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Impact of the metabolic syndrome on reproductive health in males of infertile couples

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Introduction

Metabolic syndrome (MetS) represents a constellation of abnormalities, including abdominal obesity, impaired glucose metabolism, dyslipidemia (hypertriglyceridemia, low HDL cholesterol) and hypertension, which identifies subjects at high risk for diabetes and cardiovascular diseases (Kasturi et al., 2008; Cornier et al., 2008; Eckel et al., 2010; Corona et al., 2011a).

Different diagnostic criteria for MetS have been proposed over the years (see Appendix 1). In fact, the definition of parameters to be used for the diagnosis of MetS, as well as their thresholds, is under constant debate (Corona et al., 2009a, 2011a). Nonetheless, insulin resistance and increased adiposity have been universally recognized as the main features underlying MetS (Cornier et al., 2008; Corona et al., 2009a, 2011a), although there is increasing evidence for other key components of the syndrome, such as oxidative stress, proinflammatory state or exaggerated sympathetic nervous system activation (Cornier et al., 2008).

Several pathologic conditions other than diabetes and cardiovascular diseases are associated with MetS, including non-alcoholic fatty liver disease, polycystic ovarian syndrome, obstructive sleep apnea, lipodystrophy, and microvascular disease (Cornier et al., 2008). In addition, in the male, hypogonadism, erectile dysfunction and psychological disturbances are also often comorbid with MetS (Corona et al., 2006;

2011a, 2011b). Although a possible association between MetS and male infertility has also been hypothesized (Kasturi et al., 2008), it has never been demonstrated. Available animal models of MetS, both genetically- (Vartanian et al., 2006) and high fat diet- (Mallidis et al., 2011) induced, suggest that MetS is associated with deranged spermatogenesis and poor sperm quality . Several studies report a negative effect of the individual components of MetS also in the human male, however, the association between each MetS component, but not of MetS as a "category", has been reported (see Kasturi et al., 2008).

Among the components of MetS, overweight/obesity represent the more studied. However, the impact of overweight or obesity on the reproductive health of males has been only partially elucidated. In fact, although scientific interest in this issue has grown in the last years (see for review Hammoud et al., 2008; MacDonald et al., 2010; Sermondade et al., 2012), available clinical studies investigating the relationship between obesity and male infertility are relatively poor compared to the extensive body of research investigating its association with female infertility (Pasquali et al., 2007; Zain & Norman, 2008; Loret de Mola, 2009; Wilkes & Murdoch, 2009; Brewer & Balen, 2010; Rittenberg et al., 2011). Hence, while the relationship between obesity and female infertility is more than clear, the association with male infertility is still up for discussion (Hammoud et al., 2008; MacDonald et al., 2010). In addition, a recent

meta-analysis revealed little evidence for a relationship between obesity and poor semen parameters, despite a clear association with hypogonadism (MacDonald et al., 2010). However, a more recent meta-analysis reported that overweight and obesity are both associated with an increased risk of azoospermia or oligozoospermia (Sermondade et al., 2012).

In addition, in this last decade, a growing body of evidence has also documented an independent association between benign prostatic hyperplasia (BPH)/lower urinary tract symptoms (LUTS) and obesity/MetS (Mongiu and McVary, 2008; Moul and McVary, 2010; Gorbachinsky et al., 2010; Parsons, 2011; De Nunzio et al., 2012). In particular, we previously reported a positive correlation between MetS and BPH-related chronic inflammation in patients undergoing surgery for BPH (Vignozzi et al., 2013; Gacci et al., 2013). A recently published epidemiological survey of the Boston area (BACH) confirmed an association between MetS and LUTS; however, when subjects were stratified by age, the association was confirmed only in the youngest individuals (Kupelian et al., 2013).

AIM OF THE THESIS

Since MetS is essentially based on increased adiposity and it is associated with male hypogonadism (Corona et al., 2011a), erectile dysfunction, psychological disturbances (Corona et al., 2006), and BPH/LUTS (Mongiu and McVary, 2008; Moul and McVary, 2010; Gorbachinsky et al., 2010; Parsons, 2011; De Nunzio et al., 2012), and all these factors might, in different ways, affect reproductive capacity (Hammerli et al., 2009), we investigated their possible correlations with MetS. Hence, we performed two studies.

In the first study (study 1) we evaluated possible associations between MetS, semen and hormonal parameters, as well as clinical characteristics, including sexual, ultrasound and psychological characteristics, in a cohort of men with couple infertility.

In the second study (study 2), we systematically investigated the possible associations between MetS and prostate-related symptoms and signs in a cohort of young men in infertile unions and tried to establish whether these associations correlate with fertility.

STUDY 1

METABOLIC SYNDROME AND SEMINAL, SCROTAL ULTRASOUND AND PSYCHOBIOLOGICAL PARAMETERS IN MALES OF INFERTILE COUPLES

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Seminal, ultrasound and psychobiological parameters correlate with metabolic syndrome in male members of infertile couples

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Materials and Methods

Patients

We studied a consecutive series of 376 male patients (mean age 36.0±7.5 years) attending our Outpatient Clinic for the first time from January 2008 to December 2011, seeking medical care for couple infertility. Couple infertility was defined as the inability of a sexually active couple to achieve pregnancy despite unprotected intercourse for a period greater than 12 months, according to the World Health Organization (WHO, 2000). Subjects with karyotype abnormalities (n = 3), chromosome Y micro- deletions (n = 3) and uni- or bilateral (n = 4 and n = 15, respectively) absence of vas deferens were excluded from the analysis. The socio-demographic and clinical characteristics of the sample are summarized in Table 1.

All patients were evaluated before beginning any treatment. All patients enrolled underwent the usual diagnostic protocol applied to newly referred subjects at the Andrology Outpatient Clinic for infertility. All patients underwent a complete andrological and physical examination, with measurement of blood pressure (mean of three measurements 5 minutes apart, in sitting position, with a standard sphygmomanometer), height, weight and waist. In addition, scrotal ultrasound was routinely performed. All the data provided were collected as part of a routine clinical procedure and therefore, according to Italian law, approval from the local Ethical

Committee was not required. In addition, at the time of the first visit, all patients gave their written informed consent to have their clinical records included in a dedicated database and they were aware that their data, after having been made anonymous, would be used for clinical research purposes.

Metabolic Syndrome (MetS) assessment

MetS has been defined according to the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute (IDF&AHA/NHLBI, see Appendix 1) (Alberti et al., 2009) as the presence of three or more of the following five factors: central obesity (waist circumference > 102 cm), elevated triglycerides (≥ 1.7 mmol/L or on drug treatment for elevated triglycerides), elevated blood pressure (BP ≥ 130 mm Hg systolic blood pressure or 85 mm Hg diastolic blood pressure or on antihypertensive drug treatment in a patient with a history of hypertension), elevated fasting glucose (≥ 5.6 mmol/L or on drug treatment for elevated glucose) and reduced high density lipoprotein (HDL) cholesterol (< 1.03 mmol/L or on drug treatment for reduced HDL cholesterol).

Evaluation of erectile function

Patients were asked to complete the erectile function domain (EFD) (Cappelleri et al., 1999)of the International Index of Sexual Function-15 (IIEF-15) (Rosen et al., 1997) (Appendix 2), in its Italian translation. An IIEF-15-EFD score of < 26 indicates erectile dysfunction (ED) (Cappelleri et al., 1999).

Evaluation of premature ejaculation (PE) status

Patients were asked to complete the Premature Ejaculation Diagnostic Tool (PEDT) (Symonds et al., 2007) (Appendix 3), in its Italian translation. PEDT is a brief, multidimensional, psychometrically validated, 5-item, self-reported questionnaire for diagnosing ejaculatory status, which provides scores for five subdomains, #1 control, #2 frequency, #3 minimal stimulation, #4 distress and #5 interpersonal difficulty. PEDT score was calculated as the sum of the scores of these domains. A PEDT score of ≤ 8 indicates no-PE (Symonds et al., 2007).

Screening of psychological traits

Patients were asked to complete the Middlesex Hospital Questionnaire, modified (MHQ) (Crown & Crisp, 1966) (Appendix 4), a brief self-reported questionnaire for the screening of mental disorders, which provides scores for free-floating anxiety (MHQ-A), phobic anxiety

(MHQ-P), somatization (MHQ-S), obsessive-compulsive (MHQ-O), depressive (MHQ-D) and hysterical (MHQ-H) traits and symptoms.

Colour-Doppler ultrasonography (CDU)

All patients underwent scrotal CDU, using the ultrasonographic console Hitachi H21 (Hitachi Medical System, Tokyo, Japan). In order to prevent bias on the part of the examiner, scrotal CDU was performed intermittently by 2 experienced physicians (F.L. and G.C.), unaware of the clinical data. The CDU characteristics of the sample are summarized in Table 1.

Scrotal CDU was performed in various longitudinal, transverse and oblique scans (Behre et al., 1995; Vicari, 1999, Lotti et al., 2011, 2012) using a 7.5 MHz high-frequency linear probe (L54M 6-13 MHz). Testicular and epididymal CDU features were examined according to previous studies (Behre et al., 1995; Vicari, 1999, Lotti et al., 2011, 2012; Isidori & Lenzi, 2008; Migaleddu et al., 2012). In particular, testis inhomogeneity (striated pattern) was defined as previously reported (Cohn et al., 1996; Loberant et al., 2010; Isidori & Lenzi, 2008; Migaleddu et al., 2012) (see Fig. 1, panel A, inset).

All patients underwent colour-Doppler assessment of penile arteries in the flaccid state with a 7.5-MHz high-frequency linear probe (L54M 6-13 MHz), a pulsed Doppler investigation frequency of 5 MHz and a colour

flow mapping capability, according to previous studies (Mancini et al., 2000; Corona et al., 2008a). The transducer was placed longitudinally on the ventral surface at the base of the penis. Colour flow mapping was helpful in obtaining an accurate angle corrected velocity with angle of insonation always < 45°, as previously reported (Mancini et al., 2000).

