ($P<0.0001$) decreased TAG, LDL-cholesterol and total cholesterol in plasma of the QT rats on Days 60 and 120 relative to the diabetic DC rats and normalized ($P>0.05$) them relative to the NC rats (Figures 3A-3C).

Plasma glucose and insulin concentrations and the Insulin Resistance Score (IRS)

Influenced by the HFHSD plasma glucose concentration significantly ($P \leq 0.01$) increased in the DC and QT rats on Days 0, 60 and 120 to be greater relative to the NC rats (Figure 4A).

Feeding the HFHSD significantly ($P \leq 0.01$) increased plasma insulin concentrations in the DC and QT rats on Days 0, 60 and 120 relative to the control NC rats fed the chow diet (Figure 4B). By Days 60 and 120, however, administration of quercetin decreased ($P<0.01$) plasma insulin in the QT rats compared with the DC rats, yet higher ($P<0.01$) relative to the NC rats (Figure 4B).

Shown in Figure 4C, the DC and QT rats demonstrated significantly ($P \leq 0.001$) higher ISR relative to the NC rats on Days 0, 60 and 120. Administration of quercetin lowered ($P<0.0001$) the ISR of the QT rats relative to the diabetic control DC rats on Days 60 and 120 yet failed ($P<0.01$) to normalize it relative to the nondiabetic control NC rats (Figure 4C). As shown in Table 4, the hyperglycemia correlated positively ($r \geq 0.70$; $P \leq 0.05$) with IRS and negatively ($r=-0.5519$; $P \leq 0.02$) with the number of islets of Langerhans in the pancreas of the DC, QT and NC rats on Day120. But, the IRS correlated negatively with the number of islets of Langerhans in the DC rats only on Day 120 (Table 4).

The correlation between hyperglycemia and dyslipidemia

On Day 120, the hyperglycemia correlated significantly ($r \geq 0.7000$; $P<0.05$) positive with plasma TAG concentration in the DC and NC rats (Table 5). But, under the effect of quercetin, no correlation ($r=0.3014$; $P>0.05$) was detected between hyperglycemia and TAG concentrations in the QT rats (Table 5). In contrast, the hyperglycemia did not correlate ($r \leq 0.1411$; $P>0.05$) with the LDL-cholesterol and total cholesterol in the in all experimental rats (Table 5).

Development of inflammation

The inflammatory marker CRP elevated significantly ($P<0.001$) in the plasma of the diabetic DC and QT rats relative to the NC rats on Days 0, 60 and 120 (Figure 5). Affected by quercetin, the plasma CRP concentrations decreased ($P \leq 0.001$) in the QT rats on Days 60 and 120 relative to the DC rats. Despite this, quercetin failed ($P<0.0001$) to normalize plasma CRP in the QT rats compared to the control NC rats on Days 60 and 120 (Figure 5).

Parameters	Statistics	Treatment groups		
		DC	QT	NC
PG vs. TAG	<i>r</i>	0.7000	0.30141	0.7222
	<i>P</i>	0.0244	0.3974	0.0431
PG vs. T-ch	<i>r</i>	-0.0762	-0.0223	0.1411
	<i>P</i>	0.8343	0.9512	0.7388
PG vs. LDL-ch	<i>r</i>	-0.0849	-0.2714	-0.2455
	<i>P</i>	0.8156	0.4482	0.5579

r =Pearson Correlation Coefficients (Prob>| r | under H_0 : $Rho=0$). DC stands for diabetic control group, QT stands for diabetic quercetin-treated group, NC stands for non-diabetic control group, PG stands for plasma glucose, TAG stands for triacylglycerols, T-ch stands for total cholesterol and LDL-ch stands for low-density lipoprotein cholesterol. Quercetin was gavaged at 50 mg.kg⁻¹ bw.day⁻¹ until Day 120 of the study. Statistical significance was declared at $P \leq 0.05$.

Table 5: The correlation among plasma glucose, TAG, total-cholesterol and LDL-cholesterol concentrations on Day 120 of male Wistar rats fed the standard chow diet (NC), fed the HFHSD (DC) or fed the HFHSD and gavaged with quercetin (QT).

