Treatment With Metformin Improves Erectile Dysfunction in a Murine Model of Obesity Associated With Insulin Resistance

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OBJECTIVE To evaluate the effects of treatment with metformin on a murine model of obesity-associated erectile dysfunction.

MATERIAL AND METHODS C57BL/6 male mice were fed for 10 weeks with standard chow or high-fat diet. Lean and obese mice were treated with the insulin sensitizer metformin (300 mg/kg/day, 2 weeks). Intracavernosal pressure (ICP) and in vitro corpus cavernosum (CC) relaxations to both acetylcholine and electrical field stimulation, as well as phenylephrine-induced contractions, were obtained. Levels of cyclic guanosine monophosphate in CC were detected by enzyme immunoassay.

RESULTS High-fat-fed mice exhibited higher body weight and insulin resistance. Cavernous nerve stimulation caused frequency-dependent ICP increases, which were significantly lower in obese compared with lean mice ($P < .05$). Two-week therapy with metformin reversed the decreased ICP in obese group. The maximal response to acetylcholine in CC was 35% lower ($P < .05$) in the obese compared to the lean group, which were restored by metformin treatment. Likewise, the impaired electrical field stimulation–induced CC relaxations in obese mice were also partly restored by metformin. Contractile responses to phenylephrine were significantly greater ($P < .05$) in obese compared to lean mice, which were fully restored by metformin. Basal and stimulated cyclic guanosine monophosphate productions in the erectile tissues were significantly lower ($P < .05$) in the obese group, an effect fully restored by metformin.

CONCLUSION Treatment with metformin restored the erectile function in obese mice, through improvement of in vitro endothelial and nitrergic cavernosal relaxations. Therefore, use of metformin may be a good pharmacologic approach to treat insulin resistance–associated erectile dysfunction. UROLOGY 86: 423.e1–423.e6, 2015. © 2015 Elsevier Inc.

Erectile dysfunction (ED) is characterized by a persistent inability to achieve and/or maintain an erection sufficient for satisfactory sexual performance and has been associated with an abnormal function in the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway and hence, lower NO bioavailability in the erectile tissue.[1] Epidemiologic studies show that obesity is an important risk factor for both ED and insulin resistance (IR).[2-5] Evidence supports a strong causal link between IR and ED.[6-10] In fact, ED has been considered an early clinical manifestation of risk factors for cardiovascular events including acute myocardial infarction.[11] In addition, ED may be the first clinical sign of IR in young men.[8] A recent randomized controlled trial in patients with ED with poor response to sildenafil reported that treatment with metformin improves erectile function.[7]

IR is a state of dysregulation of glucose-insulin homeostasis, in which the ability of insulin to stimulate glucose uptake in peripheral tissues is reduced.[2] Insulin is well known for inducing vascular relaxation through a mechanism that involves endothelium-dependent NO production.[12] Vasodilator action of insulin is mediated by the phosphatidylinositol 3 kinase (PI3K)-Akt pathway that phosphorylates endothelial NO synthase at Ser^{1177}.[12] IR has been strongly associated with decreased NO bioavailability and endothelial dysfunction.[13] Previous studies showed that high-fat-fed obese mice display ED, as demonstrated by reductions of intracavernous pressure (ICP) by cavernosal nerve electrostimulation and impaired endothelial and nitrergic cavernosal relaxations, as well as by increase of contractile response elicited by α1-adrenergic receptor activation.[14,15]

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Table 1. Body weight, epididymal fat mass, and insulin sensitivity of lean and obese mice treated with vehicle (water) or metformin (300 mg/kg/day, 14 days) by gavage

	Lean + V	Obese + V	Lean + Met	Obese + Met
Body weight (g)	31 ± 0.6	43 ± 2*	30 ± 0.7	42 ± 1*
Epididymal fat mass (g)	0.32 ± 0.06	1.89 ± 0.13*	0.39 ± 0.02	1.86 ± 0.10*
Kitt (min^{-1})	4.34 ± 0.30	2.06 ± 0.37*	4.38 ± 0.29	4.24 ± 0.45†

K_{itt}, constant rate for blood glucose disappearance; Met, metformin; V, vehicle.
Data are mean ± standard error of mean of 5 mice.
* $P < .05$ in comparison with lean + vehicle group.
† $P < .05$ in comparison with obese + vehicle group.

The insulin sensitizer metformin is a first-line pharmacologic treatment for patients with type 2 diabetes mellitus, which acts by decreasing hepatic glucose production and increasing insulin sensitivity in skeletal muscle and adipose tissue.[16] Recently, metformin was shown to increase the adenosine signaling in corpus cavernosum from the high-fat-fed rabbits via increases in NO production.[17] However, no detailed functional study has explored the effect of metformin treatment on a murine model of obesity-associated ED. Our hypothesis is that metformin reverses ED in the murine model of obesity associated with IR through improvement of NO bioavailability in the erectile tissue. Therefore, in the present study, in a continuing effort to investigate the effect of metformin treatment on ED, we have treated insulin-resistant obese mice with the insulin sensitizer metformin and evaluated in vivo and in vitro functional responses of the mice corpus cavernosum.

