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Gender differences in renal responses to hyperglycemia and angiotensin-converting enzyme inhibition in diabetes

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Background. Diabetes mellitus reduces female gender-mediated protection against progression of renal disease but the mechanisms responsible for this loss of protection are unknown. The impact of gender on the diabetic hyperfiltration state has not previously been studied. Since hyperfiltration is a factor in the development of diabetic renal disease, and is influenced by hyperglycemia and renin-angiotensin system (RAS) blockade, we examined gender differences in the renal response to hyperglycemia and angiotensin-converting enzyme (ACE) inhibition in young males and females with uncomplicated type 1 diabetes mellitus.

Methods. Ten male and 12 female normoalbuminuric, normotensive, adolescents with type 1 diabetes mellitus were studied before ACE inhibition during clamped euglycemia and hyperglycemia, and then after 21 days treatment with enalapril (0.1 mg/kg daily × 1 week and then 0.1 mg/kg twice a day × 2 weeks).

Results. During clamped euglycemia, males exhibited significantly higher effective renal plasma flow (ERPF) and renal blood flow (RBF) and a lower renal vascular resistance (RVR). During clamped hyperglycemia, females exhibited reductions in ERPF and RBF, and increased RVR and filtration fraction (FF). Males exhibited no significant renal hemodynamic changes during hyperglycemia. After ACE inhibition treatment, both genders exhibited significant declines in arterial pressure, but only females displayed a reduction in glomerular filtration rate (GFR) and FF.

Conclusion. The renal responses to hyperglycemia and ACE inhibition appear to differ between male and female adolescents with uncomplicated type 1 diabetes mellitus. Hyperglycemia-induced changes in RVR and FF in women may account, at least in part, for the loss of gender-based protection in diabetic renal disease.

Key words: glomerular filtration rate, renin-angiotensin system, hyperfiltration state, gender, type 1 diabetes mellitus.

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Diabetes mellitus is associated with a high risk for the development of progressive renal disease [1–8]. Although in nondiabetic renal disease, female gender is protective against the development of end-stage renal disease (ESRD), in diabetes mellitus this protection is diminished [1–6]. The mechanisms responsible for this loss of protection have not been fully elucidated. Increased renal plasma flow and glomerular hyperfiltration are known risk factors for the development of diabetic nephropathy [7–9]. Poor glycemic control is also a risk factor for the development and progression of diabetic nephropathy, partly through hyperglycemia-mediated activation of the renin-angiotensin system (RAS) [10]. Furthermore, recent studies have identified important interactions between gender and the RAS [11], but the renal hemodynamic response to hyperglycemia and blockade of the RAS has not been compared in men and women with type 1 diabetes mellitus. Accordingly, the goal of the current study was to test the hypothesis that gender differences in the renal hemodynamic response to hyperglycemia and RAS blockade might account for the loss of protection of female gender in the development of diabetic nephropathy. We studied subjects without clinical evidence of diabetic nephropathy in order to remove any confounding effect of proteinuria or renal functional impairment.

Work from several laboratories suggests that abnormal glomerular hemodynamic function may contribute significantly to initiation or progression of renal disease [7–9]. Specifically, glomerular hyperfiltration and renal hyperperfusion [7–8] and an augmented hyperfiltration response to hyperglycemia [12] are seen in diabetes mellitus and are associated with deleterious high intraglomerular pressures, and may be important in the early pathophysiology of diabetic nephropathy [13–15]. Therefore, in the first experiment, we compared renal hemodynamic function in adolescents with type 1 diabetes mellitus during short-term clamped euglycemia (capillary blood glucose 4 to 6 mmol/L) and short-term clamped hyperglycemia (blood glucose 9 to 11 mmol/L). We then studied renal hemodynamic function after a three-week

course of RAS blockade during euglycemic conditions, hypothesizing that any sex differences in the responses to these maneuvers could further our understanding of the impact of gender on the mechanisms that may underlie the predilection for developing diabetic renal disease.

