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A Mouse Model of Erectile Dysfunction Via Diet-Induced Diabetes Mellitus

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Abstract

Objective—Diabetes mellitus (DM) is a significant risk factor in the development of erectile dysfunction (ED). We sought to determine whether diet induced DM in mice would recapitulate the major features of human ED.

Methods—In total, 150 C57BL6 (bl-6) mice were divided into 6 groups (n=25/group). One hundred twenty five were fed a high fat (45% total calories) diet for the final 4 (Group 2), 8 (Group 3), 12 (Group 4), 16 (Group 5), or 22 (Group 6) weeks. Group 1 was fed a normal diet. Mice were 22–25 weeks old at study termination. Corporal tissues were harvested and studied for a) endothelium-dependent and endothelium-independent vasoreactivity, b) endothelial and smooth muscle cell content by immunohistochemistry, c) NOS expression by NADPH-diaphorase staining. d) Apoptosis by TUNEL staining.

Results—Blood glucoses were greater in Groups 2–6 vs. Group 1. Vasoreactivities, endothelial cell content, smooth muscle/collagen ratio were lower while apoptosis was higher in the DM mice (p=0.0001, p=0.10, p=0.0002, p<0.001, p<0.001, respectively). There was significant decreased NOS expression and significant increase in apoptosis (p<0.0001 each) in high fat dieted mice.

Conclusions—Corporal tissue from mice with diet induced DM demonstrate many of the major functional, structural, and biochemical changes found in humans with ED. This model should serve as a valuable tool for advancing our understanding the role DM plays in the pathogenesis of ED.

Keywords

penis; impotence; endothelial function; mice; diabetes

INTRODUCTION

Diabetes mellitus is one of the major risk factors for erectile dysfunction. It has been estimated that 50%-75% of diabetic men have some degree of erectile dysfunction and the incidence of erectile dysfunction is higher in diabetic than in age-matched nondiabetic men and this

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difference increases with age¹⁻³. Men with diabetes mellitus and erectile dysfunction have more severe erectile dysfunction than men without diabetes mellitus based upon health related quality of life measures and less durable responses to pharmacotherapy than non-diabetic men with erectile dysfunction⁴. Pathophysiological mechanisms underlying diabetes-associated erectile dysfunction are in large part due to endothelial dysfunction⁵. The precise mechanisms by which diabetes initiates and/or amplifies the disruption in the cell-cell interactions in corporal tissue in erectile dysfunction are not completely understood.

Pre-clinical models are valuable to examine potential mechanisms of human disease, as well as develop and test therapeutic approaches. Though type II diabetes mellitus can be induced in rats, this species is suboptimal for the study of erectile dysfunction because of the lower amounts of smooth muscle cells and greater amounts of collagen in the corpus cavernosum compared to humans⁶⁻⁸. Mice fed a high fat diet develop hyperglycemia, hyperinsulinemia, and glucose intolerance⁹. We sought to determine whether diet induced diabetes mellitus would recapitulate the major abnormalities in corporal tissue that underlie erectile dysfunction.

MATERIALS AND METHODS

Mouse model

In total, 150 C57BL6 mice were studied. Twenty-five (Group 1) were fed regular chow and one hundred twenty five were fed a high fat (45% total calories) diet (Research Diets, Inc, New Brunswick, NJ) for the final 4 (Group 2, n=25), 8 (Group 3, n=25), 12 (Group 4, n=25), 16 (Group 5, n=25), and 22 (Group 6, n=25) weeks of the study, using methods to those described⁹. Mice were 22-25 weeks old at study termination. All protocols were approved by the Duke University Animal Care and Use Committee.

Glucose Measurements and Glucose tolerance testing

Serum glucose measures were made using a glucometer (One Touch Profile meter, Lifescan) on tail blood. Mice were fasted for 13-15h, before ip. injection with 1g/kg of glucose. Measures were made on fasting and blood samples 30, 60, and 90 min after glucose injection.

Tissue procurement, histological section preparation, protein isolation

At study termination all mice were deeply anesthetized with ketamine and xylazine and penectomy was performed with careful dissection of the corpora cavernosa from the tunica albuginea before sacrifice, as previously described¹⁰. Tissue was cryoprotected and frozen sections (5 μ m) were prepared¹⁰. Protein lysates were prepared and concentrations determined by Bradford assay, as previously described¹⁰.

