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Metabolic syndrome-associated sperm alterations in an experimental rabbit model: Relation with metabolic profile, testis and epididymis gene expression and effect of tamoxifen treatment

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ABSTRACT

The influence of metabolic syndrome (MetS) on sperm quality and function is debated. Using a well-established high fat diet (HFD) rabbit model resembling human MetS, including development of hypogonadism, we demonstrate that HFD decreased sperm motility, morphology and acrosome reaction in response to progesterone and increased sperm cholesterol content. All the above parameters were associated with most MetS features, its severity and plasma testosterone (T) at univariate analysis. After T adjustment, sperm morphology and motility retained a significant association, respectively, with mean arterial pressure and circulating cholesterol levels. MetS modified the expression of inflammatory and tissue remodelling genes in the testis and of aquaporins in the epididymis. In a multivariate analysis, sperm morphology resulted associated with testis expression of fibronectin and collagen type 1 genes, whereas motility with epididymis aquaporin 1 gene. Administration of tamoxifen, used in the treatment of idiopathic male infertility, to HFD rabbits partially restored motility, but further decreased morphology and increased spontaneous acrosome reaction, without restoring responsiveness to progesterone. Overall our results indicate that development of MetS produces detrimental effects on sperm quality and functionality by inducing metabolic disorders leading to alterations in testis and epididymis functions and evidence a role of hypertension as a new determinant of abnormal sperm morphology, in line with a previous human study from our group.

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1. Introduction

Metabolic syndrome (MetS) affects about 20–25% of worldwide population (Grundy, 2008) and is characterised by a combination of multiple alterations (Cornier et al., 2008). In the male, MetS is associated with sexual dysfunction (Corona et al., 2008), hypogonadotropic hypogonadism (Corona et al., 2011) and, less clearly, with infertility as described in a recent meta-analysis by Sermondade et al. (2013) and in other papers (Kasturi et al., 2008; Lotti et al., 2013; MacDonald et al., 2010). Indeed, although some studies demonstrated that high BMI or waist circumference could impair sperm quality (Eisenberg et al., 2014; Fejes et al., 2005; Lotti et al., 2011; Sermondade et al., 2013), others did not (Aggerholm et al., 2008; MacDonald et al., 2010). Recently, the occurrence of a negative correlation between sperm total motility and morphology with the number of MetS components was reported in metabolic patients (Lotti et al., 2013). Moreover, an increase of sperm DNA fragmentation (SDF) has been found in MetS men (for review see Palmer et al., 2012a). Although no clear conclusions can be drawn from these studies, it appears that MetS may have effect on the fertility status of the male; however, the mechanisms and the metabolic features responsible for alterations of spermatogenesis and sperm characteristics are not fully disclosed. In particular, no studies have addressed the role of the establishment of an inflammatory status in MetS (Monteiro and Azevedo, 2010) and semen quality.

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Animal models may be of great help in understanding the relationship between MetS and pathological conditions that may affect the function of the male genital tract. Several animal models of obesity have been developed. In most (Mortazavi et al., 2014; Palmer et al., 2012b; Saez Lancellotti et al., 2010, 2013) but not all (Ouvrier et al., 2011) of these models an association between obesity and decreased sperm quality and functional parameters has been reported, although with differences depending on the species and the type of diet. Moreover, although some metabolic (glucose and insulin) and hormonal (T) parameters were evaluated at least in one study (Palmer et al., 2012b), the relationship between metabolic characteristics and semen parameters remains poorly studied. In addition, little is known about the relationship between modifications of the hormonal profile and semen quality, as well as testicular and other male genital tract alterations in these models.

Recently, we have developed an animal model that closely recapitulates the human MetS phenotype, by feeding white New Zealand male rabbits for 12 weeks with a HFD (Filippi et al., 2009; Vignozzi et al., 2011). HFD rabbits showed hyperglycaemia, reduced glucose tolerance, increased visceral fat mass, hypertension, hypercholesterolaemia, hypertriglyceridaemia, as well as hypogonadotropic hypogonadism associated with hyperoestrogenism, all characteristics similar to those developed by human MetS (Maneschi et al., 2012; Morelli et al., 2014; Vignozzi et al., 2011). In this model, we investigated the relationship between MetS features (including hormonal profile) and sperm quality and function. Moreover, we evaluated modifications of mRNA expression of some genes involved in fibrosis and inflammation in the testis and in fluid reabsorption at epididymis level of HFD animals.

In view of the increase of oestrogen levels in our model (Maneschi et al., 2012; Morelli et al., 2014) we evaluated whether the concomitant treatment of HFD rabbits with tamoxifen (Tam) – an oestrogen receptor antagonist often used to treat idiopathic male infertility (Chua et al., 2013) – could revert the HFD-induced metabolic and sperm alterations.