METHODS

Subjects

The protocol was approved by the Research Ethics Board of the Hospital for Sick Children, Toronto, Canada. All patients and/or their parents gave informed consent. Twenty-two adolescents (10 males and 12 females) with type 1 diabetes mellitus were recruited from an ongoing longitudinal cohort study of ambulatory blood pressure monitoring at the Hospital for Sick Children. Participants who fulfilled the following inclusion criteria were asked to participate: duration of type 1 diabetes mellitus longer than 5 years, Tanner stage 2 to 5 puberty, normoalbuminuria [albumin excretion rate (AER) < 20 μg/min on two of three overnight urine collections obtained during the month prior to study], normal clinic blood pressure, and absence of chronic illness other than treated hypothyroidism or mild asthma.

Protocol and evaluations

During the 7-day prestudy phase of each experiment, subjects adhered to a sodium-replete (150 to 200 mmol/day) and protein-replete (1 to 1.5 g/kg/day) diet. Compliance was determined by measuring the urinary sodium and urea in overnight urine collections on days 5 and 6. Protein intake was calculated from the urea excretion using standard equations. Subjects were studied if the excretion of sodium and urea were 2 to 3 mmol/kg and 3 to 6 mmol/kg, respectively, in 24 hours. All subjects met these study criteria.

Euglycemic or hyperglycemic conditions were maintained for 10 to 12 hours preceding and during the investigations. Female subjects were studied during the early follicular phase of the menstrual cycle, determined by counting days of the menstrual cycle and measuring 17β-estradiol levels. None of the female subjects were taking oral contraceptive medications.

Studies were carried out on 3 separate days. Subjects were studied on day 1 during clamped euglycemia (blood glucose = 4 to 6 mmol/L) and on day 2 during clamped hyperglycemia (blood glucose = 9 to 11 mmol/L). After 21 days of enalapril (0.1 mg/kg daily ×1 week and then 0.1 mg/kg twice a day ×2 weeks), subjects were studied on day 3 using the same procedure during euglycemic conditions.

Subjects were admitted to the Clinical Investigation Unit at the Hospital for Sick Children the evening before each phase of the study. A 16-gauge peripheral venous cannula was inserted into a vein in one arm for infusion of glucose and insulin and a second blood sampling line was inserted into a vein in the contralateral arm. Blood glucose was measured every hour and the insulin infusion was adjusted to maintain euglycemia (4 to 6 mmol/L). Studies were conducted the following morning after an overnight fast with subjects lying in the supine position in a warm quiet room. A third intravenous line was inserted into the arm contralateral to the insulin infusion and was connected to a syringe infusion pump for infusions of inulin and paraminohippurate (PAH). Mean arterial pressure (MAP) and heart rate were measured prior to each blood sample throughout the study by an automated sphygmomanometer (Dinamapp). After collecting blood for inulin and PAH blank, a priming infusion containing 25% inulin (60 mg/kg) and 20% PAH (8 mg/kg) was administered. Thereafter, inulin and PAH were infused continuously at a rate calculated to maintain their respective plasma concentrations constant at 20 and 1.5 mg/dL. Subjects remained supine at all times. After a 90-minute equilibration period, blood was collected for inulin, PAH, and hematocrit. Blood was further collected each 20 minutes for 60 minutes, and glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were estimated by steady state infusion of inulin and PAH according to the calculation method described by Schnurr et al [16].

Sample collection and analytical methods

Blood samples collected for inulin and PAH determinations were immediately centrifuged at 3000 rpm for 10 minutes at 4°C. Plasma was separated, placed on ice, and then stored at −70°C before the assay. Inulin and PAH were measured in serum by colorimetric assays using anthrone and N-(1-naphthy) ethylenediamine, respectively. The mean of the final two baseline clearance periods represent GFR and ERPF, expressed per 1.73 m^2. Filtration fraction (FF) was determined as the ratio of GFR to ERPF. Renal blood flow (RBF) was calculated by dividing the ERPF by 1 hematocrit. Renal vascular resistance (RVR) was derived by dividing MAP by the RBF. Renal hemodynamic measurements were adjusted for body surface area (BSA).

Urinary AER was determined from three timed overnight urine collections. Urinary albumin concentration was determined by immunoturbidimetry [17]. Hemoglobin A_{1c} (HbA_{1c}) was measured by high-performance liquid chromatography (HPLC). The nondiabetic range for our laboratory is 4% to 6.3% [18].