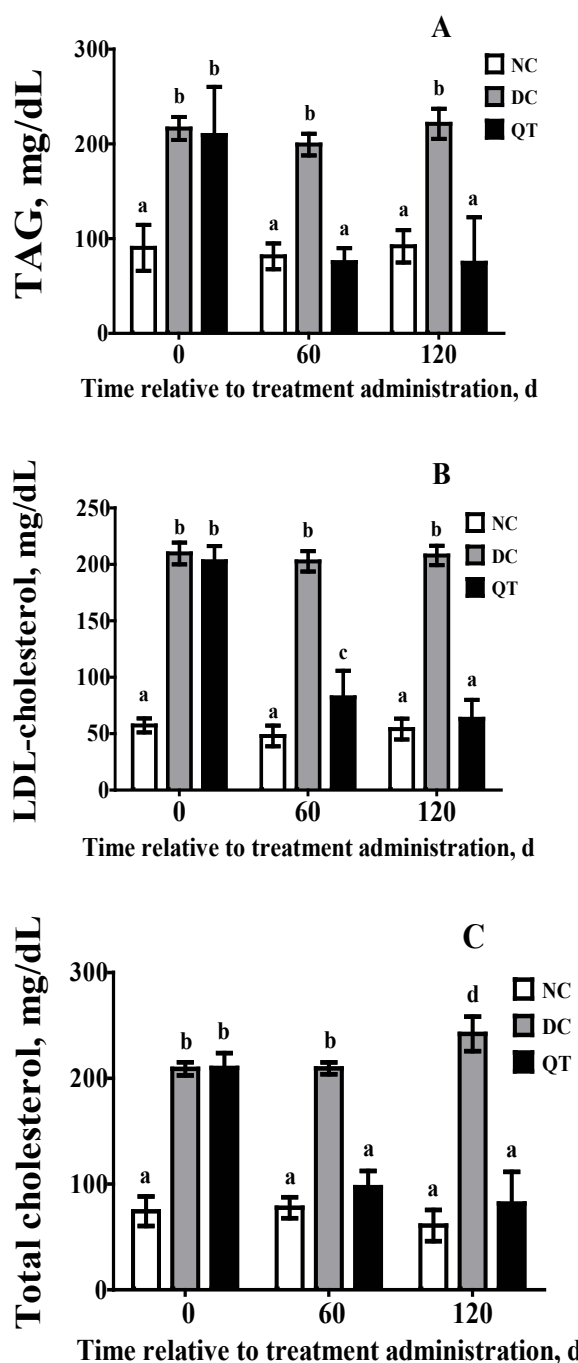


Figure 3: Plasma TAG, LDL-cholesterol and total cholesterol concentrations on Days 0, 60 and 120 of male Wistar rats fed the standard chow diet (NC), fed the HFHSD (DC) or fed the HFHSD and gavaged with quercetin (QT) at 50 mg.kg⁻¹ bw.day⁻¹ until day 120 of the study. Panel A: Plasma TAG concentrations, mg/dL. Plasma TAG concentrations significantly (all $P < 0.0001$) increased in the diabetic DC rats on Days 0, 60 and 120 (216 ± 12.2 ; 199.2 ± 11.5 ; 221.1 ± 15.9) and in the QT rats on Day 0 (209.4 ± 16.0) compared with the normal control NC rats (90.3 ± 8.6 ; 81.2 ± 4.6 ; 91.9 ± 6.1 mg/dL, respectively). Administration of quercetin significantly ($P < 0.0001$) decreased TAG in plasma of the QT rats on Days 60 and 120 relative to the diabetic DC rats (75.2 ± 4.6 ; 74.5 ± 15.2 vs. 216 ± 11.5 ; 199.2 ± 15.9 mg/dL) and normalized ($P > 0.05$) it relative to the NC rats (75.2 ± 4.6 ; 74.5 ± 15.2 vs. 81.2 ± 4.8 ; 91.9 ± 6.1 mg/dL, respectively). Panel B: Plasma LDL-cholesterol, mg/dL. Plasma LDL-cholesterol concentrations significantly (all $P < 0.0001$) increased in the diabetic DC rats on Days 0, 60 and 120 (239.6 ± 12.8 ; 202.6 ± 9.0 ; 207.8 ± 11.7) and in the QT rats on Day 0 (232.7 ± 23.5) compared with