

Quercetin Supplementation Reduces Maternal Hyperglycemia in a Type 2 Diabetes Mellitus Mouse Model

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Authors' contributions

This work was carried out in collaboration between all authors. Author JCG designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MRP and SDH managed the analyses of the study and reviewed the manuscript. All authors read and approved the final manuscript.

Short Research Article

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ABSTRACT

Aims: Pregnancy may be complicated by diabetes mellitus. The use of natural antioxidants may alleviate the increased glucose levels in the diabetic pregnancy.

Methodology: The combination of high Fat Diet (HFD) and streptozocin (STZ) was used pre-breeding in CD1 female mice to generate maternal hyperglycemia. A subgroup of the females received quercetin (Q) supplementation in their HFD.

Results: Hyperglycemic females supplemented with Q displayed significantly decreased blood glucose levels at gestation days 10 and 17.

Conclusion: These results suggest quercetin could have utility as a supplement for diabetic women during pregnancy, and could represent a protection for the offspring of diabetic mothers.

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1. INTRODUCTION

Pre-pregnancy maternal diabetes, particularly type 2 diabetes (insulin resistant) can severely complicate the course of pregnancy. The mother suffering type 2 diabetes either does not produce enough insulin, or the target cells of insulin display reduced responsiveness to insulin (defective insulin receptors) [1]. Human type 2 diabetes is often the combination of both resistance to insulin action and an inadequate compensatory insulin secretory response.

Quercetin is a common, biologically active polyphenolic pharmaceutical plant product (phytochemical) in the flavonoid family that imparts color to onions, kale, broccoli, cabbage, apples, plums, berries, red grapes, squash, seeds, and nuts [2]. Because of its natural antioxidant activity, quercetin has been used for treatment of allergic conditions, asthma, gout, pancreatitis and prostatitis [3,4]. Quercetin treatment similarly has been associated with decreased risk of cataracts [5]. Quercetin has also a positive effect in lowering glucose levels of male diabetic rats [6]. Considering that quercetin can be easily added to the diet of a diabetic patient, the present experiments were performed to determine if dietary quercetin may reduce hyperglycemia in pregnant type 2 diabetic mice.

2. MATERIALS AND METHODS

Six- to seven-week-old CD1 female mice were purchased from Charles River. Mice were acclimated to the rodent facility for 1 week on standard mouse chow, then were fed either with a HFD (Table 1) or continued on the standard diet (SD). Tap water was given *ad libitum*.

Control group (C): SD fed CD1 female mice (n = 6) were injected intraperitoneally (IP) with a citrate buffer (0.05 M, pH: 4.5) and served as the main control group.

Type 2 diabetes group (T2): CD1 female mice (n = 24) were fed a HFD for 4 weeks. At the end of the fourth week they were injected with a moderate dose of STZ (100 mg/kg IP) dissolved in a citrate buffer (0.05 M, pH: 4.5) [7,8]. Four weeks after the STZ injection, hyperglycemic females (n = 5) with levels of hyperglycemia ≥ 250 mg/dl (determined by tail venipuncture using an Accu-Check compact glucometer, Roche laboratories, distributed by www.americandiabeteswholesale.com) were separated and bred overnight. The plug-positive females were then continued on a HFD throughout pregnancy. This combination of STZ + HFD was recently found to produce a type 2 diabetes-like blood glucose profile in mice [8].

Type 2 diabetes group supplemented with quercetin (T2Q): CD1 female mice (n = 22) were fed a HFD supplemented with 66 mg quercetin/kg chow for 4 weeks, as previously described (Prater et al., 2008). At the end of the fourth week the mice were injected with 100 mg/kg STZ (IP) as above. Four weeks after the STZ injection, hyperglycemic females (n = 5) with levels of hyperglycemia ≥ 250 mg/dl were bred and then continued on a HFD during pregnancy.

Maternal body weight, blood glucose, and plasma insulin levels (as determined by ELISA mouse ultrasensitive kit, ALPCO diagnostics, Windham, NH) were then followed. Blood

samples were taken from the tail vein using capillary tubes at breeding day 1 (BD1) and gestation days (GD) 10 and 17 to have an overview of glucose levels close to the end of the first and second half of pregnancy. Maternal weight was determined at GD 17. Female mice were then euthanized on gestation day (GD) 17 by CO₂ inhalation. All procedures were reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee (IACUC) before experiments were initiated.

2.1 Statistical Analysis

Statistical software SAS 9.1 was used to run one-way ANOVA to detect differences among groups. When a significant difference was observed ($p < .05$), a Scheffes statistical test was used to further analyze differences among groups.

Table 1. High fat diet composition

High Fat Diet	gm%	Kcal%
Protein	26.2	20
Carbohydrate	26.3	20
Fat	34.9	60
Fat composition:		%
Saturated		37.1
Monounsaturated		46
Polyunsaturated		16.9

3. RESULTS AND DISCUSSION

Four weeks after the STZ injection, the percentage of hyperglycemic females (defined as ≥ 250 mg/dl) in the T2 or T2Q HFD groups ranged from 25 to 30 %, similar to previous results in our laboratory (Gutierrez et al., 2010). These hyperglycemic females only were then used for breeding. Blood glucose levels at breeding day (BD) 1, GD10 and GD17 were significantly different among groups ($p < .001$). As expected, the control group (C) showed significantly lower levels of blood glucose compared to the diabetic groups at BD1. However, at GD 10 and GD 17, glucose levels from the T2Q group were not significantly different than the control groups (Fig. 1).