Statistical analysis

The data were analyzed on the basis of gender. Results are presented as mean ± SEM. GFR, ERPF, and RBF were corrected for BSA. Between-group comparisons of

Table 1. Baseline characteristics (mean ± SEM)

Parameter	Female	Male	*P* value
Age *years*	14.32 ± 1.64	15.40 ± 2.30	0.21
Body mass index *kg/m²*	24.59 ± 3.56	22.15 ± 1.88	0.07
Hemoglobin A_{1c}%	8.56 ± 0.93	8.50 ± 1.2	0.89
Diabetes duration *years*	10.02 ± 2.61	11.54 ± 3.05	0.22
Protein intake *g/kg/day*	1.17 ± 0.04	1.21 ± 0.05	0.89
Urine Na^+ *mmol/day*	144 ± 21	152 ± 18	0.23
Plasma renin activity *ng angiotensin I/L/min*	0.09 ± 0.002	0.08 ± 0.001	0.64
17β-estradiol *pmol/L*	62 ± 3	—	—
Mean arterial blood pressure *mm Hg*	76.23 ± 5.65	78.30 ± 9.16	0.64

Table 2. Mean systemic and renal hemodynamic responses to hyperglycemia (mean ± SEM)

	Female			Male		
Parameter	Euglycemia	Hyperglycemia	*P* value	Euglycemia	Hyperglycemia	*P* value
Mean arterial blood pressure *mm Hg*	76.87 ± 7.51	78.04 ± 10.09	0.52	78.38 ± 7.36	74.62 ± 8.82	0.41
Glomerular filtration rate *mL/min/1.73 m²*	135.59 ± 43.96	144.37 ± 21.44	0.07	155.20 ± 59.89	145.96 ± 25.28	0.39
Effective renal plasma flow *mL/min/1.73 m²*	647.61 ± 122.65	589.35 ± 77.98	0.001	796.67 ± 206.16	761.25 ± 119.77	0.43
Filtration fraction	0.21 ± 0.05	0.24 ± 0.04	0.01	0.20 ± 0.08	0.20 ± 0.03	0.73
Renal blood flow *mL/min/1.73 m²*	1039.93 ± 171.34	935.37 ± 99.14	0.004	1339.63 ± 373.09	1285.98 ± 194.52	0.45
Renal vascular resistance *mm Hg/L/min*	75.89 ± 12.15	84.49 ± 14.28	0.01	61.92 ± 13.58	59.39 ± 9.11	0.83

all parameters at baseline were made using parametric methods (unpaired *t* test). Within-subject-and-between-group differences in the response to hyperglycemia and ACE inhibitors were determined by analysis of variance (ANOVA). All statistical analyses were performed using the statistical package SAS (SAS Institute Inc., Cary, NC, USA).

RESULTS

Baseline parameters: Clamped euglycemia and hyperglycemia

At baseline, the male and female groups were not significantly different in terms of age, HbA_{1c}, body mass index (BMI), MAP, diabetes duration, urine sodium excretion, protein intake, or plasma renin activity (PRA). In females, 17β-estradiol levels were consistent with the early follicular phase (Table 1). During clamped euglycemia (Table 2), males exhibited significantly higher ERPF ($P = 0.048$) and RBF ($P = 0.037$), after correction for BSA. RVR was significantly lower in males ($P = 0.019$). Although the GFR in males was numerically higher than in females, the values did not differ significantly. FF and MAP did not differ significantly between the two groups.

During clamped hyperglycemia compared to clamped euglycemia (Table 2), females exhibited a trend toward an elevation in GFR ($P = 0.07$), a significant reduction in ERPF, and RBF with a significant rise in RVR and FF. In response to hyperglycemia, males did not display any significant alterations in renal hemodynamic function. The hyperglycemia-mediated renal hemodynamic changes in GFR ($P = 0.023$), FF ($P = 0.014$), and RVR ($P = 0.01$) were significantly different in males compared to females (Figs. 1 to 3).