Semen analysis, urine and seminal cultures and hormone evaluation

All patients underwent, after the ultrasound session, semen analysis, performed according to the WHO criteria. Leukocytospermia was defined as leukocytes in seminal plasma $\geq 1 * 10^6$/ml (WHO, 2010). In addition, urine and seminal cultures were routinely assessed in all men.

Blood samples were drawn in the morning, after an overnight fast, for determination of blood glucose (by glucose oxidase method, Aeroset Abbott, Rome, Italy), HDL cholesterol and triglycerides (by automated enzymatic colorimetric method; Aeroset Abbott, Rome, Italy), LH, FSH, PRL,TSH, total testosterone (TT) by electrochemiluminescent method (Modular Roche, Milan, Italy) and sex hormone binding globulin (SHBG) by modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). Calculated free testosterone was derived according to Vermeulen's formula (available at http: //www.issam.ch/freetesto.htm) (Vermeulen et al., 1999). Biochemical hypogonadism has been defined for testosterone levels < 12 nmol/L (Wang

et al., 2009). Secondary or primary hypogonadism has been defined for LH ≤ 9.4 or > 9.4 U/L, respectively, according to Tajar et al. (2010).

Identification of case patients and controls

MetS was defined as described above. Subjects with 3 or ≥ 4 MetS components (n=15 and n= 12, respectively) were compared with controls selected from the same cohort with a 1:3 ratio (n=45 and n=36, respectively). For each case, the first three following patients within the same series with the same age (±4 years), body mass index (±2 kg/m^2), TT levels (±4 nmol/L), smoking status (current/non-smoker), alcohol consumption (current/no consumption), past or present cryptorchidism (presence/absence), leukocytospermia (presence/absence), current positive urine and/or semen cultures (presence/absence) were taken as a control. Associations with a p value < 0.05 were considered as significant.

Data analysis

Data were expressed as mean ± standard deviation (SD) when normally distributed, as median (quartiles) for parameters with non-normal distribution, and as percentages when categorical. Correlations were assessed using Spearman's or Pearson's method whenever appropriate. Differences between more than two groups were assessed with one-way ANOVA or Kruskal-Wallis test, whenever appropriate. Unpaired two-sided

Student’s t tests were used for comparisons of means of normally distributed parameters. In all other cases, Mann–Whitney U-test was used for comparisons between groups. Relative risk and 95% confidence interval were calculated for association of categorical parameters, and chi-squared test was used for comparisons. Stepwise multiple linear or logistic regressions were applied for multivariate analysis, whenever appropriate. All statistical analysis was performed on SPSS (Statistical Package for the Social Sciences, Chicago, USA) for Windows 17.0.

Results

Among the patients selected (mean age 36.0±8.0), 178 (50.7%) had no MetS components, 99 (28.2%) one, 47 (13.4%) two, 15 (4.3%) three, 8 (2.3%) four and 4 (1.1%) five components. Overall, 27 (7.7%), satisfied MetS criteria, according to the IDF&AHA/NHLBI criteria. Subjects with MetS were older (43.7±10.4 vs 35.5±7.4; $p<0.0001$) and had significantly lower levels of TT (13.8±6.5 vs 16.7±6.2; $p<0.05$) compared to the rest of the sample. No difference in the percentage of subjects with current smoking was found comparing MetS and no-MetS subjects (18.5 vs 28.5%, $p=0.178$). In addition, no difference in the frequency of those consuming alcohol was observed between the two groups (22.2 vs 25.6%, for MetS and no-MetS subjects, respectively; $p=0.671$). Subjects with diabetes mellitus were 3 (1.1%), representing 11.1% of the MetS group. The percentage of subjects under specific medications for MetS components (hypoglycaemic, n=3; lower-lipid, n=6; antihypertensive, n=14) in the whole sample and in the MetS group is reported in Table 1.

Erectile dysfunction (ED), assessed by the IIEF-15-EFD score, was complained of by 69 (19.9%) patients, in particular by 46.2% and 16.7% of the MetS and no-MetS subjects, respectively. Premature ejaculation (PE), assessed by the PEDT score, was observed in 53 (15%) patients, particularly in 22.2% and 14.4% of the MetS and no-MetS subjects, respectively.

Etiological factors involved in male infertility: classification, prevalence and associations with MetS.

Table 2 shows a patient stratification according to etiological factors involved in male infertility (as in Krausz, 2011), and the prevalence of MetS for each infertility factor. Possible associations between MetS and different causes of infertility have been investigated. A higher prevalence of secondary hypogonadism was found in subjects with MetS compared to the rest of the sample (51.9 vs 17.6%, respectively; $p< 0.0001$). In contrast, a lower frequency of varicocele was observed in MetS subjects, according to both clinical and CDU criteria (Table 2). In particular, the presence of severe CDU varicocele was negatively associated with MetS (RR=0.28 [0.08-0.97], $p<0.05$). No further association with other clinical abnormalities was observed (Table 2). In particular, no statistically significant difference in the prevalence of past or present cryptorchidism, leukocytospermia or current positive urine and/or semen cultures was observed comparing subjects with and without MetS (Table 2).

In the same Table it is also reported the prevalence of MetS subjects under each clinical condition involved in male infertility. It is evident that secondary hypogonadism is the most common MetS-associated condition.

Ultrasonography testis parameters

At univariate analysis, both testis and epididymal inhomogeneity were associated with MetS components (RR= 1.43 [1.16-1.76], p=0.001 and 1.27 [1.03-1.57], p<0.05, respectively for each increment in the number of MetS components). In an age-adjusted logistic modelling, only testis inhomogeneity retained a significant association, and the HR for testis inhomogeneity as a function of MetS components was 1.36 [1.09-1.70], p<0.01 (for each increment in the number of MetS components, see Figure 1A and 2A). These results were confirmed even after exclusion of subjects with diabetes mellitus (n=3) from the analysis (data not shown). Figure 2A shows associations between individual MetS components and testis inhomogeneity. After adjusting for age, the only factor significantly associated with testis inhomogeneity was increased waist (HR=2.25 [1.23-4.09], p<0.01).

No further association between MetS and other testicular ultrasound parameters, including volume, was observed (not shown).

Hormonal and seminal parameters

Figure 1, panel B, shows that TT declines as a function of MetS components (p for trend at ANOVA <0.0001). This result was confirmed even after exclusion of subjects with diabetes mellitus (n=3) from the analysis (data not shown). Differences in TT levels between those having 2

or 3 MetS components were not statistically significant (p=0.263; Figure 1, panel B).

Table 3 reports associations between MetS and hormonal parameters before and after adjusting for age. In an age-adjusted model, both TT and also calculated free T were negatively associated with MetS components, whereas gonadotropins, TSH and PRL were not (Table 3). These results were confirmed even after exclusion of subjects with diabetes mellitus (n=3) from the statistical analysis (data not shown). By using an age-adjusted, iterative logistic modelling, we calculated the HR for hypogonadism (TT < 12 nmol/L) as a function of MetS components, taken together or individually (Figure 2B). Factors significantly associated with hypogonadism were increased waist (HR=3.1 [1.6-5.9], p=0.001), low HDL cholesterol (HR=2.4 [1.16-4.96], p<0.02), and high triglycerides (HR=2.4 [1.12-5.1], p<0.05). We also found that testis inhomogeneity was a risk factor for hypogonadism, even after adjusting for age (HR=2.99 [1.71-5.24], p<0.0001). We therefore introduced TT levels in the logistic model describing the association between MetS factors and testis inhomogeneity, as previously described (see before). Adding TT as a further covariate, besides age, significantly attenuated the relationship between number of MetS components and testis inhomogeneity (HR=1.24 [0.98-1.56], p=0.07).

Table 1 shows semen parameters of the whole sample and of subjects with or without MetS. Since sexual abstinence had a wide variation in our population, eventually affecting semen parameters, the possible association between sexual abstinence and semen parameters has been investigated by univariate analysis, finding no correlation either in the whole sample or in MetS-stratified groups (data not shown). At univariate analysis, progressive motility and normal morphology were negatively related to number of MetS components (both $p<0.0001$ at ANOVA and Kruskal-Wallis, respectively). Since diabetes mellitus or current male genital infections may affect semen parameters, statistical analyses have been performed excluding iteratively subjects with diabetes mellitus (n=3), leukocytospermia (n=29) or current positive urine and/or semen cultures (n=25). After the exclusion of these subjects, previous associations were confirmed at ANOVA for progressive motility and Kruskal-Wallis for normal morphology (data not shown). When age and TT were introduced as covariates in a multivariate model describing the relationships between MetS components and sperm parameters, only sperm morphology retained a significant association ($B=-1.418\pm0.42$; $p=0.001$) (see Figure 1, panel C). In addition, as smoking habit/alcohol consumption and use of medications might negatively affect sperm parameters, these confounders were iteratively introduced as further covariates in the aforementioned multivariate model. Neither smoking/drinking habit nor use of medications

were associated with abnormal sperm morphology while MetS still resulted a statistically significant determinant in both models (B=-1.318±0.45; p=0.003 and B=-1.333±0.24; p<0.005, respectively).

Figure 2, panel C, reports, as a Forest Plot the HR for abnormal morphology (< 4%, WHO 2010) as a function of individual MetS components and MetS overall. In an age- and testosterone-adjusted logistic model, only hypertension was associated with abnormal morphology (HR=1.99 [1.11-3.54], p=0.02). The percentage of normal sperm morphology (≥4%, WHO 2010) in subjects with or without IDF&AHA/NHLBI-defined hypertension was 3.0 [1.0-7.0] vs 6.0 [2.0-10.0] (p<0.01). Since testis inhomogeneity was an important risk factor for abnormal morphology, even in an age- and TT-adjusted logistic model (HR=3.68 [1.99-6.82], p<0.0001), we tested the effect of introduction of testis inhomogeneity on the relationship between IDF&AHA/NHLBI-defined hypertension and abnormal morphology. In an age- and TT-adjusted logistic model, both testis inhomogeneity and hypertension were found to be independent risk factors for poor sperm morphology (HR=3.44 [1.82-6.53], p<0.0001 and HR=1.86 [1.02-3.39], p<0.05, respectively). No other associations between MetS and semen parameters were observed (data not shown).

