ORIGINAL RESEARCH—BASIC SCIENCE

Estrogen Mediates Metabolic Syndrome-Induced Erectile Dysfunction: A Study in the Rabbit

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ABSTRACT

Introduction. Estrogen receptor (ER) α is critical in mediating the harmful effects of hyperestrogenism in fetal or neonatal life on the developing penis. In contrast, little is known on the impact of an excess of estrogens on penile function in adulthood.

Aim. To investigate the effect of estrogens on metabolic syndrome (MetS)-associated erectile dysfunction (ED).

Methods. We employed a recently established animal model of high fat diet (HFD)-induced MetS. Subgroups of MetS rabbits were dosed with either testosterone (T) or tamoxifen. We evaluated penile responsiveness to acetylcholine (Ach) as well as the expression of genes related to penile smooth muscle relaxation and contractility.

Main Outcome Measure. Associations between MetS-induced penile alterations and sex steroids were investigated in an animal model of HFD-induced MetS. To understand the role of either androgen deficiency or estrogen excess on ED, we treated subgroups of MetS rabbits with either T or tamoxifen, a classical ER antagonist.

Results. Feeding an HFD-induced MetS was associated to elevated estradiol (E2) and low T levels. E2, but not T, was independently and negatively associated with genes able to affect penile erection. Smooth muscle-related markers decreased as a function of E2 and were positively associated with all the variables investigated. Increasing concentrations of circulating E2 were negatively associated with Ach-induced relaxation. In HFD rabbits, in vivo T dosing significantly improved MetS and completely normalized circulating E2. Conversely, in vivo tamoxifen dosing reduced visceral adiposity and partially restored T level. Ach-induced relaxation was severely impaired by HFD and significantly restored, up to the control level, by both tamoxifen and T dosing. In rabbit smooth muscle cells cultures 17β-E2 (1 nM) significantly reduced the expression of α-smooth muscle actin, transgelin, and phosphodiesterase type 5. The effects of 17β-E2 were completely reverted by tamoxifen (100 nM).

Conclusions. This study demonstrates, for the first time, that HFD-induced ED is more associated with a high E2, rather than to a low T, milieu. HFD-induced ED is partially restored by in vivo treatment not only with T but also with the nonsteroidal ER antagonist, tamoxifen. **Vignozzi L, Filippi S, Comeglio P, Cellai I, Morelli A, Marchetta M, and Maggi M. Estrogen mediates metabolic syndrome-induced erectile dysfunction: A study in the rabbit. J Sex Med 2014;11:2890–2902.**

Key Words. Erectile Dysfunction; Hyperestrogenism; Metabolic Syndrome

Introduction

Estrogens mediate their effects by binding to estrogen receptors (estrogen receptor [ER]α and ERβ), which are both present in several districts of the male reproductive tract [1–4], including the penis [5–9]. In human [7] and animal [8,9] penile tissues, ERα is abundantly expressed, with a concentration similar to androgen receptor (AR) but two log-units higher than ERβ.

An excess of estrogen has a detrimental effect on many facets of male health, especially on male reproductive biology [10]. Epidemiological studies have shown a link between hyperestrogenism, due to a perinatal exposure to environmental estrogens, and an increasing frequency of reproductive abnormalities in offspring [11]. Accordingly, more than two million male offsprings of mothers exposed during pregnancy to the synthetic estrogen diethylstilbestrol have a higher incidence of cryptorchidism, hypospadias, and small penis [12,13]. American alligators (*Alligator mississippiensis*) living in Florida's Lake Apopka—which is severely contaminated with industrial effluents containing estrogenic chemicals—exhibit altered plasma hormone concentrations and a reduced phallus size [14]. Similarly, laboratory animals exposed either neonatally or perinatally to estrogens developed reproductive abnormalities, including microphallus and hypospadias [15,16]. Interestingly, ERα-knockout (ERα –/–) male mice, but not (ERβ –/–) ones, are resistant to these estrogen-induced reproductive abnormalities [17].

In humans, up to now, a total of 23 men have been reported to have familial hyperestrogenism (aromatase excess syndrome), a clinical condition characterized by an excessive conversion of C19 androgens into estrogens. This condition in male individuals is characterized by markedly elevated E2/testosterone (T) ratio, along with increased E2 levels, prepubertal or peripubertal gynecomastia (heterosexual precocity), advanced bone maturation, small testis, and micropenis [18,19].

Taken together, these data indicate that ERα is critical in mediating the harmful effects of hyperestrogenism in fetal or neonatal life on the developing penis. In contrast, little is known on the impact of an excess of estrogens on penile function in adulthood.

Obesity, a pandemic problem worldwide, represents an interesting model of naturally occurring male hyperestrogenism due to an increased expression of aromatase in adipose tissue. Visceral obesity is the core component of metabolic syndrome (MetS), a constellation of metabolic and cardiovascular (CV) alterations increasing the risk of major adverse CV events and type 2 diabetes. MetS, in the male, is also involved in the development of several nonmetabolic alterations, including central hypogonadism [20,21] and erectile dysfunction (ED) [20–22].

The objective of the present study is to investigate the possible role of estrogens on MetS-associated ED. As an experimental model, we employed a recently established animal model of high fat diet (HFD)-induced MetS, which recapitulates the human MetS phenotype (visceral obesity, dyslipidemia, hypertension, and glucose intolerance), along with an impaired erectile function, central hypogonadism, and hyperestrogenism [8,23–30]. In this nongenomic model, associations between MetS-induced penile alterations and sex steroids were investigated. In addition, subgroups of MetS rabbits were dosed with either T or tamoxifen, a classic ERs antagonist [31], to understand the role of either androgen deficiency or estrogen excess on ED, respectively.

Materials and Methods

Animal Treatment

Male New Zealand White rabbits (Charles River, Calco, Lecco, Italy), weighing about 3 kg, were individually caged under standard conditions in a temperature- and humidity-controlled room on a 12-hour light/dark cycle. Water and food were unrestricted throughout the study. After 1 week of standard rabbit diet, the animals were randomly assigned to a control or a treatment group. The control group continued to receive a standard diet (regular diet [RD]), while the treatment group was fed an HFD made up of 0.5% cholesterol and 4% peanut oil (HFD rabbit) for 12 weeks, slightly modified according to a previously described protocol [8,23–30]. The composition of the RD and HFD diets has been previously reported [26]. A subset of HFD rabbits received tamoxifen treatment (0.25 mg/kg/day) according to a previously described protocol [8]. A second subset of HFD animals was supplemented with a pharmacological dose of T (30 mg/kg weekly i.m.), used as a treatment known to ameliorate MetS features, as well as erectile function in HFD rabbit models [30]. Blood samples for glucose, total cholesterol, triglycerides, T, and 17β-estradiol (E2) analyses were obtained from the animals via marginal ear vein at week 0 (baseline) and at week 12 in all groups. The blood was immediately centrifuged at 3,000 rpm for