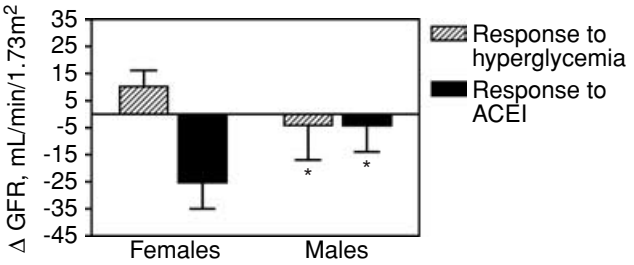

Fig. 1. Gender difference in glomerular filtration rate (GFR) responses to hyperglycemia and angiotensin-converting enzyme inhibitors (ACEI). The change (Δ) in GFR in response to hyperglycemia and ACE inhibitors in females and males with uncomplicated type 1 diabetes mellitus. $^*P < 0.05$ versus response in females.

Response to ACE inhibitors

Males and females both exhibited significant reductions in MAP during clamped euglycemia post-ACE inhibition (Table 3). Females experienced a significant fall in GFR and FF with no significant change in ERPF, RBF, or RVR. In contrast, no significant change in any renal hemodynamic parameter was observed in males. The changes in GFR ($P = 0.04$), FF ($P = 0.01$), and RVR ($P = 0.01$) responses to ACE inhibitors were significantly different in males compared to females (Figs. 1 to 3).

DISCUSSION

The protective effect of female gender in nondiabetic renal disease is diminished in diabetes mellitus [1–6], and

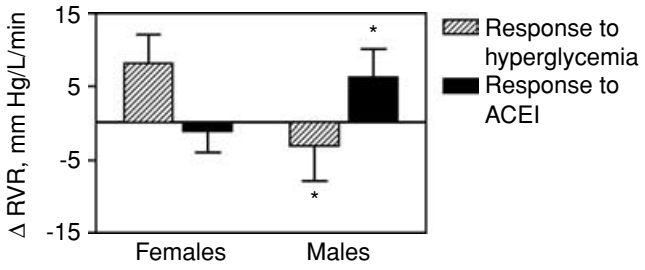

Fig. 2. Gender difference in renal vascular resistance (RVR) responses to hyperglycemia and angiotensin-converting enzyme inhibitors (ACEI). The change (Δ) in RVR in response to hyperglycemia and ACE inhibitors in females and males with uncomplicated type 1 diabetes mellitus. $^*P < 0.05$ versus response in females.

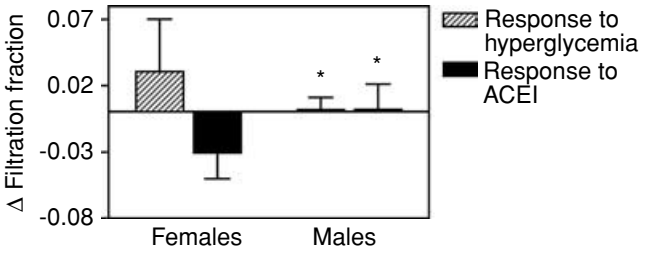

Fig. 3. Gender difference in filtration fraction (FF) responses to hyperglycemia and angiotensin-converting enzyme inhibitors (ACEI). The change (Δ) in FF in response to hyperglycemia and ACE inhibitors in females and males with uncomplicated type 1 diabetes mellitus. $^*P < 0.05$ versus response in females.

the goal of the current study was to identify mechanisms that might be responsible for the loss of female gender protection. Our major findings were (*1*) although males exhibited significantly higher ERPF and RBF and lower RVR at baseline during euglycemia, the renal hemodynamic response to hyperglycemia was augmented in women compared to men; (*2*) RAS blockade decreased GFR and FF in females but not in males during clamped euglycemia.

To our knowledge there are no previous studies examining gender differences in the renal hemodynamic profile that characterize the early, clinically silent phase of type 1 diabetes mellitus. The finding that males exhibited significantly higher baseline ERPF and RBF and lower RVR during euglycemia than did females suggests that renal hyperperfusion and hyperfiltration may be more common at this stage of type 1 diabetes mellitus in young males. In animal models, female nondiabetic rats have been shown to have higher afferent and efferent arteriolar resistances (and higher RVR) and lower RBF compared to male rats [19], which is consistent with the findings from our study. Conceivably, increased RVR might indicate increased afferent arteriolar tone, which in turn could protect the kidneys from hyperfiltration and hyperperfusion injury [20], delaying the progression to overt nephropathy. Similar hemodynamic observations were made in large-scale human studies in the 1940s and 1950s, which examined gender-based differences in renal hemodynamic function in healthy nondiabetic humans, and also found that women exhibit lower RBF [21–23], which is again consistent with findings from our current study. It is therefore tempting to speculate that the loss of afferent arteriolar tone, which is considered characteristic of the diabetic kidney [24], may be primarily a male phenomenon.

