Testosterone Partially Ameliorates Metabolic Profile and Erectile Responsiveness to PDE5 Inhibitors in an Animal Model of Male Metabolic Syndrome

Sandra Filippi, PhD,* Linda Vignozzi, MD, PhD,† Annamaria Morelli, PhD,† Aravinda K. Chavalmane, PhD,† Erica Sarchielli, PhD,‡ Benedetta Fibbi, MD, PhD,† Farid Saad, MD,§ Peter Sandner, MD,¶ Peggy Ruggiano, MD,† Gabriella B. Vannelli, MD,‡ Edoardo Mannucci, MD,** and Mario Maggi, MD†,††

*University of Florence Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction—Departments of Pharmacology and Clinical Physiopathology, Florence, Italy; †University of Florence—Andrology Unit Department of Clinical Physiopathology, Florence, Italy; ‡University of Florence—Department of Anatomy, Histology and Forensic Medicine, Florence, Italy; §Bayer Schering Pharma AG—Scientific Affairs Men's Healthcare, Berlin, Germany; ¶Bayer Schering Pharma AG—Global Drug Discovery, Wuppertal, Germany; **University of Florence—Diabetes Section Geriatric Unit, Department of Critical Care, Florence, Italy; ††CIRMAR (Centro Interuniversitario di Ricerca sulle basi molecolari delle Malattie della Riproduzione), Milan, Italy

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ABSTRACT

Introduction. Metabolic syndrome (MetS) is a clustering of cardio-metabolic risk factors (hyperglycemia, hypertension, dyslipidemia, visceral fat accumulation) that is also associated with hypogonadism and erectile dysfunction (ED).

Aim. To clarify the relationships among MetS, hypogonadism, and ED, we developed an animal model of MetS.

Methods. Male rabbits fed a high-fat diet (HFD), with or without testosterone (T) supplementation, were compared with control rabbits (fed a standard chow) and with rabbits made hypogonadal by a single injection of a long-acting GnRH-analog, triptorelin.

Main Outcome Measures. Evaluation of metabolic disturbances (plasma glucose, cholesterol, triglycerides, testosterone, LH, FSH level, glucose tolerance, mean arterial pressure, visceral fat accumulation), and corpora cavernosa (CC) relaxant capacity (in vitro contractility study) in HFD animals as compared with control, GnRH analog-treated rabbits, and T-supplemented HFD rabbits.

Results. HFD rabbits showed all the features of MetS. HFD induced hypogonadotropic hypogonadism is characterized by a reduction of plasma T, FSH, LH levels, testis and seminal vesicles weight, and testicular steroidogenic enzymes. Such a phenotype is similar to that induced by triptorelin administration. A reduced GnRH immunopositivity in hypothalamus suggests a central origin of HFD-related hypogonadism. HFD also induced penile alterations, as demonstrated by a reduction of acetylcholine-and electrical field stimulation-induced CC relaxation, hyper-responsiveness to the NO donor, SNP, and unresponsiveness to PDE5 inhibitors. Similar penile alterations were observed in triptorelin treated rabbit. In HFD, as well as in triptorelin treated rabbits, PDE5 and eNOS mRNA expression quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) were significantly decreased. T administration prevented almost all penile alterations observed in HFD rabbits. T treatment dramatically reduced HFD-induced visceral obesity, partially ameliorating also the metabolic profile.

Conclusion. We have developed an animal model of MetS associated with hypogonadotropic hypogonadism and penile alterations including unresponsiveness to PDE5 inhibitors. T supplementation was able to partially revert HFD-induced phenotype. **Filippi S, Vignozzi L, Morelli A, Chavalmane AK, Sarchielli E, Fibbi B, Saad F, Sandner P, Ruggiano P, Vannelli GB, Mannucci E, and Maggi M. Testosterone partially ameliorates metabolic profile and erectile responsiveness to PDE5 Inhibitors in an animal model of male metabolic syndrome. J Sex Med 2009;6:3274–3288.**

S. Filippi and L. Vignozzi equally contributed.

Key Words. Metabolic Syndrome; Androgen Deficiency; Erectile Dysfunction; Visceral Obesity; Testosterone; Animal Model

Introduction

Metabolic syndrome (MetS) is a constellation of medical disorders that increase the overall risk of developing cardiovascular (CV) and metabolic diseases. Features of this syndrome include: insulin resistance (hyperinsulinemia, impaired glucose tolerance, and type 2 diabetes mellitus), dyslipidemia (hypertriglyceridemia and low serum high-density lipoprotein cholesterol), visceral fat accumulation, and hypertension [1]. Although obesity is a risk factor for insulin resistance, type 2 diabetes mellitus (T2DM), and a significant risk factor for CV disease, not every obese patient is insulin resistance, and the recommendation to measure waist circumference rather than body mass index (BMI) recognized the important role played by visceral obesity in metabolic syndrome [1].

MetS, and in particular, visceral obesity, is often associated with testosterone (T) deficiency [2–4]. Epidemiological studies show that T2DM and MetS could predict the development of male hypogonadism [5–7]. On the other hand, hypogonadism per se represents a risk factor for T2DM, MetS [8–10] and CV diseases [11,12]. Animal studies demonstrated that low endogenous T is associated with both fatty streak formation within the aortic root and a proatherogenic circulating lipid profile in mice after feeding on a cholesterol-enriched diet [13].

Recently, it has been recognized that subjects with MetS have a higher prevalence of sexual dysfunction, and in particular erectile dysfunction (ED) [14–15]. In subjects with sexual dysfunction, the prevalence of MetS is an age-dependent phenomenon that, at midlife, could affect almost half of the patient population [16]. ED, the most commonly referred symptom of male hypogonadism [12,17], is considered a warning sign of MetS [10,15]. Moreover, because of shared factors impairing both penile and systemic vascular blood flow, ED has been recognized as a sentinel sign of forthcoming CV diseases [16,18–19]. Hence, studying ED and hypogonadism in MetS may help to elucidate their pathogenetic relationships.

The aim of the present study is the development of an experimental animal model of MetS, in order to investigate the association between MetS, hypogonadism, and ED.

A non-genomic model of MetS was developed by exposing rabbits to a high-fat diet (HFD). Such a model, conceptually similar to diet-induced obesity in mice [20], could resemble human MetS phenotype.

Materials and Methods

Chemicals

Phenylephrine (Phe) HCl, sodium nitroprusside (SNP), acetylcholine (Ach), guanethidine, indomethacin, and atropine were purchased from Sigma-Aldrich (St. Louis, MO, USA). T supplementation was performed using a mix of 110 mg of T enanthate and 25 mg of T propionate, corresponding to 100 mg of T (supplied by Bayer-Schering Pharma, Berlin, Germany). Triptorelin pamoate was obtained by Ipsen (Milan, Italy). Special diet for rabbits was purchased from Mucedola (Settimo Milanese, Milan, Italy). Sildenafil was supplied by Pfizer (Pfizer Italia, Rome, Italy), and vardenafil was supplied by Bayer Schering Pharma AG, Global Drug Discovery (Wuppertal, Germany).

All the substances, except of indomethacin and sildenafil, were dissolved daily in double-distilled water, and further dilutions to the final concentrations were made in saline solution. Stock solutions of indomethacin and sildenafil were made in ethanol and further dilutions to the final concentrations were made in Krebs' solution. Control experiments showed that the concentrations of ethanol used modified neither the vasoconstrictor response to Phe nor the relaxation induced by the different agents. The mouse monoclonal GnRH primary antibody (HU11B, sc-32292) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Animal Treatments

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