Sexual parameters

When subjects with ED (IIEF-15-EFD score < 26) were divided according to the presence of MetS, those satisfying MetS criteria had a higher ED prevalence (46.2% vs. 16.7%, p=0.001). These results were confirmed after exclusion of subjects with diabetes mellitus (n=3) from the analysis (data not shown). The association was confirmed in a logistic model, after adjustment for age and TT (HR=2.86[1.18-7.31], p<0.05). At univariate analysis, the risk of ED increased as a function of MetS components (RR=1.56 [1.22-2.01] p<0.0001 for each increment in the number of MetS components), and this association was confirmed after adjusting for the aforementioned confounders (HR=1.45[1.08-1.95], p<0.02, for each increment in the number of MetS components, Figure 1 D). In an age- and TT-adjusted logistic model, the MetS factors positively associated with ED were increased waist (HR=2.92 [1.28-6.63], p<0.02) and blood pressure (HR=2.33 [1.1-4.9], p<0.05, Figure 2D).

When penile basal CDU was considered, arterial peak systolic velocity and acceleration were negatively related to MetS components, even after adjusting for age and TT (B=-0.748±0.314 , p<0.02 and B=-0.201±0.092, respectively, both p < 0.05). These results were confirmed after exclusion of subjects with diabetes mellitus (n=3) from the analysis (data not shown).

No association between PEDT score and MetS was observed (not shown) and no difference in PE prevalence was found comparing subjects with or without MetS (22.2% vs 14.4%, p=0.283).

Psychological parameters

At univariate analysis, patients with MetS showed a significantly higher score in MHQ-somatization (MHQ-S; 2.962±2.821 vs. 1.857±2.266, p=0.022) and depression scales (MHQ-D; 4.578±2.845 vs. 3.004±2.516, p=0.011). Scores for these two MHQ subscales were associated with numbers of MetS factors in both an unadjusted and in age- and TT- adjusted models (MHQ-S: unadjusted r = 0.126, adjusted B=0.66 ± 0.03, for each increment in the number of MetS components, both $p<0.05$; MHQ-D unadjusted r = 0.197, p=0.001, adjusted B= 0.69 ± 0.03 for each increment in the number of MetS components, $p<0.02$).

Case-control analysis

The correlations between MetS and seminal, ultrasound and sexual parameters resulting statistically significant were further assessed by comparing subjects with MetS with a 1:3 ratio matched controls (matched for age, body mass index, TT, smoking habit, alcohol consumption, past or present cryptorchidism, leukocytospermia, current positive urine and/or semen cultures; see Table 4). Even in the case-control analysis, subjects

with 3 or more MetS components showed a significantly increase in prevalence of abnormal morphology and testis inhomogeneity (Table 4). In addition, subjects with ≥ 4 MetS factors had more often ED (Table 4).

Effect of increasing number of MetS factors on reproductive health

In order to assess if, in MetS subjects, having ≥ 4 components has a more negative impact on reproductive health than having only 3 components, a comparison of the aforementioned parameters was performed between the two groups (Table 4). Subjects with ≥ 4 MetS factors were older, had a higher body mass index, were more often affected by ED and characterized by a lower TT levels compared to those with 3 components only (Table 4). No significant difference in normal sperm morphology and prevalence of testis ultrasound homogeneity was observed between the two groups (Table 4).

Discussion

This study demonstrates, for the first time, a component-dependent, stepwise association between the presence of MetS and abnormal semen parameters, along with several psychobiological factors which might further negatively impact male reproductive potential. In particular, we confirm, in subjects seeking medical care for male infertility, a clear-cut association between increasing numbers of MetS components and severity of male hypogonadism, previously reported in other patient populations (Muller et al., 2005; Corona et al., 2006, 2009a). Both total and calculated free T were reduced as a function of MetS factors. In particular, we found that having ≥ four MetS components exert a much more negative impact on TT levels than having only three. The finding that gonadotropins did not rise according to the androgen fall, indicates an hypogonadotropic nature of the hypogonadism, as previously described in studies performed on animal models of MetS (Filippi et al., 2009; Corona et al., 2011b) and in human cohorts (Corona et al., 2009b; Dandona et al., 2011). Accordingly, MetS triplicates the risk of having secondary hypogonadism, and secondary hypogonadism resulted the most common, infertility-associated, condition in subjects with MetS. When the contribution of individual MetS components was investigated, we found that increased visceral adiposity (waist) and dyslipidemia (reduced HDL-cholesterol and elevated triglycerides) were the main determinants of testosterone decline.

Interestingly, increased waist was found as the main determinant for testis inhomogeneity, indicating a specific pathogenetic role for adiposity-related factors in testicular abnormalities. Testis inhomogeneity at ultrasonography is suggestive of atrophy and fibrosis (Cohn et al., 1996; Loberant et al., 2010) and may reflect changes in the functional activity of the testis (Lenz et al., 1993; Behre et al., 1995). In fact, in the aging testis, atrophy of the tubular elements and proliferation of interstitial ones produce an exaggeration of the normally-unapparent septal and interstitial architecture, resulting in a striated appearance (Loberant et al., 2010). While a similar appearance is often observed in the elderly and considered normal, in young subjects it is recognized in a variety of pathological conditions, including hypogonadism (Cohn et al., 1996; Loberant et al., 2010; Migaleddu et al., 2012). Present data suggest that a secondary hypogonadism is predominantly associated to MetS, and in particular to visceral adiposity. Visceral adiposity is also the main determinant of testis inhomogeneity. The latter can derive from a decreased testicular stimulation, because of a central defect, and/or from a direct effect of visceral adiposity on testis itself (mixed hypogonadism), as previously reported in other patient cohorts (Corona et al., 2008b, 2009b). Several molecules, such as insulin, estrogens, leptin, tumor necrosis factor α (TNFα) or other adipokines, mainly related to increased adiposity, have been hypothesized to induce MetS-related hypogonadism, acting both at a

central or at a peripheral level (see for review Corona et al., 2011b). In contrast to the positive association between hypogonadism and MetS, varicocele was less often found in subjects fulfilling MetS criteria, thus confirming the view that obesity is a protective factor against varicocele (Handel et al., 2006; Nielsen et al., 2006; Tsao et al., 2009).

We here describe that there is a specific association between MetS and some sperm parameter abnormalities, including abnormal forward motility and morphology. These are two well recognized sperm parameters for successful fertilization both *in vitro* and *in vivo* (Chan et al., 1989; Joshi et al., 1996; Donnelly et al., 1998). However, after adjusting for testosterone levels, only abnormal morphology retained a specific association with MetS, suggesting that hypogonadism, more than MetS itself, is responsible for the decreased progressive motility. We also reported that testis inhomogeneity was associated with abnormal morphology, as previously described (Lenz et al., 1993). The MetS factor specifically associated with this abnormality was hypertension, and not visceral adiposity or other metabolic disturbances (hyperglycemia, dyslipidemia). Hypertension increased by a factor of two the risk of abnormal morphology, independently from other factors, such as testis inhomogeneity and testosterone levels. Mechanisms by which hypertension might affect sperm morphology are far from being understood (Kasturi et al., 2008). Akagashi et al. (1997) found an extreme spermatogenic impairment in the form of

atrophic seminiferous tubules devoid of spermatids in stroke-prone spontaneously hypertensive rats. Very recently, a study of semen quality in hypertensive men demonstrated an association between hypertension with more fragmented/abnormal sperm DNA and with increased expression of clusterin, an apoptosis-associated protein, which identifies a subset of morphologically altered spermatozoa not necessarily dead (Muciaccia et al., 2012). In that study (Muciaccia et al., 2012) the link between elevated blood pressure and abnormal morphology/DNA fragmentation was not clarified, but it was hypothesized that hypertension induces, within the testis, a generally altered vascular status characterized by enhanced ROS generation and limited antioxidant defense (Sharma et al., 1999; Brownlee, 2005; Amaral et al., 2008). Another recently published report (Mbah et al., 2012) indicates that treating normotensive, oligospermic men with a low-dose angiotensin converting enzyme (ACE) inhibitor (lisinopril, 2.5 md/daily) ameliorates, in a rigorous placebo-controlled, crossover design, semen parameters, including sperm number, motility and morphology. In a rabbit model of MetS, also characterized by hypertension, we observed an increase in advanced glycation end products and their receptor (RAGE) in the seminiferous epithelium and in the cytoplasm of Sertoli cells, spermatocytes and spermatids, respectively (Mallidis et al., 2009). Accumulation of advanced glycation end products and their receptor RAGE are thought to be capable of generating, promoting and/or

amplifying oxidative stress and its detrimental consequences, including DNA damage (Sakkas and Alvarez, 2010). More studies are advisable to verify whether hypertension is associated with DNA fragmentation in subjects with MetS.

ED (IIEF-15-EFD score < 26) was present in ~20% of the study cohort. This prevalence is essentially in agreement with previous reports in infertile men (Jain et al., 2000; O'Brien et al., 2005; Shindel et al., 2008; Lotti et al., 2012), but is at least double that observed in the general Italian population, when subjects with a similar mean age are considered (Parazzini et al., 2000; Mirone et al., 2004). The prevalence of ED in our patient population was further increased by the MetS condition, with hypertension and visceral adiposity as the main determinants. In addition, we found that having ≥ four MetS components exerts the maximal negative impact on sexual function. Also PCDU-measured penile blood flow was decreased as a function of MetS severity. Similar results were reported in other studies (Corona et al., 2006; Demir et al., 2009; Tomada et al., 2011). Interestingly, in this study, the association between an increasing number of MetS components and both ED and reduced penile blood flow were independent from low testosterone. The lack of association between MetS and premature ejaculation (PE or PEDT score) was expected according to previous studies of our group (Corona et al., 2008c, 2011c, 2012), where

hypogonadism was more prevalent in delayed than in premature ejaculation.