Interestingly, although studies in nondiabetic female animal and human models usually conclude that females exhibit significantly lower arterial pressure levels than do males [25], in our study, there were no significant gender differences in MAP. It has been demonstrated that, after the onset of puberty, boys exhibit higher blood pressure than do age-matched girls, but at age 13 to 15 years (the age range in which the majority of our subjects fell) the difference is only approximately 4 mm Hg [26], but even this difference was not evident in our study. We are not aware of any large-scale studies that have examined blood pressure differences in males and females with type 1 diabetes mellitus, but since some of the protective effect of gender is purported to stem from lower arterial pressure in females [27], it would be appropriate to examine in larger samples whether the usual gender differences are lost in this population.

During the clamped hyperglycemic phase of the study, females experienced significant decreases in ERPF and RBF, and an elevation in FF. The rise in FF suggests that the acute alteration in renal hemodynamic function was that of a greater increase in efferent relative to afferent arteriolar resistance. Such an effect, perhaps secondary to hyperglycemia-mediated RAS activation [10] would be associated with an increase in intraglomerular hydrostatic pressure. Interestingly, men exhibited little change in renal hemodynamic function in response to short-term hyperglycemia. We have previously shown that the vasoconstrictive response to RAS activation is blunted in females during the high estrogen phases of the menstrual cycle [11]. The augmented renal hemodynamic response to hyperglycemia in females in this study may have been partially because the studies in females were carried out during the follicular, low estrogen phase of the menstrual cycle. Although the mechanisms responsible for the increased renal hemodynamic response to hyperglycemia in females compared to males were not determined in this study, the predicted increase in glomerular capillary pressure may account for the clinical observation that female gender is not protective in diabetic nephropathy, glomerular capillary hypertension being a critical hemodynamic determinant of diabetic kidney disease progression [28–31].

The finding that ACE inhibitors resulted in beneficial reductions in GFR and FF only in females is consistent with studies in animals with and without experimental

Table 3. Mean systemic and renal hemodynamic responses to angiotensin-converting enzyme (ACE) inhibitors (mean ± SEM)

Parameter	Female			Male		
	Euglycemia	Post-ACE inhibitor	*P* value	Euglycemia	Post-ACE inhibitor	*P* value
Mean arterial blood pressure *mm Hg*	76.87 ± 7.51	71.37 ± 6.35	0.0002	78.38 ± 7.36	70.13 ± 6.23	0.002
Glomerular filtration rate *mL/min/1.73 m²*	135.59 ± 43.96	112.65 ± 24.17	0.04	155.20 ± 59.89	151.16 ± 44.10	0.64
Effective renal plasma flow *mL/min/1.73 m²*	647.61 ± 122.65	639.15 ± 134.46	0.55	796.67 ± 206.16	777.72 ± 150.60	0.58
Filtration fraction	0.21 ± 0.05	0.18 ± 0.04	0.05	0.20 ± 0.08	0.20 ± 0.07	0.71
Renal blood flow *mL/min/1.73 m²*	1039.93 ± 171.34	1013.04 ± 188.43	0.34	1339.63 ± 373.09	1297.12 ± 257.20	0.58
Renal vascular resistance *mmHg/L/min*	75.89 ± 12.15	74.74 ± 15.69	0.69	61.92 ± 13.58	55.79 ± 12.36	0.19