the normal control NC rats (57.3 ± 6.2 ; 48.0 ± 3.7 ; 54.1 ± 9.2 mg/dL, respectively). Quercetin significantly ($P < 0.0001$) decreased LDL-cholesterol in the QT rats on Days 60 and 120 relative to the diabetic DC rats (82.7 ± 4.6 ; 63.1 ± 16.8 vs. 202.6 ± 9.0 ; 207.8 ± 11.7 mg/dL) and normalized ($P > 0.05$) it relative to the NC rats (82.7 ± 4.6 ; 63.1 ± 16.8 vs. 202.6 ± 9.0 ; 207.8 ± 11.7 mg/dL, respectively). Panel C: Plasma total-cholesterol, mg/dL. Influenced by HFHSD, plasma total cholesterol concentrations significantly (all $P < 0.0001$) increased in the diabetic DC rats on Days 0, 60 and 120 (208.9 ± 6.2 ; 209.4 ± 5.5 ; 241.9 ± 6.2) and in the QT rats on Day 0 (209.7 ± 4.5) compared with the normal control NC rats (74.3 ± 4.9 ; 77.6 ± 3.5 ; 60.8 ± 5.2 mg/dL, respectively). On Days 60 and 120, quercetin significantly ($P < 0.0001$) decreased total cholesterol in the QT rats on compared with the diabetic DC rats (97.0 ± 4.9 ; 81.6 ± 9.5 vs. 209.4 ± 5.5 ; 241.9 ± 16.3 mg/dL) and normalized ($P > 0.05$) it relative to the NC rats (97.0 ± 4.9 ; 81.6 ± 9.5 vs. 77.6 ± 3.5 ; 60.8 ± 5.2 mg/dL, respectively). Values are the means $\pm$ SEM. Means with different superscripts differ significantly. Statistical significance declared at $P \leq 0.05$.