Not only ED, but also depressive symptoms and dysfunctional sexual relationship have been previously reported in subjects with couple infertility (Shindel et al., 2008; Smith et al., 2009; Lotti et al., 2012). It is well known that infertile men may develop feelings of inadequacy, anxiety, guilt and depression (Irvine & Cawood, 1996; Seidman & Roose, 2000). We now report that MetS severity further increases these negative emotions. In fact, both depressive symptoms and somatized anxiety were more prevalent in infertile men with MetS. MetS is highly prevalent among patients with a history of depression, especially those with current major depression (Heiskanen et al., 2006), perhaps because of increased body fatness or lifestyle alterations. However, previous prospective data indicated that depression, hostility and anger predict increased risk of MetS (Goldblacher & Matthews, 2007). Association between MetS and somatized anxiety was previously described by our group in subjects with sexual dysfunction (Corona et al., 2006). Somatization, which is the expression of physical symptoms in the absence of medically explained physical illness, might be viewed as a part of maladaptive thought or behaviour, or just as a dysfunctional response, to the infertility condition itself – an often unaccepted or unrecognised medical condition. We here report that having MetS increases somatization and depression scoring,

thus increasing the high attention devoted by these subjects to their body and possible bodily malfunctioning and therefore freezing several aspect of couple's sexual behaviour, including sexual desire and intercourse frequency (Corona et al., 2008d), further exacerbating the infertility condition. A large body of evidence indicates that infertility evokes more general psychological distress in women than in men (Jordan & Revenson, 1999). Accordingly, supportive psychosocial interventions are thought to be more beneficial for the female than for the male partner of the infertile couple (Hammerli et al., 2009). It is possible that MetS-associated negative emotions and sexual dysfunctions reduce this gender-related gap in psychological distress. Further studies are advisable to clarify this point.

Study 1 conclusions

MetS is a set of metabolic risk factors originally proposed as a diagnostic tool because of its potential in identifying subjects at risk for diabetes and cardiovascular diseases, with the idea that its components could somehow have a synergistic effect on predicting unfavourable events. Later on, it became clear that other medical conditions are comorbid with MetS, including male hypogonadism and erectile dysfunction (Corona et al., 2009a, 2011a, 2011b). We now report that an increasing number of MetS factors are dose-dependently associated with relevant organic (poor sperm quality, hypogonadism, ED) and psychological (depression, somatization) features that might affect reproductive outcomes of men seeking medical care for couple infertility. This might tailor ad hoc therapeutic intervention. Behavioural interventions targeting lifestyle factors, such as dietary practice and physical activity, might ameliorate not only metabolic and psychological parameters but also male infertility, as has been demonstrated for female infertility. Recognizing the umbrella condition termed “metabolic syndrome” might therefore represent a unique occasion to improve not only reproductive health, but also psychological, sexual and overall health.

STUDY 2

METABOLIC SYNDROME AND PROSTATE ABNORMALITIES IN MALE SUBJECTS OF INFERTILE COUPLES

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Metabolic syndrome and prostate abnormalities in male subjects of infertile couples

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Materials and Methods

We retrospectively evaluated a consecutive series of 187 male patients (age: 36.5±8.3 years) who attended our Outpatient Clinic initially between January 2010 and December 2011 seeking medical care for infertility. Based on guidelines of the World Health Organization, infertility was defined as the inability of a sexually active couple to achieve pregnancy despite unprotected intercourse over a period of greater than 12 months (WHO, 2000). Subjects with karyotype abnormalities (*n*=3), chromosome Y micro-deletions (*n*=2) or an absence of at least one vas deferens and/or one seminal vesicle (*n*=11) were excluded from the analysis. Hence, a cohort of 171 selected patients was used for the analyses. The socio-demographic and clinical phenotype of the sample population is summarised in Table 1.

All patients were evaluated prior to any treatment. All enrolled patients underwent the typical diagnostic protocol used for infertility in newly referred subjects at the Andrology Outpatient Clinic. They underwent a complete andrological and physical examination and blood pressure (mean of three measurements taken 5 min apart in the sitting position using a standard sphygmomanometer), height, weight and waist circumference were measured. In addition, routine scrotal and transrectal ultrasounds were performed because our Regional Health Care System does not allow any genetic analysis on infertile patients unless a suspected

obstruction has been evaluated. All data were collected as part of the routine clinical procedure; therefore, based on Italian law, approval from the local Ethical Committee was not required. In addition, at the time of the initial visit, all patients provided written, informed consent to have their clinical records included in a dedicated database to be used, anonymously, for clinical research purposes.

MetS assessment

MetS was defined, based on the National Cholesterol Education Program Third Adult Treatment Panel (NCEP-ATPIII, 2001) (see Appendix 1), as the presence of three or more of the following five factors: central obesity (waist circumference >102 cm), elevated triglycerides (≥1.7 mmol/L or treated for elevated triglycerides), elevated blood pressure (systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg or treated for hypertension), elevated fasting glucose (≥6.1 mmol/L or treated for diabetes) and reduced high-density lipoprotein (HDL) cholesterol (<1.03 mmol/L or treated for dyslipidaemia).

Biochemical parameters

Blood samples were drawn in the morning after an overnight fast to determine blood glucose (using the glucose oxidase method; Aeroset Abbott, Rome, Italy), HDL cholesterol and triglycerides (using the

automated enzymatic colourimetric method; Aeroset Abbott, Rome, Italy), total testosterone (TT, using the electrochemiluminescent method; Modular Roche, Milan, Italy), insulin levels and sex hormone binding globulin (SHBG) using an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). Free testosterone was calculated based on Vermeulen's formula (available at http: //www.issam.ch/freetesto.htm) (Vermeulen et al., 1999).

Semen analyses and determination of seminal plasma interleukin 8 (sIL-8) levels

On the same day as the ultrasound, all patients underwent semen analysis, which was performed based on World Health Organization criteria (WHO, 2010). In addition, routine urine and seminal cultures were assessed in all men. Furthermore, sIL-8, a reliable surrogate marker of prostatitis (Penna et al., 2007) was also quantified. Seminal plasma aliquots were frozen and stored for later quantification of sIL-8 levels using conventional two-site ELISA (human IL-8 ELISA set; BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions (Penna et al., 2007). Each seminal plasma sample was diluted from 1:5 to 1:625. Assay sensitivity for sIL-8 was <1 pg/ml.

Screening of prostate-related symptoms and LUTS

Patients were asked to complete the Italian translation of the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) (Litwin et al., 1999) (Appendix 5), which is a brief self-reported questionnaire for screening prostatitis symptoms and that scores for pain, voiding symptoms and quality of life (QoL). The NIH-CPSI total score was calculated as the sum of the scores of these domains. LUTS were evaluated using the Italian translation of the International Prostate Symptom Score (IPSS) (Appendix 6), which is a brief self-administered questionnaire for screening symptoms related to BPH and that comprises seven questions on symptoms and one question on QoL (Barry et al., 1992).

Transrectal colour-Doppler ultrasonography (CDU)

We previously reported an association between MetS and scrotal parameters in a larger cohort of subjects who attended our unit for infertility (Lotti et al., 2013a). The characteristics of that cohort were not different from the cohort in this study; thus, in this study, we focused on the possible associations between MetS and transrectal ultrasound features.

All patients underwent scrotal and transrectal CDU; the latter before and after ejaculation. To prevent bias on the part of the examiner, scrotal and transrectal CDU was performed intermittently by two experienced physicians who were unaware of the clinical data and who used the same

ultrasonographic console (Hitachi H21, Hitachi Medical System, Tokyo, Japan). Prostate and seminal vesicle CDU features were studied by scanning the organs at 5 mm intervals at various longitudinal, transverse and oblique scans based on previous studies (Behre et al., 1995; Vicari, 1999; Lotti et al., 2011a,b, 2012) using a transrectal biplanar probe (linear transducer U533L 7.5 MHz; convex transducer U533C 6.5 MHz) and an "end fire" probe (V53W 6.5 MHz, field of view 50°–200°). Based on: 1) antero-posterior (APD) and 2) transverse (TD) diameters at the maximal dimensions and 3) the superior-inferior or longitudinal diameter (LD) at the maximal length from the base to the apex of the midline sagittal plane, prostate volume was calculated using the formula of the ellipsoid (APD x TD x LD x $\pi/6$) based on previous publications (Lotti et al., 2011b; Collins et al., 1995; St Sauver et al., 2006). During this exam, similar methods were also used to determine the transitional zone volume (TZV) based on a previous publication (St Sauver et al., 2006). Prostate echogenicity was defined based on previous studies (Behre et al., 1995; Vicari, 1999; Lotti et al., 2011a,b). The severity of prostate texture non-homogeneity was classified using an arbitrary Likert scale and scores of 0–3 (0=homogeneous texture, 1=mild, 2=moderate and 3=severe texture non-homogeneity). Prostate vascularisation, prostate hyperaemia and arterial prostatic peak systolic velocity (APPSV) were defined based on previous studies (Lotti et al., 2011a,b; Cho et al., 2000; Lotti et al., 2012b) and

evaluated prior to ejaculation to avoid post-ejaculatory changes in vascular flow pattern as has been previously reported (Keener et al., 2000). Seminal vesicle ultrasound features and abnormalities were defined based on previous studies (Colpi et al., 19997; Vicari, 1999; Lotti et al., 2011a, 2012a; 2013b). Ejaculatory duct CDU characteristics were evaluated after ejaculation to better emphasise indirect CDU signs of subobstruction (Colpi et al., 1997; Lotti et al, 2011a, 2012a).

Scrotal CDU was performed systematically using a 7.5 MHz high-frequency linear probe (L54M 6-13 MHz) at various longitudinal, transverse and oblique scans with patients lying in a supine position (Behre et al., 1995; Vicari, 1999; Lotti et al., 2011a). Testicular and epididymal CDU features were examined based on previous studies (Behre et al., 1995; Vicari, 1999; Lotti et al., 2011a).