MATERIAL AND METHODS

Animals

All animal procedures and experimental protocols are in accordance with the Ethical Principles in Animal Research adopted by the Brazilian College for Animal Experimentation and followed the Guide for the Care and Use of Laboratory Animals. Four-week-old male C57BL/6J mice were provided by Central Animal House Services of the University of Campinas.

Diet-induced Obesity and Treatment

Mice were housed 3 per cage on a 12-hour light-dark cycle and fed for 10 weeks with either a standard chow diet (carbohydrate, 70%; protein, 20%; fat, 10%) or a high-fat diet that induces obesity (carbohydrate, 29%; protein, 16%; fat, 55%), as previously described. Lean and obese mice were treated with vehicle (water) or the insulin sensitizer metformin (300 mg/kg/day) by gavage from the eighth to the 10th week. In our protocols, we used a total of 70 mice divided into 4 experimental groups, namely lean plus vehicle (N = 16), lean plus metformin (n = 19), obese plus vehicle (n = 16), and obese plus metformin (n = 19). Tail-cuff pressure was measured by using a modified tail-cuff method in conscious animals in a quiet room.

In Vivo Insulin Sensitivity

After 6 hours of fasting, systemic insulin sensitivity was analyzed by the insulin tolerance test. Tail blood samples were collected before (0 min) and at 5, 10, 15, 25, 30, and 60 minutes after an intraperitoneal injection of 1.00 U/kg of regular insulin (Novolin R; NovoNordisk, Bagsværd, Denmark). Glucose concentrations were measured using a glucometer (ACCUCHEK Performa; Roche Diagnostics, Indianapolis, IN), and the values were used to calculate the constant rate for blood glucose disappearance (Kitt), based on the linear regression of the Napierian logarithm of glucose concentrations obtained from 0 to 30 minutes of the test. Kitt was calculated using the formula $0.693/(t_{1/2})x - 1 \times 100$.

ICP Measurement

Mice were anesthetized with an intraperitoneal injection of urethane (1.8 g/kg). To perform ICP measurement, right penile crus was exposed and cannulated using a 26-gauge needle. The cannula was filled with sterile heparinized saline solution and attached to a pressure detector for continuous ICP monitoring. The bladder and prostate were exposed through a midline abdominal incision. The right major pelvic ganglion and cavernous nerve were identified posterolateral to the prostate on one side. The cavernous nerve was electrically stimulated with 2 platinum electrodes connected to a Grass S48 stimulator (Astro-Med Industrial Park, West Warwick, RI). The cavernous nerve stimulation was conducted at 6 V, with 1 ms pulse width and trains of stimuli lasting 60 seconds at varying frequencies, with intervals of 3 minutes between the stimulation trains. Changes in ICP were recorded using a PowerLab 400 data acquisition system (LabChart software, version 7.0; ADInstruments, Colorado Springs, CO). Mice undergoing ICP measurements were not used for other experimental protocols.[15]

Functional Studies in Cavernosal Strips and Concentration-Response Curves

Mice were anesthetized with isoflurane and exsanguinated. Strips of mice CC were mounted in a 10-mL organ system containing Krebs solution at 37°C continuously bubbled with a mixture of 95% O_2 and 5% CO_2 (pH 7.4) and vertically suspended between 2 metal hooks. One hook was connected to a force transducer and the other acted as a fixed attachment point. Tissues were allowed to equilibrate for 60 minutes under a resting tension of 2.5 mN. Isometric force was recorded using a PowerLab 400 data acquisition system (LabChart, version 7.0; ADInstruments, MA).

Cumulative concentration-response curve was constructed for the muscarinic agonist acetylcholine (ACh; 10^{-9} to 3×10^{-5} M) in cavernosal strips precontracted with the α_1-adrenergic receptor agonist phenylephrine (PE; 10^{-5} M). Cumulative concentration-response curves of PE (10^{-8} to 3×10^{-4} M) were also obtained in the cavernosal tissue. Nonlinear regression analysis to determine the pEC_{50} was carried out using GraphPad Prism (GraphPad Software, San Diego, CA) with the constraint that $\Phi = 0$. All concentration-response data were evaluated for a fit to a logistics function in the form: $E = E_{max}/[1 + (10^c/10^x)^n] + \Phi$, where E is the maximum response produced by agonists; c is the logarithm of the EC_{50}, the concentration of drug that produces a half-maximal response; x is the logarithm of the concentration of the drug; the exponential term, n, is a curve-fitting parameter that defines the slope of the concentration-response line, and Φ is the response observed in the absence of added drug.