There were no significant differences among groups in maternal weight at GD17 (Fig. 2). Plasma insulin levels likewise did not show significant difference among groups ($p > .05$) (Table 2).

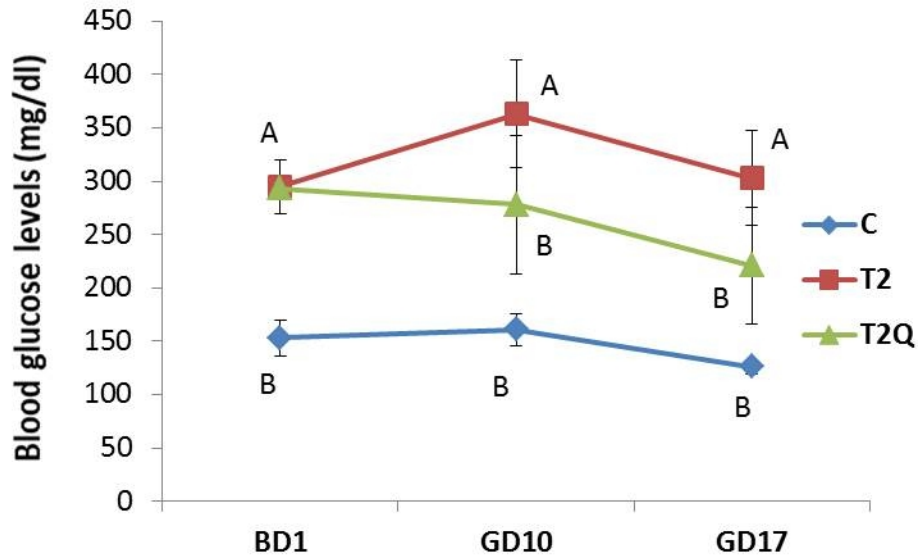


Fig. 1. Maternal glucose levels. C: control group, T2: T2 diabetic group, T2Q: T2 diabetic group supplemented with Q, BD: breeding day, GD: gestation day. Groups sharing the same letter are not significantly different. $P < 0.05$

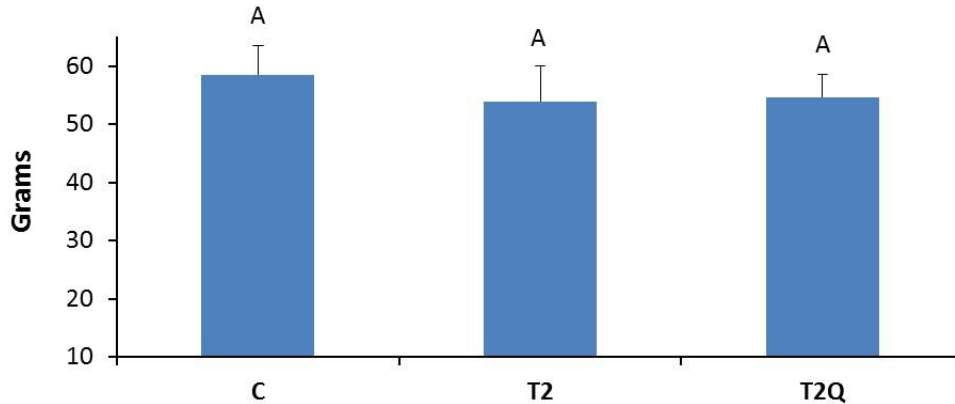


Fig. 2. Maternal weight at GD 17. C: control group, T2: T2 diabetic group, T2Q: T2 diabetic group supplemented with Q

Table 2. Maternal plasma insulin levels (ng/ml). C: control group T2: T2 diabetic group, T2Q: T2 diabetic group supplemented with Q. No differences were found among groups

Insulin	N (at GD17)	BD1	GD 10	GD 17
T2	5	1.36 ± 0.4	1.53 ± 0.4	1.2 ± 0.4
T2Q	5	1.28 ± 0.9	1.46 ± 0.7	1.45 ± 0.9
C	6	0.86 ± 0.03	1.23 ± 0.3	1.32 ± 0.5

Moderate pre-gestational diabetes mellitus is an important human health problem that complicates pregnancy, and has been related to consumption of a HFD [8]. To study this condition, hyperglycemic female mice were prepared using a HFD and low-dose STZ. Dietary supplementation of these pregnant hyperglycemic mice with quercetin resulted in reduction of blood glucose levels during the course of gestation (GD10 and GD17).

In a study conducted by Vessal [6], diabetic male rats were supplemented with IP quercetin. In these experiments, quercetin did not have an effect on plasma glucose levels of the normal rats. However, quercetin significantly and dose-dependently reduced the blood glucose levels of the diabetic male rats. Studies by Su [9] used dietary supplementation with the red wine natural antioxidant resveratrol (another member of the polyphenolic family). They also observed reduced plasma glucose levels in STZ-diabetic rats. The latter authors further reported that resveratrol ameliorated common diabetes symptoms such as body weight loss, polyphagia and polydipsia.

4. CONCLUSION

The present results are the first demonstration of efficacy of dietary quercetin for reducing maternal hyperglycemia in a type 2 diabetic mouse model. These results suggest quercetin could have utility as a supplement for diabetic women during pregnancy, and could represent a protection for the offspring of diabetic mothers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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