Identification of case patients and controls

MetS was defined as described above. Subjects with ≥3 MetS components (*n*=22) were compared with controls selected from the same cohort at a 1:2 ratio (*n*=44). For each case, the first two patients following those with MetS within the same series who were the same age (±4 years old) and who showed a similar TT level (±4 nmol/L), smoking habit (current/non-smoker) and moderate-severe alcohol consumption (current/no consumption of ≥4 drinks per day based on a previous

publication; Boddi et al., 2010). For statistical analyses that compared cases with age-, TT-, smoking habit-, moderate-severe alcohol consumption-matched controls, associations with $P<0.05$ were considered significant.

Data analyses

Data were expressed as the mean±s.d. when normally distributed, the median (quartiles) for parameters with non-normal distributions, and as percentages when categorical. Correlations were assessed using Spearman's or Pearson's methods as appropriate. Differences between more than two groups were assessed using one-way ANOVAs. Unpaired two-sided Student's *t* test was used to compare means of normally distributed parameters. Relative risks and 95% confidence intervals were calculated for correlations of categorical parameters, and chi-squared tests were used for comparisons. Stepwise multiple linear, logistic binary or ordinal regressions were applied for multivariate analyses as appropriate. All statistical analyses were performed in SPSS (Statistical Package for the Social Sciences, Chicago, USA) for Windows 20.0.

Results

Among the 171 patients studied (age: 36.6±8.4), 44.4% (*n*=76) showed no components of MetS whereas one, two, three, four and five MetS factors were present in 47 (27.5%), 26 (15.2%), 16 (9.4%), 3 (1.75%) and 3 (1.75%) subjects, respectively. Twenty-two subjects (12.9%) fulfilled the criteria of NCEP-ATPIII MetS. Subjects with MetS were older (43.8±10.6 *vs.* 35.5±7.5 years for MetS and no-MetS subjects, respectively; *P*<0.0001). No difference in the percentage of subjects who smoked currently or consumed moderate-severe amounts of alcohol was found when MetS and no-MetS subjects were compared (21.1% *vs.* 28.6%, *P*=0.496; 16.7% *vs.* 24.8%, *P* = 0.233; MetS *vs.* no-MetS subjects, respectively). In addition, no difference in the prevalence of leukocytospermia or current positive urine and/or seminal cultures was observed when men with and without MetS were compared (5.3 *vs.* 8.8%, *P* 0.508; 5.6 *vs.* 8.1%, *P*=0.575; MetS *vs.* no-MetS subjects, respectively).

Correlations of MetS with hormonal, clinical and semen parameters

Age-adjusted logistic ordinal models showed that insulin levels increased as a function of MetS components (Wald=29.5 [0.09–0.18], *P*<0.0001; Figure 1A). Figure 1B–1D also showed the age-adjusted relationships between insulin and TT, SHBG and calculated free testosterone: all of these parameters decreased as a function of increasing

insulin levels (adjusted r=-0.359, P<0.0001; adjusted r=-0.200, P<0.001; adjusted r=-0.320, P<0.0001, respectively). In view of these associations, all the following analyses were adjusted for age, insulin and TT levels.

Digito-rectal examinations revealed that an enlarged prostate was positively associated with the number of MetS components (HR=1.47 [1.03–2.14] for each increment in the number of MetS components, P<0.05). Of the semen parameters, only normal semen morphology was negatively associated with an increasing number of MetS components (Wald=5.59 [-0.15–-0.01], P<0.02).

No association between MetS components and other physical or seminal parameters including leukocytospermia was observed (not shown). Finally, no association between MetS components and current positive urine and/or seminal cultures was detected (not shown).

Correlations of MetS with prostate-related symptoms and signs (sIL-8)

No association was found between MetS and prostate-related symptoms as captured by both NIH-CPSI and IPSS (not shown). A stepwise, positive correlation between the number of MetS components and sIL-8 levels was observed (Wald=4.32 [0.04–1.48], P<0.05; Figure 2A). In particular, among the MetS components, only waist circumference was positively associated with sIL-8 (Figure 2B).

Correlations of MetS with transrectal ultrasound parameters

A progressively higher prostate volume was detected by ultrasound as a function of an increasing number of MetS components (Wald=17.6 [0.05–0.13], $P<0.0001$; Figure 2C). In a logistic iterative analysis, of the MetS factors, waist size and reduced HDL cholesterol were significantly associated with prostate volume (Figure 2D). Interestingly, TZV was positively correlated with total prostate volume even after adjusting for the aforementioned confounders (adjusted $r=0.757$, $P<0.0001$). Thus, TZV also increased as a function of an increasing number of MetS components (Wald=12.5 [0.07–0.24], $P<0.0001$). In addition, TZV of MetS subjects was significantly higher than other subjects (10.7±10.8 *vs.* 3.9±2.6 ml; $P<0.0001$; subjects with MetS *vs.* those without MetS, respectively). Finally, similarly to total prostate volume results, TZV was significantly associated with reduced HDL cholesterol levels (HR=1.15[1.01-1.31], $P<0.05$). Conversely, only a trend towards statistical significance was observed for increased waist (HR=1.11 [0.99–1.24], $P=0.08$).

A positive association between an increasing number of MetS components was also observed for some ultrasonographic prostate features, such as arterial peak systolic velocity (APPSV, Wald=9.57 [0.05-0.24], $P=0.002$; Figure 3A) and diameter of the major calcification (Wald=3.11 [0.01–0.13], $P<0.05$; Figure 3C). Using a binary logistic model, moderate-severe prostate texture non-homogeneity was also associated with an

increase in the number of MetS components (HR=1.87 [1.05–3.33] for each increment in the number of MetS components, $P<0.05$; Figure 3C). Of the MetS components, only increased waist circumference was significantly associated with APPSV (Figure 3B) and with moderate-severe texture non-homogeneity (Figure 3D). After adjusting for confounders, no associations between MetS related-prostate CDU abnormalities and standard semen parameters were observed (Table 2). Finally, no associations between the number of MetS components and seminal vesicle features or the mean diameter of the deferential ampulla were observed (data not shown).

Case-control analyses

Correlations between MetS and seminal or ultrasound parameters showing statistical significance were further assessed by comparing subjects with MetS with matched controls (matched for age, TT, smoking habit and moderate-severe alcohol consumption; Table 3) at a 1:2 ratio. Even in the case-controlled analyses, subjects with three or more MetS components showed a lower percentage of normal sperm morphology, higher sIL8 levels and more frequent prostate abnormalities, such as greater volume, higher arterial peak systolic velocity, greater calcification size and prevalence of moderate-severe non-homogeneity (Table 3). No difference in the prevalence of leukocytospermia or current positive urine and/or seminal cultures was observed when MetS subjects were compared with

controls (Table 3).

Correlations of insulin levels with clinical, seminal and transrectal ultrasound parameters

Univariate analyses revealed positive associations between insulin levels and increased prostate volume detected by either digito-rectal examination (RR=1.08 [1.03–1.14], P=0.002 for each insulin mU/L increment) or ultrasound (r= 0.294, P<0.0001), moderate-severe texture non-homogeneity (RR=1.08 [1.00–1.17], P=0.05 for each insulin mU/L increment), hyperaemia (RR=1.05 [1.00–1.11], P<0.05 for each insulin mU/L increment) and arterial peak systolic velocity (r=0.286, P<0.0001) and a negative correlation with the prostatic venous plexus diameter (r=-0.180, P<0.02). When a multivariate regression model was applied that included age and TT, the significant associations between insulin levels and prostate volume detected both by digito-rectal examination (HR=1.07 [1.01–1.13], P<0.05 for each insulin mU/L increment) and ultrasound (adjusted r=0.327, P<0.0001; Figure 4A) and arterial peak systolic velocity (adjusted r=0.229, P=0.002; Figure 4B) were confirmed. However, when the number of MetS components was introduced into the same model, only prostate volume detected by ultrasound was significantly associated with insulin levels (adjusted r=0.171, P<0.05).

Even after adjusting for the aforementioned confounders, no

associations between insulin levels and semen parameters, sIL-8 levels, seminal vesicle features or diameters of the deferential ampulla were observed (data not shown).

Discussion

This study demonstrates that in a cohort of relatively young male subjects examined for infertility, a stepwise, component-dependent association was observed between an increase in MetS severity and prostate enlargement and/or inflammatory signs (including sIL-8 levels and CDU abnormalities) but not with current infection of the male genital tract. No association between MetS-related prostate CDU abnormalities and semen parameters was detected. However, in this cohort, MetS was associated with poor sperm morphology. Reduced HDL levels and increased abdominal adiposity were the main correlates of prostate enlargement in this young, asymptomatic cohort.

The association between MetS and prostate enlargement is consistent with several previous reports (Gacci et al., 2013; Hammarsten et al., 1998; Hammarsten and Högstedt 1999, 2001; Ozden at al., 2007; Sohn et al., 2007; Park and Park 2007; Koo et al., 2008; Byun et al., 2012). Thus far, only a few studies have examined relatively young adults, and they offer conflicting results (Jeong et al., 2011; Yim et al., 2011). Here, we report a novel association in a relatively young population (mean age 36.6±8.4 years) of males of infertile couples. Increased central obesity and reduced HDL cholesterol were the parameters that most closely correlated with prostate enlargement. A potential relationship between BPH/prostate enlargement and obesity or increased waist circumference has been widely

reported in several (Mongiu and McVary, 2009; Parsons, 2013), but not all previous studies (Gacci et al., 2013). Moreover, low HDL cholesterol has been previously reported as a risk factor for the development of BPH (Gacci et al., 2013; Hammarsten et al., 1998; Hammarsten and Högstedt 1999, 2001; Ozden at al., 2007). Our data suggest that MetS, and particularly high waist circumference and reduced HDL cholesterol, may play an important role in prostate growth onset at a young age.