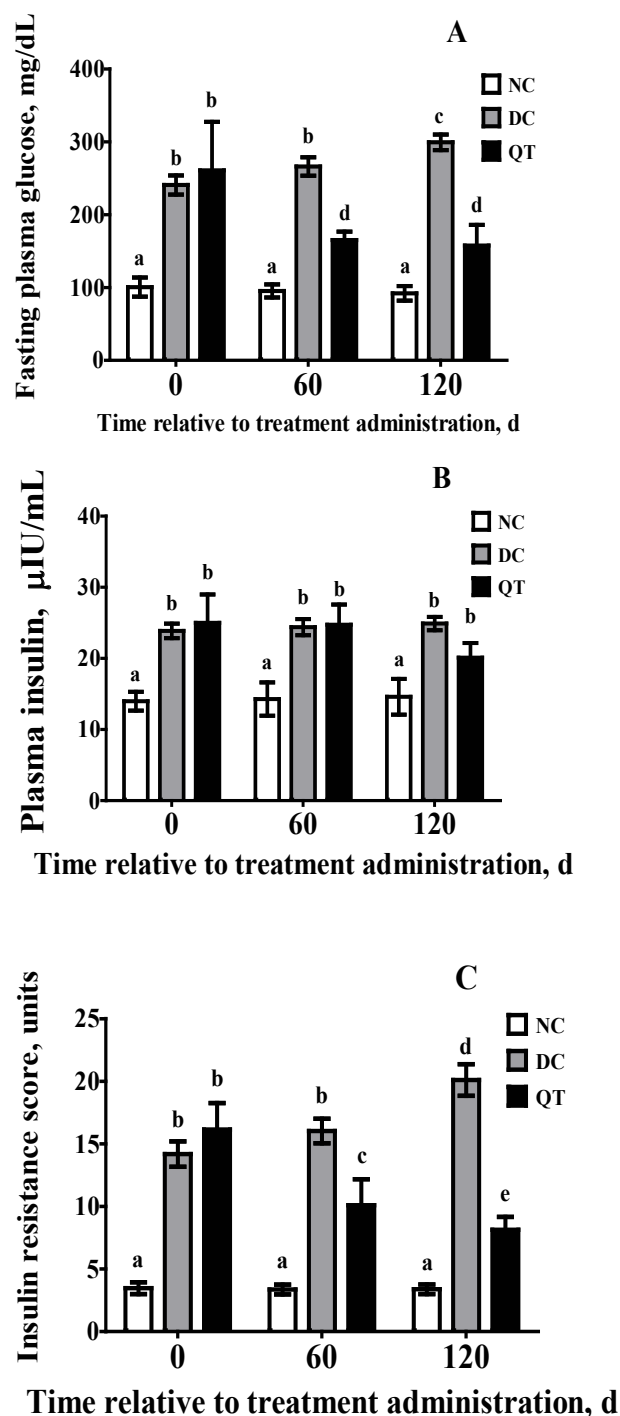
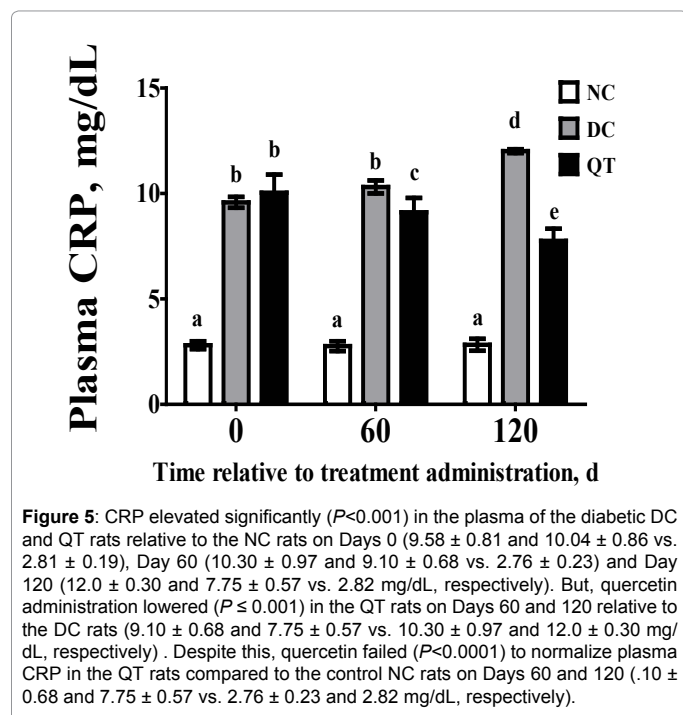