Vasoreactivity Studies

Endothelium-dependent relaxation was assessed using acetylcholine, while direct nitric oxide mediated corporal smooth muscle relaxation was assessed by sodium nitroprusside, on corporal strips and the data analyzed and expressed as a percent of the active tension generated norepinephrine, as previously described¹⁰.

Histological Evaluation

Vascular smooth, endothelial cells and collagen content in the corporal tissue was assessed by immunohistochemistry or Masson staining, similar to those previously described¹⁰⁻¹³. Terminal deoxynucleotidyl transferase biotin-d-UTP nick-end labeling (TUNEL) was performed to label apoptotic cells, similar to those previously described¹⁴. Areas were quantified on 3 randomly selected 200x/50x• magnification fields in each of 2 sections as described^{10,12}. Measurement of total NOS (nitric oxide synthase) by NADPH (nicotinamide

adenine dinucleotide)-diaphorase staining was performed as previously described¹⁰. The positive signal is blue and positive area was quantitated by NIH image J software

Assessments of Akt, p-Akt

Both p-Akt and Akt, was assessed with ELISA kits (BioSource International, Inc, Camarillo, CA, #KHO0101 and #KHO0111), using methods described¹².

Measurement of VEGF protein

VEGF was determined using a solid-state ELISA system with a Quantikine VEGF ELISA Kit (R & D Systems, Minneapolis, MN), as previously described¹².

Statistical analysis

Results are expressed as the mean $\pm$ SD. The mean values were compared using analysis of variance (ANOVA). If the overall F-test for the ANOVA was at least of borderline significant ($p \leq 0.10$), post hoc comparisons between groups were performed. A Dunnett's multiple comparison was used to test for differences between each of the high fat diet groups vs. the normal diet group, repeated measures analysis of variance was used to test for glucose difference. A $p < 0.05$ was considered statistically significant.

RESULTS

High fat feeding, body weights and blood sugars

The mean body weight was 28.32 ± 1.68 g for Group 1. The weights were 33.95 ± 2.71 , 35.21 ± 4.19 , 36.95 ± 4.04 , 38 ± 5.2 , and 39 ± 4.31 for Group 2-6, respectively, all $p < 0.001$ vs. Group 1. Differences among groups over time was highly significant, $p < 0.0001$. At each time point, the mice in Group 2-6 had higher glucose measurements, all $p < 0.05$, compared to Group 1. The changes in serum glucose following glucose loading was higher in Group 2-6 compared to Group 1 and Group 5 and 6 had significantly higher values over the 90 minute test compared to Groups 2 and 3 (Figure 1, $n=15$ /group).

Diabetes mellitus is associated with abnormalities in endothelium-dependent and endothelium-independent vasoreactivity

The F-test from the analysis of variance was significant for an overall difference among means for ACH ED₅₀ ED₅₀[-log(M)], $p=0.0001$. Endothelium-dependent vasoreactivity was significantly lower in the Groups receiving 12, 16, and 22 weeks of high fat diet feeding, Groups 4-6, respectively, $p < 0.05$. The F-test from the analysis of variance was significant for an overall difference among means for SNP ED₅₀, $p=0.01$. Endothelium-independent vasoreactivity was significantly lower in Groups 5 and 6, $p < 0.05$ vs. Group 1 (Figure 2A, B and Table 1, $n=7$ /group).

Diabetes mellitus is associated with a reduction in endothelial cell content and smooth muscle/collagen ration in corporal tissue

The F-test for the content of CD-31 positive cells from the analysis of variance was significant for an overall difference among means, $p=0.0002$. As was found for abnormalities in endothelium-dependent vasoreactivity, the endothelial content was significantly lower in Groups 4-6 vs. Group 1, $p < 0.05$. The F-test for smooth muscle content was not significant for an overall difference among the groups ($p=0.11$). The F-test for smooth muscle/collagen ratio from the analysis of variance was significant for an overall difference among means, $p < 0.0001$. The means of the group receiving the 12 week, 16 week, and 22 week high fat diet were significantly lower than that for the normal diet, $p < 0.01$, $p < 0.0001$, and $p < 0.0001$, respectively (Table 1, Figure 3A-F, $n=6$ /group).

Diet Induced diabetes mellitus is associated with a reduction in NOS expression

The F-test for the area of NADPH-diaphorase positive staining from the analysis of variance was significant for an overall difference among the means, $p < 0.0001$. Diaphorase staining was significantly lower in the Groups 4-6, $p < 0.01$ vs. Group 1. The F-test for the intensity of NADPH-diaphorase positive staining from the analysis of variance was significant for an overall difference among the means, $p < 0.0001$. The intensity of staining was significantly lower in Groups 3-6, $p < 0.001$ vs. Group 1 (Table 1, Figure 3 G, H, I, $n = 8/\text{group}$).