We also noted a significant, stepwise correlation between the number of MetS components and seminal IL-8 (sIL-8), which has been proposed as a surrogate marker of prostate inflammation (Penna et al., 2007; 2009; Fibbi et al., 2009; Lotti and Maggi, 2013). IL-8 is a proinflammatory chemokine that is secreted by several cell types and that contributes to inflammation by acting in concert with IL-1β and IL-6 (Steiner et al., 2002; Baggiolini et al., 1995; Feldmann et al., 2001). Higher IL-8 levels have been reported in the expressed prostatic secretions of subjects with BPH, bacterial prostatitis and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) (Hochreiter et al., 2000; Orhan et al., 2001; Liu et al., 2009). Because IL-8 in seminal plasma is considerably higher when compared with serum levels (Shimoya et al., 1993; Koumantakis et al., 1998), local (within the male genital tract) production has been suggested (Koumantakis et al., 1998; Hochreiter et al., 2000; Lotti and Maggi, 2013). Of different cytokines and chemokines, sIL-8 appears to be the most reliable and

predictive surrogate marker of prostatitis (Penna et al., 2007; Fibbi et al., 2009), and it is associated with CDU features suggestive of prostate inflammation in patients with male accessory gland infection (MAGI) (Lotti et al., 2011a). IL-8 is actively involved in BPH-associated chronic inflammation and mediates epithelial and stromal cell proliferation (Fibbi et al., 2009). In BPH tissues, epithelial and stromal cells secrete IL- 8 actively (Fibbi et al., Konig et al., 2004) in response to varying stimuli including the proinflammatory cytokines IFNγ and IL-17 that are produced by prostate-infiltrating Th1 and Th17 cells, respectively (Penna et al., 2007; Steiner et al., 2003; Kramer et al., 2007; Adorini et al., 2010). In particular, human stromal prostatic cells actively contribute to the organ-specific inflammatory process by acting as targets of bacterial or viral toll-like receptors agonists and as antigen-presenting cells capable of activating antigen-specific CD4+ T cells (Penna et al., 2009b). In BPH cells, toll-like receptor activation leads to the production of proinflammatory cytokines (IL-6) and chemokines (IL-8 and CXCL10) capable of recruiting CXCR1 and CXCR2-positive leukocytes and CD15+ neutrophils (Penna et al., 2007). Finally, IL-8 stimulates overgrowth of prostate stromal and epithelial cells by directly promoting the proliferation of senescent epithelial cells (Castro et al., 2004), stromal trans-differentiation of myofibroblasts (Schauer et al., 2008) and by increasing secretion of fibroblast growth factor 2 (Giri et al., 2001). Hence, IL-8 appears to be the

link between T-cell-mediated inflammatory responses and cell proliferation in the pathogenesis of BPH (Fibbi et al., 2009; Adorini et al., 2010).

A higher prevalence of MetS components was also associated with other CDU features of prostate inflammation including texture non-homogeneity, major calcification size and elevated APPSV. Increased waist size is the common determinant of all of these CDU abnormalities. Prostate non-homogeneity is typically considered a CDU abnormality related to inflammation (Behre et al., 1995) and has been previously associated with elevated sIL-8 levels in infertile subjects with MAGI (Lotti et al., 2011a). Moreover, prostatic hyperechogenicity, which is associated with areas of calcification, has been previously proposed to be a CDU feature suggestive of MAGI (Vicari, 1999). In particular, prostatic high-density echoes are considered the sonographic correlates of prostatic calculi and corpora amylacea, which as confirmed by histology performed on ultrasound-guided biopsies of the prostate (Doble and Carter, 1989). A recent report indicated that prostatic calculi and corpora amylacea comprised acute inflammatory proteins including lactoferrin, calprotectin, mieloperoxidase and α-defensins, all of which are in neutrophil granules (Sfanos et al., 2009). Prostatic calcifications are common in patients with CP and have been associated with the maintenance or enhancement of prostate inflammation, bacterial colonisation and duration of symptoms (Meares, 1974; Shoskes et al., 2007). A positive correlation between sIL-8 levels and

calcification size has also been previously reported by our group (Lotti et al., 2011a). Finally, we noted that APPSV correlated with MetS severity. Arterial PSV reflects tissue inflammation at various sites including the thyroid (Lagalla et al., 1998; Corona et al., 2008e), exocrine glands (Giovagnorio et al., 2000; Carotti et al., 2001) and synovial membrane/joints (Versamidis et al., 2005; Carotti et al., 2012). Thus far, APPSV has been studied for varying purposes including evaluation of BPH (Berger et al., 2006), prostate cancer (Turgut et al., 2007), varicocele-related prostate CDU changes (Lotti et al., 2009) and premature ejaculation (Lotti et al., 2012b). More recently, elevated APPSV has been proposed as a CDU parameter that correlates with prostate inflammation (Lotti et al., 2011a,b; 2012b), and in the prostate, APPSV is closely related to sIL-8 (Lotti et al., 2011a).

No correlation was found between the number of MetS components and current positive urine and/or seminal cultures suggesting that MetS is not associated with current infection of the male genital tract but is rather associated with chronic inflammation.

The relationship between central obesity and dyslipidaemia with prostate overgrowth and inflammation, even in young subjects, is the main finding of this study. We previously reported a clear-cut association between MetS severity and prostate size (Gacci et al., 2013) and inflammation19,85 in cohorts of aged subjects. In the study of Gacci et

al.(2013), reduced HDL cholesterol and increased triglyceride levels were also noted to be the main determinants of MetS-related prostate alterations. However, previous studies were performed in old individuals undergoing surgery for BPH (Vignozzi et al., 2012a, 2013). By culturing BPH stromal cells obtained from those patients, we demonstrated that in addition to TNF-α and LPS, oxidised LDL (oxLDL) was capable of increasing IL-8, IL-6 and bFGF secretion (Vignozzi et al., 2013). In addition, TNF-α sensitised BPH cells to oxLDL by inducing its receptor (LOX-1) (Vignozzi et al., 2013). In a rabbit model of MetS we recently demonstrated that a 3-month high-cholesterol diet (HFD) induced severe prostatic inflammation characterised by increased corpora amylacea, fibrosis and hypoxia (Vignozzi et al., 2012b). In addition, the mRNA expression of several proinflammatory cytokines including IL-8 and T lymphocyte, macrophage, neutrophil and fibrosis/myofibroblast activation markers were significantly increased in the prostate of HFD animals (Vignozzi et al., 2012b). Together, all of these data suggest that BPH may be viewed as a complex disorder that also involves a metabolic component that may begin early in the life of the male, and although asymptomatic, it is likely detectable even in the early stages of the disease as suggested by this study. The mechanisms underpinning the relationship between MetS and prostate inflammation are likely to be similar in young and old men but chronic exposure to elevated inflammation may contribute to BPH in the long term.

Because hyperinsulinaemia and insulin resistance represent the cornerstone of all definitions of MetS (Reaven, 2004; Eckel et al., 2010), all of the data reported here were adjusted for insulin levels. When the specific contribution of hyperinsulinaemia was considered, after adjusting for MetS components and total testosterone levels and age, we observed a specific effect of increased insulin levels only on prostate volume and not on prostate inflammation. This finding is in apparent contrast with results published recently by our group that showed that insulin increased IL-8 release from myofibroblastic hBPH cells (Vignozzi et al., 2013). However, in that study, the effect of insulin was negligible when compared with oxLDL (six-fold lower) (Vignozzi et al., 2013). The growth-promoting activity of insulin on the prostate gland is well-documented in several experimental and epidemiological studies (Vikram et al., 2010).

In this study, no association between an increase in the number of MetS components and prostate-related symptoms was observed, using either NIH-CPSI or IPSS scores. The lack of correlation between MetS and LUTS is consistent with some studies but contrasts with most previous studies (Moul and McVary, 2010; Gorbachinsky et al., 2010; De Nunzio et al., 2012) . However, all previous studies were performed in aged cohorts whereas our data were obtained from young subjects with relatively small prostates as assessed by ultrasound.

Finally, even after adjusting for confounders including the

hypogonadal status, we observed an association between the number of MetS components and poor sperm morphology. These results confirmed previous findings obtained using a larger cohort of males of infertile couples where we assessed the possible correlations of MetS with scrotal parameters (Lotti et al., 2013). The possible impact of MetS on sperm morphology was discussed in detail in that study. Here, we extend our investigation to possible associations between MetS-related prostate CDU features and semen parameters and note no correlation. This finding suggests that the effect of MetS on sperm quality is independent of MetS-related prostate abnormalities. Although a possible association between CP or MAGI and sperm quality has been proposed and some prostate CDU features have been proposed as suggestive of MAGI (Vicari, 1999; Weidner et al., 1999; Krause, 2008; Rusz et al., 2012) a specific association between prostate CDU features and sperm parameters alterations has not been demonstrated. Here, we here report that the number of MetS components, but not related prostate CDU abnormalities, are associated with poor sperm morphology.

This study has several limitations. First, the results were derived from patients consulting an Italian Andrology Clinic for infertility, and our population could have different characteristics from the general male population or those males consulting general practitioners for reasons other than infertility. Second, a true control group comprising age-matched,

apparently healthy, fertile men is lacking and therefore true normative data on sonographic parameters cannot be inferred. Third, the data cannot distinguish between men who are clinically infertile and those who are not, and whether this feature confounds the results is unknown. Fourth, this study relies on a relatively small and young sample. Fifth, markers of prostate inflammation other than sIL-8 are lacking. Furthermore, parallel mechanistic studies to determine the consequences of IL-8 secretion on prostate growth and differentiation are warranted. Finally, the link between MetS or insulin levels and prostate enlargement in this study are correlative only. Statistically significant associations in a cross-sectional study do not infer causality.

However, this study also has several strengths. First, this study systematically evaluates several hormonal, seminal, laboratory and ultrasound parameters in a consecutive series of males of infertile couples. Second, during the same sonographic session, this study examined both scrotal and transrectal ultrasound features before and after ejaculation. Third, this study was performed on a sample of relatively young, infertile men and investigated a population that is poorly studied in the scientific literature. Fourth, this study considers several possible confounders, such as age, testosterone and insulin levels, smoking habits, alcohol consumption, leukocytospermia and positive semen and urine cultures of the patients. Fifth, a statistical analysis comparing patients with MetS with

age-, total testosterone-, smoking habits-, alcohol consumption-matched controls was performed. Finally, the study examined several end points simultaneously within the same population, which enabled a valid comparison of the co-prevalence of the examined parameters and supported their possible association with the number of MetS components.