diabetes and humans with and without diabetes mellitus that examined the interaction between the RAS and estrogen [20, 32–37]. Animal models have demonstrated a reduction in serum ACE levels, tissue ACE activity and renal medullary ACE mRNA in response to estrogen [32]. The same study demonstrated an estrogen-mediated decrease in blood pressure, which was attributed to the observed reduction in RAS activity [32]. Studies in rat models have demonstrated that estrogen attenuates the responses to infusions of angiotensin II (Ang II) [33], and that 17β-estradiol replacement in ovariectomized diabetic rats reduces functional renal impairment, pathologic indices of glomerulosclerosis and interstitial fibrosis, urinary AER and transforming factor-β protein expression [38]. Gender-based differences in RAS function have also been shown in nondiabetic humans, including a blunted ERPF and FF response to Ang II infusion in women compared to men, especially in high estrogen states of the menstrual cycle [34]. In addition, alterations in components of the RAS and responsiveness of the RAS at the level of the peripheral vasculature [20] and renal vasculature [35] have been previously demonstrated. In humans with type 2 diabetes mellitus, hormone replacement therapy has been associated with a reduction in proteinuria and an increase in renal function [37]. Data from the Ramipril Efficacy in Nephropathy (REIN) study in nondiabetic renal disease showed that women experienced a significantly increased antiproteinuric effect from ACE inhibitors compared to men [36]. Women maintained on ramipril also exhibited a smaller decline in GFR over time, and a lower incidence of ESRD. This female gender-mediated augmented renal response to ACE inhibitors may be due to a synergistic effect of ACE inhibitors and estrogen on components of the RAS. Animal models examining the physiologic interaction between RAS blockade and estrogen have demonstrated synergistic effects leading to a reduction in deleterious vascular remodeling [39]. It is tempting to speculate that this phenomenon is also relevant to the hemodynamic effects of RAS activation. In the case of the present study, even though the experiments were carried out during the follicular phase, we noted an augmented response to ACE inhibitors in females, suggesting that any level of estrogen may act synergistically with RAS blockade.

The influence of gender on progression of diabetic nephropathy has been examined in at least six outcome studies by different investigators [1–6]. The studies by Mangili et al [1], Orchard et al [2], and Jacobsen et al [3] found associations between male gender and worse outcomes as measured by albuminuria in the first two studies and the change in GFR in the third. The study by Orchard et al [2] reported a higher risk of nephropathy in women with a short duration of diabetes, and a lower risk with longer duration. Three other studies in a total of 1301 patients found no impact of gender on progression of diabetic renal disease [4–6]. From these studies it is apparent that women with diabetes mellitus as the cause of their chronic kidney disease do not have the same degree of protection against progression as women with nondiabetic renal disease [1–6, 40]. The present study has demonstrated that although females appeared to exhibit a protective renal hemodynamic response to ACE inhibitors, their response to hyperglycemia was deleterious, resulting in a physiologic profile that could favor the development and progression of disease.

This study has some important limitations. The sample size was small which probably contributed to the failure to reach statistical significance in some measurements (for example the GFR response to hyperglycemia in females). We attempted to correct for this by constructing homogenous groups and by careful prestudy preparation. In addition we used a paired design that allowed each subject to act as his/her own control, thereby decreasing variability and allowing us to reach some significant conclusions. Responses to RAS blockade can be influenced by prestudy differences in effective circulating volume [41]. We avoided this confounding effect by ensuring that our subjects were sodium replete, and by monitoring compliance with 24-hour urine collections for sodium excretion. Further, extreme hyperglycemia can activate the RAS through volume contraction induced by glucosuria. We protected against this by clamping blood glucose at 9 to 11 mmol/L in the hyperglycemic phase, below the threshold for glucosuria. Women exhibit variations in renal hemodynamic and RAS function throughout the menstrual cycle [11] or while taking oral contraceptive medications [42]. We reduced the impact of these factors by studying only female subjects who

were non-users of oral contraceptive medications, and only during the follicular (low estrogen) phase of the menstrual cycle. Finally, extrapolating from short-term physiologic studies to a long-term condition which progresses over years can be tenuous. However, our aim was to determine whether there are gender differences in the renal physiologic profile in response to the major factors that could conceivably promote or protect against progression of renal disease rather than to examine progression of disease over time, a project that would require a much larger, long-term study.

CONCLUSION

We have demonstrated gender differences in baseline renal hemodynamic function, the renal hemodynamic response to short-term hyperglycemia and to ACE inhibitors, in subjects with uncomplicated type 1 diabetes mellitus. These differences may be important in understanding the initiation and progression of diabetic renal disease in men and women. Our study suggests that women with type 1 diabetes mellitus respond more favorably to ACE inhibitors, with a beneficial reduction in GFR and FF, but that hyperglycemia may impact more negatively on their renal function, and may mitigate some of the protective effects of female gender. Glycemic control, although crucial in the management of all patients with type 1 diabetes mellitus, may be particularly important in women.

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