2. Materials and methods

2.1. Chemicals

Human tubal fluid (HTF) medium and human serum albumin (HSA) were purchased from Technogenetics (Milan, Italy). Diff-Quick kit was purchased from CGA, Diasint (Florence, Italy). The other chemicals were from Sigma Chemical.

2.2. 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 12-hour light/dark cycle. Water and food were unrestricted throughout the study.

After 1 week of standard diet (water 12%, protein 16.5%, fibres 15.5%, vegetable fat 3.5%; Harlan-Global Diet 2030, Mucedola, Settimo Milanese, Milan, Italy), the animals were randomly assigned to two groups: (1) control ($n = 23$), fed a regular diet for 12 weeks; (2) HFD ($n = 16$), fed a high-fat diet implemented with 4% peanut oil and 0.5% cholesterol with respect to regular diet (water 12%, protein 12.6%, fibres 21.2%, vegetable fat 6%, animal fat 0.5%; Mucedola) for 12 weeks.

To evaluate the effect of Tam, 10 additional animals were concomitantly treated for 12 weeks with HFD + Tam (0.25 mg/kg/die dissolved in drinking water (Filippi et al., 2002; Orgebin-Crist et al., 1983)).

Blood samples for glucose, total cholesterol, triglycerides, testosterone (T) and oestradiol (E_2) analyses were obtained early in the morning after an overnight fasting from the animals via marginal ear vein at week 12 in all groups. The blood was immediately centrifuged at 3.000 rpm for 20 minutes and collected plasma was stored at −20 °C until assayed. When obtained plasma was not sufficient for all the analyses, we opted to measure first all metabolic parameters and T levels. Mean arterial blood pressure (MAP) was measured by using a polyethylene catheter inserted into a femoral artery at week 12, after pentobarbital (45 mg/kg) sedation. Afterward, the rabbits were sacrificed by a lethal dose of pentobarbital. After sacrifice, an entire epididymis and the controlateral cauda epididymis were collected in 1 ml of warmed PBS (pH 7.4), whereas other epididymis and testis were harvested and appropriately stored for the subsequent analyses. All animal experiments and tissues collection were performed in accordance to D.L. 116/92 and approved by the Institutional Animal Care and Use Committee of the University of Florence.

2.3. Measurement of cholesterol, triglycerides, glycaemia, T in rabbits

Plasma cholesterol, triglycerides and glucose levels were measured by using an Automated System (ADVIA 2004 Siemens Chemistry System; Siemens Science Medical Solution Diagnostic, Tarrytown, NY, USA). Plasma T and E_2 levels were measured by using an automated chemiluminescence system (Immunolite 2000 Siemens, Siemens Healthcare Diagnostics, Deerfield, IL, USA), after appropriate extraction. For extraction, samples were mixed with four volumes of diethyl ester for 15 minutes, centrifuged for 5 minutes at 2.000 rpm and the aqueous phase frozen in dry ice. The organic phase was recovered, evaporated to dryness under a nitrogen stream and reconstituted in the assay buffer.

2.4. Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed in accordance with the published method (de Roos et al., 2001; Kawai et al., 2006). After an overnight fasting, a 50% glucose solution was orally administered to the animals, at a dose of 1.5 g/kg. Blood samples were collected via the marginal ear vein before and 15, 30, and 120 minutes after glucose loading. Samples were stored on ice and centrifuged at 3.000 rpm for 20 minutes at 4 °C to obtain plasma. Plasma glucose was measured as described above. The incremental area under the curve (iAUC) was calculated by using the GRAPHPAD PRISM software version 4.0 for Windows.

2.5. Semen samples collection and preparation

Sperm were collected from the entire epididymis and from cauda epididymis by squeezing out in PBS and centrifuged at 2.000 rpm for 5 minutes at room temperature (RT). Sperm from entire epididymis were used for evaluation of sperm number, viability, motility, morphology and DNA fragmentation, whereas sperm from cauda were used for evaluation of acrosome reaction. It was possible to evaluate all the parameters only when the number of recovered sperm exceeds 15 million.

Sperm samples were suspended in 1 ml of HTF medium supplemented with 10% HSA and counted after appropriate dilution by Neubauer camera. After counting, sperm were re-suspended at the concentration of 10×10^6/ml and analysed for motility, viability and morphology. Sperm motility was evaluated by determining the percentage of progressive, non-progressive and immotile sperm by scoring at least 100 sperm/slide, according with WHO criteria (World Health Organization, 2010). Sperm viability (as percentage of live sperm) was calculated after eosin–nigrosin staining by scoring of at least 200 sperm/slide (World Health Organization, 2010). Sperm morphology was evaluated by determining the percentage of normal

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