Diet-Induced diabetes mellitus is associated with an increase in apoptosis in corporal tissue

The F-test from the analysis of variance was significant for an overall difference among means, $p < 0.0001$. Apoptosis was significantly higher in the Groups 4-6, $p < 0.001$ vs. Group 1. In addition apoptosis was significantly higher in Group 3, compared to Group 1, $p < 0.05$ (See Table 1, Figure 3 J, K, L, $n = 8/\text{group}$).

Diet-Induced diabetes mellitus was not associated with changes in VEGF protein or VEGF signaling in corporal tissue

The F-test for the levels of VEGF protein or pAkt/Akt from the analysis of variance was not significant for a difference among the means (See Table 1, $n = 6/\text{group}$).

DISCUSSION

Type II diabetes mellitus is a major cause of erectile dysfunction. The purpose of this study was to determine whether diet-induced type II diabetes mellitus would result in any abnormalities in corporal tissues that are relevant to erectile dysfunction. The first major finding of our study was that diet induced diabetes mellitus resulted, sequentially, in abnormalities in endothelium-dependent and endothelium-independent vasoreactivity. Abnormalities in vasoreactivity in corporal tissue are the result of dysfunctional physiological interactions between endothelium and vascular smooth muscle, and to a smaller extent adventitial cells and nerves, in penile tissue². Reports have shown evidence of abnormalities in endothelium-dependent vasoreactivity in the aorta of hyperglycemic mice and in the penile tissue of patients with diabetes mellitus^{15,16}. These findings suggest a direct role for diet induced diabetes mellitus in the pathogenesis of erectile dysfunction in the absence of ischemia or occlusive vascular injury.

Corpus cavernosal tissue from men with erectile dysfunction secondary to diabetes mellitus exhibited impairments in both endothelium-dependent and endothelium-independent smooth-muscle relaxant capacity that was significantly correlated with diabetes mellitus duration¹⁷. Abnormalities in endothelium-dependent vasoreactivity occurred prior to the development of a derangement in vascular smooth muscle mediated vasoreactivity. Abnormalities in vasoreactivity directly contribute to the pathogenesis of vascular injury and this creates a paradigm for a self-perpetuating vascular injury in diseases states¹⁸. In our study abnormalities in endothelium-independent vasoreactivity occurred despite the fact that vascular smooth content was not different across the groups. However, we did notice a significant decrease in smooth muscle/collagen ratio following high fat diet, which may account for the change in endothelium-independent vasoreactivity, similar to the findings in diabetic rabbits or hypercholesterolemic rabbits^{19,20}. Diabetes mellitus renders the corpus cavernosum less compliant and the tunica albuginea less elastic and supportive, thus attenuating the filling and storage processes^{2,17,19}. Moreover, the possible alteration of the neural function due to diabetes mellitus may also play an important role^{2,17}.

Nitric oxide is the principal mediator of arterial dilation and smooth muscle relaxation which bring about tumescence of penile erectile tissue^{2,21}. We found a reduction in the intensity of

NADPH diaphorase staining prior to changes in the area of NADPH diaphorase staining, endothelial content, and endothelium-dependent vasoreactivity. We found no changes VEGF protein expression or Akt phosphorylation. These data are different from what others have shown in a genetic model of diabetes mellitus and alloxan induced diabetes-associated erectile dysfunction in rats ^{6, 22}. Defects in VEGF signaling, result in reductions in the activation of the protein kinase Akt, which promotes both endothelial cell survival and eNOS activation, are well known to play a role in the angiogenesis defect that is known to occur in type I diabetes mellitus, hypercholesterolemia and castration induced erectile dysfunction ^{6,12,21-23}, thus diet-induced erectile dysfunction appears to be different from these other forms of erectile dysfunction. Finally, we found increases in apoptosis at late time points following high fat diet, which is similar to what had been shown previously in diabetes mellitus and other erectile dysfunction models^{6,24-26}. Simple diet manipulations can cause type II diabetes mellitus, which if combined with the array of murine genetic models should provide powerful tools to advance our understanding of the mechanisms of diabetes mellitus induced erectile dysfunction.