Figure 4: Plasma glucose, plasma insulin and the insulin resistance score values on Days 0, 60 and 120 of male Wistar rats fed the standard chow diet (NC), fed the HFHSD (DC) or fed the HFHSD and gavaged with quercetin (QT) at 50 mg.kg⁻¹ bw.day⁻¹ until day 120 of the study. Panel A: Plasma glucose concentrations, mg/dL. Feeding the HFHSD significantly ($P \leq 0.01$) increased plasma glucose concentration in the DC and QT rats on Days 0, 60 and 120 to be greater relative to the NC rats (240.7 $\pm$ 13.1 and 260.7 $\pm$ 21.0 vs. 100.5 $\pm$ 4.6) on Day 0, (266.2 $\pm$ 12.7 and 165.2 $\pm$ 3.7 vs. 95.3 $\pm$ 3.2) on Day 60 and (299.3 $\pm$ 10.7 and 157.6 $\pm$ 9.0 vs. 92.0 $\pm$ 3.2 mg/dL, respectively) on Day 120. Panel B: Plasma insulin concentrations, μ U/mL. Influenced by the HFHSD plasma insulin concentrations significantly ($P \leq 0.01$) increased in the DC and QT rats on Day 0 (23.9 $\pm$ 3.20 and 25.0 $\pm$ 4.0 vs. 14.0 $\pm$ 1.31), Day 60 (24.4 $\pm$ 3.60 and 24.7 $\pm$ 2.84 vs. 14.3 $\pm$ 2.35) and Day 120 (24.6 $\pm$ 0.71 and 20.1 $\pm$ 0.47 vs. 14.6 $\pm$ 0.41 μ U/mL, respectively) relative to the control NC rats fed the chow diet. By Day 120, however, administration of quercetin decreased ($P < 0.01$) plasma insulin in the QT rats compared with the DC rats, yet higher ($P < 0.01$) relative to the NC rats (20.1 $\pm$ 2.07 vs. 24.9 $\pm$ 2.96 and 14.6 $\pm$ 2.51 μ U/mL, respectively). Panel C: Insulin resistance score (ISR), units. The DC and QT rats demonstrated significantly ($P \leq 0.001$) higher ISR relative to the NC rats on Day 0 (14.2 $\pm$ 3.22 and 16.1 $\pm$ 3.1 vs. 3.47 $\pm$ 0.46), Day 60 (16.0 $\pm$ 2.01 and 10.1 $\pm$ 1.08 vs. 3.36 $\pm$ 0.30) and Day 120 (20.1 $\pm$ 1.10 and 8.14 $\pm$ 1.65 vs. 3.38 $\pm$ 0.05 units, respectively). Administration of quercetin lowered ($P < 0.0001$) the ISR of the QT rats relative to the diabetic control DC rats on Days 60 and 120 yet failed to normalize it relative to the nondiabetic control NC rats (10.1 $\pm$ 2.08; 8.14 $\pm$ 1.02 vs. 16.0 $\pm$ 3.10; 20.1 $\pm$ 4.0 and 3.36 $\pm$ 0.39; 3.38 $\pm$ 0.39 units, respectively).



Discussion

This study describes the ability of quercetin to alleviate the obesity and dyslipidemia, but not the insulin resistance risk factors for T2D [17] induced by long-term (12 mo) HFHSD feeding in male Wistar rats. Induction of T2D by the HFHSD (Table 2) in the DC rats lead to significant increase in their BMI, AC:TC ratio, dyslipidemia, hyperglycemia, hyperinsulinemia and insulin resistance score (Figures 1-4). The HFHSD also decreased their RPW (%) and its content of islets of Langerhans (Figure 2) relative to the control NC rats consuming the normal chow diet (Table 1).

Rodent diets high in sucrose and fats increase BMI of Wistar rat [13,14]. On Days 60 and 120, we found that quercetin administered at $50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{bw} \cdot \text{day}^{-1}$ normalized ($P > 0.05$) BMI and the AC:TC ratio of the QT rats relative to the NC rats (Figures 1A and 1B). Consistent with previous findings, administration of quercetin decreases the body weight and adiposity in rats [18] and decreases liver and visceral fat in C57BL/6J mice via inhibiting adipogenesis and activating fatty acid β -oxidation in the mitochondria [19]. Promotion of energy expenditure by quercetin is mediated via upregulation of adiponectin gene expression (Authors' manuscript accepted for publication).

Consistent with previous report [20], induction of T2D by HFHS diet in our rats was accompanied by significant dyslipidemia (Figures 3A-3C). Curiously enough, under quercetin effect, the QT rats demonstrated normal plasma TAG, LDL-cholesterol and total cholesterol concentrations compared to the normal control NC rats on Day 120. The anti-dyslipidemic effects of quercetin are well documented in mice [9] and male Wistar rats [21]. Quercetin alleviates dyslipidemia via induction of the insulin-independent AMP-protein kinase and glucagon-like peptide-1 (GLP-1) promoting GLUT-4 gene expression. As a result, glucose uptake by myocytes is significantly enhanced increasing the energy expenditure. Thus, the carbon source for hepatic lipogenesis decreases [22]. Additionally, quercetin also promotes cholesterol-to-bile conversion [12] to lower hypercholesterolemia.