Study 2 conclusions

This study demonstrates that in a cohort of men with infertility, a component-dependent, stepwise association was observed between an increase in the number of MetS components and the total and transitional zone prostate enlargement and prostate related-inflammatory signs but not symptoms or current infection of the male genital tract, which suggests a sub-clinical inflammation of the prostate. Relative prostate overgrowth may also correlate with MetS-related hyperinsulinaemic state. In addition, MetS but not MetS's related prostate CDU abnormalities was associated with poor sperm morphology.

FINAL CONCLUSIONS

In men with couple infertility, MetS is associated with hypogonadism, poor sperm morphology, testis ultrasound inhomogeneity, erectile dysfunction, somatization and depression.

In addition, MetS is positively associated with prostate enlargement, biochemical (seminal interleukin 8) and ultrasound-derived signs of prostate inflammation but not with prostate-related symptoms, which suggests that MetS is a trigger for a subclinical, early-onset form of benign prostatic hyperplasia.

Recognizing MetS could help patients to improve not only fertility but also sexual and overall health.

STUDY 1 FIGURES AND TABLES

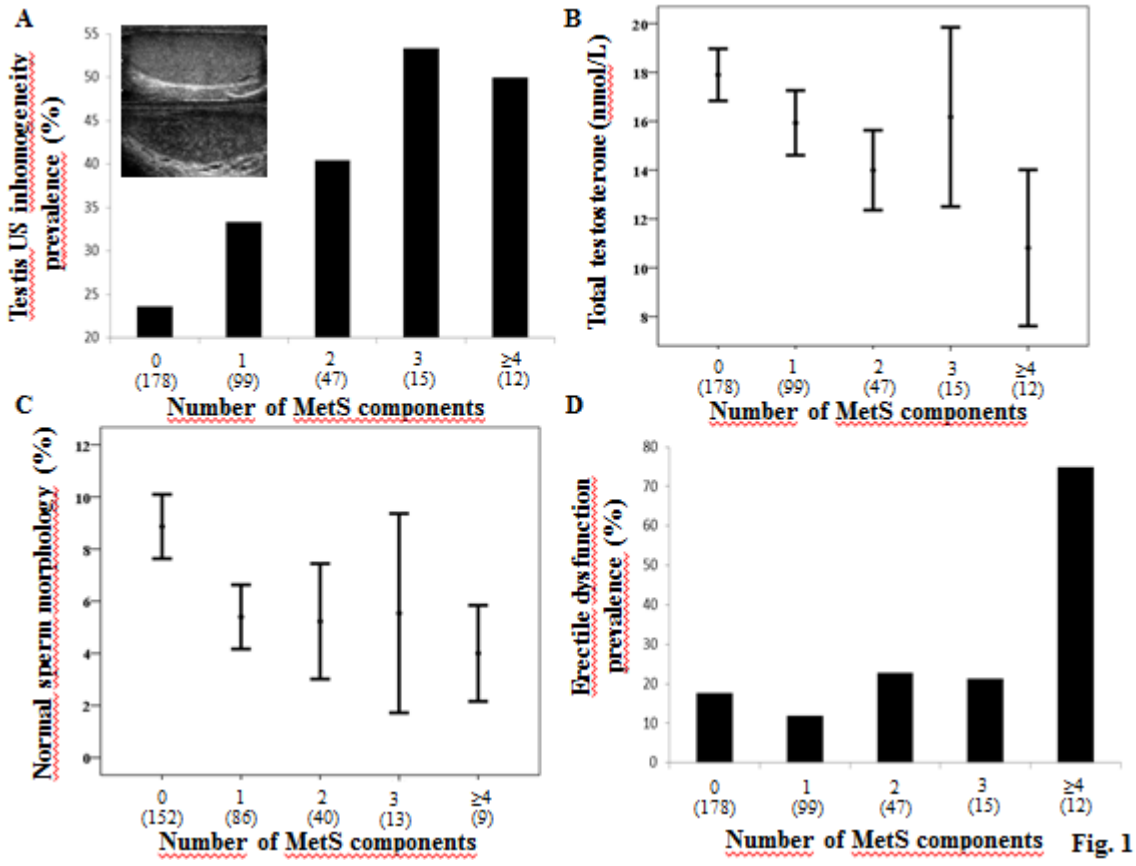

Figure 1. Association between the prevalence of testis ultrasound (US) inhomogeneity (panel A), total testosterone levels (panel B), normal sperm morphology (panel C) and prevalence of erectile dysfunction (panel D) with the number of metabolic syndrome (MetS) components (IDF&AHA/NHLBI classification). Panel A, inset: ultrasonographic images of homogeneous (upper picture) and inhomogeneous (lower picture) testis. In every panel, the number of subjects with no, one or more MetS components is indicated.

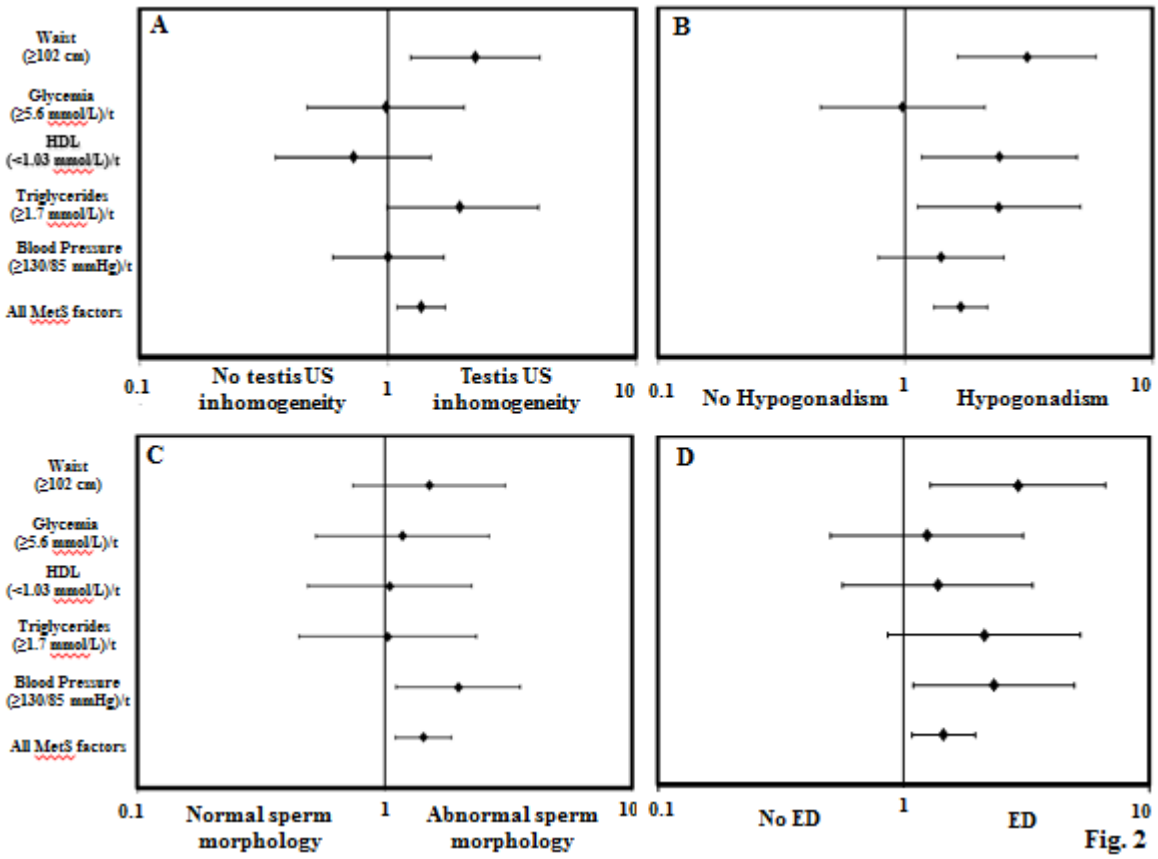

Figure 2. Hazard ratio (95% confidence interval) for testis ultrasound (US) inhomogeneity (panel A), hypogonadism (total testosterone <12 nmol/L) (panel B), abnormal sperm morphology (< 4%, WHO 2010) (panel C) and erectile dysfunction (ED; IIEF-15-EFD < 26) as detected by logistic regression analysis considering metabolic syndrome (MetS) components (IDF&AHA/NHLBI classification) as putative predictors. Panels A, C and D: data have been adjusted for age and total testosterone levels. Panel B: data have been adjusted for age. MetS components are defined by abnormal parameters or by specific therapy (t), according to IDF&AHA/NHLBI classification.

Table 1. Clinical and scrotal colour-Doppler ultrasound characteristics of the whole sample and of subjects with or without (w/o) metabolic syndrome (MetS) (*see below, page 62*).

Data are expressed as mean ± standard deviation or as median (quartiles), when appropriate, and as percentage, when categorical. HDL, high density lipoprotein; BP, blood pressure; FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, prolactin; TSH, thyroid stimulating hormone. Tp, therapy § echographic defined severe varicocele = basal venous reflux increasing after Valsalva's manoeuvre at sonography (according to Isidori & Lenzi, 2008; Lotti et al., 2009).