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Blood Glucose Changes

Time P.G.I.	0 min (mg/dl)	30 min (mg/dl)	60 min (mg/dl)	90 min (mg/dl)
Group 1	98.25±17.93	209.8±27.9	170.8±28.9	162.6±23.3
Group 2	165±41.9	309.5±78	291.4±84.6	236.9±60.2
Group 3	161.8±32.7	338.4±66.4	307.1±56	251.3±50.8
Group 4	204.4±54.6	371.5±101.1	343.1±90.5	292.4±84
Group 5	215.3±46.3	374.7±81.1	383.3±113.3	341.3±97.1
Group 6	223.6±33.7	385.4±89.8	400.4±86.1	381.9±74.7

Figure 1.

Graph and table demonstrate the results of fasting and post glucose infusion (P.G.I.) serum glucose values from mice. Values were significantly higher in Groups 2, 3, 4, 5 and 6 vs. Group 1. The response to intra-peritoneal glucose is shown both graphically and in tabular form. HF = high fat.

Figure 2.

The results of the vasoreactivity studies are shown for endothelium-dependent (A) and endothelium-independent vasoreactivity (B). In A, the response of pre-contracted corpus cavernosum to acetylcholine shown as the $-\text{Log}[\text{ED}_{50}]$ are shown and these revealed that the endothelium-dependent vasoreactivity in the hyperglycemic mice was gradually lower in high fat diet groups (Group 1 vs. Group 4 or Group 5 or Group 6, * $p < 0.05$ each). In B, the response to sodium nitroprusside is shown as the $-\text{Log}[\text{ED}_{50}]$ and this value was gradually lower in high fat diet groups (Group 1 vs. Group 5 or Group 6, * $p < 0.05$). HF = high fat.

Figure 3.

Representative immunohistochemical stains of penile tissue from normal Diet (Group 1) and a mouse fed a high fat diet for 22 weeks (Group 6) showing endothelial cell (CD31, A and B, C, red), smooth muscle/ collagen ratio (Masson staining. D, E, F. Smooth muscle, pink; Collagen, blue), NOS expression (NADPH-diaphorase, G, H and I, blue), and apoptosis (TUNEL, J, K and L, brown). Photos for Masson staining were taken at 50x magnification while all other photos were taken at 200x magnification. Note the decreased endothelial cell content, smooth muscle/collagen ratio, NOS expression and an increase in apoptosis that occurs with diet induced diabetes. HF = high fat and NC = negative control.

TABLE 1
Results of the Isometric Tension Studies, Histology, and VEGF and VEGF signaling Assays in the Study Groups.

	ACH EDS0	SNP EDS0	Endo Cells %	Smooth Muscle cells %	SMA/ Collagen (ratio)	VEGF (pg/ mg)	p/t Akt (Ratio)	TUNEL (+)%	NADPH (+)%
Group 1	5.15±0.52	5.58±0.41	14.9±1.38	33.4±3.9	0.19±0.023	8.4±2.8	0.41±0.32	0.57±0.06	54.6±6.90
Group 2	4.91±0.22	5.31±0.30	14.3±1.50	33±6.0	0.19±0.015	7.5±5.6	0.42±0.47	0.6±0.10	52.3±6.99
Group 3	4.4±0.62	5.16±0.57	13±2.20	31±6.3	0.17±0.019	8.2±4.7	0.38±0.20	0.71±0.08	48.6±5.04
Group 4	4.2±0.91	4.95±0.58	12.3±1.90	30±5.0	0.15±0.019	13.8±11.9	0.24±0.23	0.84±0.09	43.5±4.99
Group 5	3.53±0.38	4.78±0.58	12±1.40	28.8±3.6	0.13±0.017	11.3±9.2	0.24±0.09	0.98±0.10	37.8±4.77
Group 6	3.34±0.42	4.66±0.24	11.2±0.81	27.8±2.9	0.11±0.015	7.6±3.8	0.19±0.15	1.27±0.15	30.5±3.66
p value for F Test	p=0.0001	p =0.01	p=0.0002	p =0.11	p<0.0001	p =0.7	p =0.59	p<0.0001	p<0.0001

ACH = acetylcholine; SNP = sodium nitroprusside; Endo = endothelial; SMA = smooth muscle actin; VEGF = vascular endothelial growth factor; p/t Akt = phosphorylated Akt/ total Akt; TUNEL = terminal deoxynucleotidyl transferase biotin-d-UTP nick-end labeling; NADPH, nicotinamide adenine dinucleotide phosphate.