Here, we show for the first time that the hyperglycemia correlated significantly positive with plasma TAG, but not with plasma cholesterol concentrations in the diabetic DC rats (Figure 3A and Table 5). This correlation implies that plasma TAG is a predictor of T2D-associated dyslipidemia in male Wistar rats (Table 5).

Consistent with previous findings [9,11,12,23], we observed that quercetin administration attenuated the concentration of the inflammatory marker CRP in plasma of the QT rats on Day 120. Quercetin mediates its anti-inflammatory property via down-regulation gene expression of glycoprotein (gp91phox) component of NADPH oxidase in hepatocytes, and decrease of plasma 8-isoprostane, a mediator and a marker of oxidative stress in rodents [9].

During evaluation of emerging antidiabetic drug, its effect on pancreatic mass is seriously considered [24]. Illustrated in Figures 2A and 2B, we noted significant decrease of the RPW (%) and number of islets of Langerhans in the pancreas of diabetic DC rats (Days 0, 60 and 120) and in QT rats (Day 0) as evidence of pancreatic tissue alteration induced by HFHSD and T2D. In contrast, quercetin normalized RPW (%) and enhanced number of islet of Langerhans in the QT rats (Figures 2A and 2B) lending support to previous findings *in vitro* in INS-1E β -cell line [25] and *in vivo* in rats [26,27] and mice [28], that quercetin prevents pancreatic tissue injury induced by chemicals and proinflammatory cytokines insults.

In humans, the pancreatic weight and size correlate positively with BMI and body weight [29]. In our study, RPW (%) did not correlate with BMI, but correlated with AC:TC ratio and hyperglycemia in T2D male Wistar rats (Table 3).

One of the strengths of this study is its ability to determine preliminary data about the usefulness of the RPW (%) as marker of the obesity-related T2D induction. We clearly showed that while long-term HFHSD induced obesity-associated T2D and significantly decreases RPW (%) and number of islets of Langerhans, quercetin increases reverses this effect.

Additionally, because body weight does not increase under *ad libitum* feeding in all experimental rats [30] body weight parameter might not precisely predict obesity in rats. We found that the AC:TC ratio correlated positively with the hyperglycemia and negatively with RPW (%), suggesting that the abdominal circumference (visceral fats) component of the AC:TC adequately predicts obesity-associated T2D in rats (Table 3).

The significant ($P \leq 0.05$) decrease of the RPW (%) and number of islets of Langerhans in the pancreas of diabetic DC rats fed the HFHSD on (Days 0, 60 and 120) and QT rats (Day 0) implies pancreatic tissue alteration. Chronic hyperglycemia in T2D induces glucotoxicity leading to β -cell mitochondrial damage, β -cell necroptosis [25,31] that could decrease the RPW. Our observation that quercetin normalized the RPW (%) and enhanced number of islet of Langerhans in the QT rats (Figure 2A and B) agrees with previous findings *in vitro* in INS-1E β -cell line [25] and *in vivo* in rats [25,26] and mice [30], which shows that quercetin prevent pancreatic tissue injury induced by chemicals and proinflammatory cytokines insults.

Thus, quercetin sufficiently normalized obesity, dyslipidemia and RPW, but failed to normalize the number of islets of Langerhans and insulin resistance in the QT rats. As a result, the hyperglycemia was not normalized.

In conclusion, quercetin cured the long-term obesity and dyslipidemia, which are long considered the underlying etiology of T2D

in obese male Wistar rats. However, cure of obesity and dyslipidemia by quercetin did not lead to normalization of insulin resistance implying that other aberrant homeostatic alterations might have developed and blunted effect of quercetin in long-term T2D rats.

Acknowledgements

The authors thank the Faculty of Pharmacy, University of Khartoum for providing housing of the Wistar rats at their Restricted Animal Facility. Our appreciation is extended to Professor Hamid Suliman Abdulla, Faculty of Veterinary Medicine, University of Khartoum for assisting with the animal dissection. The authors also thank the Department of Statistics, University of Iowa, USA for helping with the statistical analysis.

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