	All patients n = 351	W/o MetS n = 324	With MetS n = 27
Clinical and laboratory parameters			
Age (years)	36.0±8.0	35.5±7.4	43.7±10.4
Current smoker, %	27.4	28.5	18.5
Current alcohol consumption (≥2 drinks/die), %	25.4	25.6	22.2
Diabetes mellitus (%)	1.1	0	11.1
Waist (cm)	95.5±11.6	94.1±9.9	111.4±16.7
Glycaemia (mmol/L)	4.97±0.68	4.89±0.64	5.56±0.75
Triglycerides (mmol/L)	1.13 [0.79-1.58]	1.05 [0.77-1.47]	1.77 [1.11-2.62]
HDL cholesterol (mmol/L)	1.28±0.33	1.32±0.32	1.06±0.35
Systolic BP (mm Hg)	124.7±12.3	123.8±11.2	135.0±18.5
Diastolic BP (mm Hg)	80.0±7.6	79.5±7.1	85.2±10.2
Mean testis volume (Prader) (ml)	18.4±4.9	18.3±4.8	19.7±5.4
History of cryptorchidism (%)	9.9	9.5	14.8
FSH (IU/L)	4.9 [3.1-7.7]	4.8 [3.0-7.7]	5.6 [3.3-9.2]
LH (IU/L)	3.7 [2.6-5.1]	3.7 [2.6-5.2]	3.9 [2.8-4.5]
PRL (pmol/L)	294 [228-435]	294 [226-435]	282 [234-489]
TSH (mIU/L)	1.53 [1.11-2.11]	1.51 [1.08-2.09]	1.84 [1.13-2.26]
Total testosterone (nmol/L)	16.4±6.3	16.7±6.2	13.8±6.5
Sex hormone binding globulin (nmol/L)	28.8 [22.9-37.7]	28.9 [23.3-38.3]	28.0 [16.8-34.7]
Calculated free testosterone (nmol/L)	0.344±0.120	0.349±0.117	0.299±0.139
MetS components			
central obesity (waist circumference > 102 cm)	18.5	13.2	77.7
elevated fasting glucose (≥ 5.6 mmol/L) or tp	18.5	13.2	55.5
elevated triglycerides (≥ 1.7 mmol/L) or tp	20.6	12.9	70.3
reduced HDL cholesterol (< 1.03 mmol/L) or tp	24.2	16.3	74.0
elevated BP (BP ≥ 130/85 mm Hg) or tp	40.2	35.6	88.8
Specific medications			
Hypoglycemic drugs	2.7	0	11.1
Lipid-lowering drugs	5.4	0	22.2
Antihypertensive drugs	12.6	0	51.8
Seminal parameters			
Azoospermic subjects, %	14.0	13.9	14.8
Normozoospermic subjects, %	33.0	33.4	25.9
Sexual abstinence (days)	4.2±1.9	4.2±2.0	4.0±1.3
pH	7.5±0.3	7.5±0.3	7.6±0.2
Semen volume (ml)	3.0 [2.0-4.2]	3.0 [2.0-4.2]	2.8 [1.3-3.8]
Sperm concentration, $*10^6$/ml	13.0 [1.65-46.0]	13.0 [1.60-46.0]	16.1 [3.9-49.5]
Spermatozoa per ejaculate, $*10^6$/ml	34.1 [4.78-131.78]	34.0 [4.50-136.0]	43.8 [11.5-92.4]
Sperm progressive motility, %	36.5±20.4	36.2±20.7	39.3±16.9
Sperm morphology, % normal forms	5.0 [2.0-10.0]	5.0 [2.0-10.0]	4.0 [2.0-6.3]
History of infertility			
Duration of infertility (years)	1.9±1.6	1.8±1.5	2.3±2.4
Primary infertility	80.6	81.0	77.7
Secondary infertility	19.4	19.0	22.2
Female partner age (years)	33.8±6.4	33.7±6.4	35.9±6.7
Colour-Doppler ultrasound parameters			
Testis			
Mean testis volume (ml)	14.6±4.8	14.5±4.6	15.8±5.9
Testicular inhomogeneity	31.1	29.3	51.8
Testicular hypoechogenicity	21.1	21.3	18.5
Testicular microcalcifications	8.8	8.9	7.4
Epididymis and vas deferens			
Mean size of the head (mm)	9.1±2.1	9.0±1.8	10.0±4.1
Mean size of the tail (mm)	4.4±1.3	4.3±1.3	4.7±1.3
Mean size of the vas deferens (mm)	3.9±0.9	3.9±0.9	4.1±1.1
Inhomogeneous head	30.9	29.4	48.1
Inhomogeneous tail	27.8	26.3	44.4
Hypoechoic tail	15.1	16.4	3.7
Hyperechoic tail	15.4	15.8	11.1
Coarse tail calcifications	8.2	8.9	3.7
Hyperaemia	3.9	4.3	3.7

Etiological factors involved in male infertility	% of the cohort (n = 351)	W/o MetS n = 324	With MetS n = 27	% of subjects with MetS for each etiological factor
Secondary hypogonadism, % §	20.2	17.6	51.9**	19.7
Primary hypogonadism, % §	1.1	1.2	0	0
Previous testicular trauma, %	1.8	1.6	0	0
Previous testicular torsion, %	1.5	1.6	3.7	0
Post-inflammatory forms -History of orchitis, % -History of epididymitis, %	 2.4 5.6	 2.6 6.2	 0 0	 0 0
Subobstruction of proximal urogenital tract, %	4.8	4.9	3.7	6.3
History of urogenital infection, %	28.6	28.9	22.2	7.1
Current urogenital infection (positive urine/semen culture), %	7.8	8.1	3.7	4
Asymptomatic leukocytospermia, %	8.7	8.4	11.1	10.3
Exogenous factors (previous chemo/radiotherapy, pesticides, heat, solvents, etc.), %	12.5	12	18.5	11.4
Clinical varicocele, %^	35.1	36.5	18.5*	4.1
Severe CDU varicocele, %^^	29	30.6	11.1*	3.1
Past or present cryptorchidism, %	9.9	9.5	14.8	11.8

Table 2: Etiological factors involved in male infertility in the whole sample and in subjects with or without (w/o) metabolic syndrome (MetS). % of subjects with MetS for each infertility factor. Data are expressed as percentage. CDU, colour-Doppler ultrasound. § Biochemical hypogonadism has been defined for total testosterone levels < 12 nmol/L, according to Wang et al. (2009). Secondary or primary hypogonadism has been defined for LH ≤ 9.4 or > 9.4 U/L, respectively, according to Tajar et al. (2010). ^ clinical varicocele: any degree of varicocele (grade 1-3) according to Dubin & Amelar classification (Dubin & Amelar, 1970). ^^ CDU defined severe varicocele = basal venous reflux increasing after Valsalva's manoeuvre at CDU (according to Isidori & Lenzi, 2008; Lotti et al., 2009). A statistical analysis comparing patients with and without MetS has been performed. * $p < 0.05$; ** $p < 0.0001$

	Univariate analysis	Multivariate analysis	
	p for trend	B ± SD	p
Total testosterone (nmol/L)	< 0.0001	-1.25 ± 0.33	< 0.0001
cFT (nmol/L)	0.001	-0.16 ± 0.01	<0.02
SHBG (nmol/L)	0.435	1.72 ± 0.74	0.02
LH (U/L)	0.562	- 0.15 ± 0.16	0.350
FSH (U/L)	<0.05	0.09 ± 0.36	0.793
PRL (mU/L)	0.503	4.47 ± 5.87	0.447
TSH (mU/L)	0.908	0.13 ± 0.67	0.054

Table 3: Univariate and multivariate (age-adjusted) associations between metabolic syndrome components and hormonal parameters. cFT, calculated free testosterone; SHBG, Sex Hormone Binding Globulin; LH, luteinizing hormone; FSH, follicle stimulating hormone; PRL, prolactin; TSH, thyroid stimulating hormone.

	Case patients (with 3 MetS factors, n=15)	Controls (matched 1:3, n=45)	p	Case patients (with≥4 MetS factors, n=12)	Controls (matched 1:3, n=36)	p	Patients with ≥4 vs 3 MetS factors, p
Age	39.9±8.5	39.3±7.2	0.815	48.5±10.9	41.5±10.1	0.055	**0.035**
Total testosterone (nmol/L)	16.1±6.6	16.8±5.4	0.735	10.8±5.0	13.5±4.8	0.137	**0.027**
BMI (kg/m^2)	29.5±4.7	27.6±4.6	0.181	34.5±6.0	30.8±5.0	0.071	**0.030**
Current smoker, %	21.4	47.4	0.118	9.1	28.6	0.194	0.404
Current alcohol consumption, %	28.6	18.9	0.454	11.1	16.0	0.723	0.322
Past or present cryptorchidism	26.7	25	0.898	0	9.4	0.272	0.053
Leukocytospermia, %	20	7.5	0.185	0	6.9	0.418	0.151
Current positive urine and/or semen cultures, %	0	6.8	0.299	12.5	3.2	0.289	0.161
Sperm concentration,*10^6/ml	10 [3.5-27]	42 [5-120]	0.126	29 [4-60]	31 [3.4-62]	0.685	0.585
Spermatozoa per ejaculate,*10^6/ml	43 [10.9-68]	74 [21-245]	0.114	56 [12-111]	103 [11-244]	0.183	0.659
Sperm progressive motility, %	42.1±19.2	42.3±18.6	0.975	35.0±13.9	36.9±17.8	0.741	0.305
Sperm morphology, % normal forms	3 [1.5-6.5]	12 [6-18]	**0.001**	4 [2-6]	9 [3.8-16]	**0.036**	0.973
Testis inhomogeneity at ultrasound, %	53.3	20	**0.015**	50.0	10.7	**0.006**	0.863
ED prevalence (IIEF-15-EFD<26), %	21.3	26.3	0.746	75.0	29.4	**0.016**	**0.006**

Table 4. Comparisons between subjects with metabolic syndrome (MetS) and 1:3 ratio matched controls (matched for age, body mass index, total testosterone, smoking habit, alcohol consumption, past or present cryptorchidism, leukocytospermia, current positive urine and/or semen cultures). Comparison between subjects with ≥ 4 MetS components and 3 MetS components. Data were expressed as mean ± standard deviation when normally distributed, median (quartiles) when not normally distributed, and as percentages when categorical. BMI, body mass index. ED, erectile dysfunction. IIEF-15-EFD, International Index of Sexual Function-15 erectile function domain.

STUDY 2 FIGURES AND TABLES

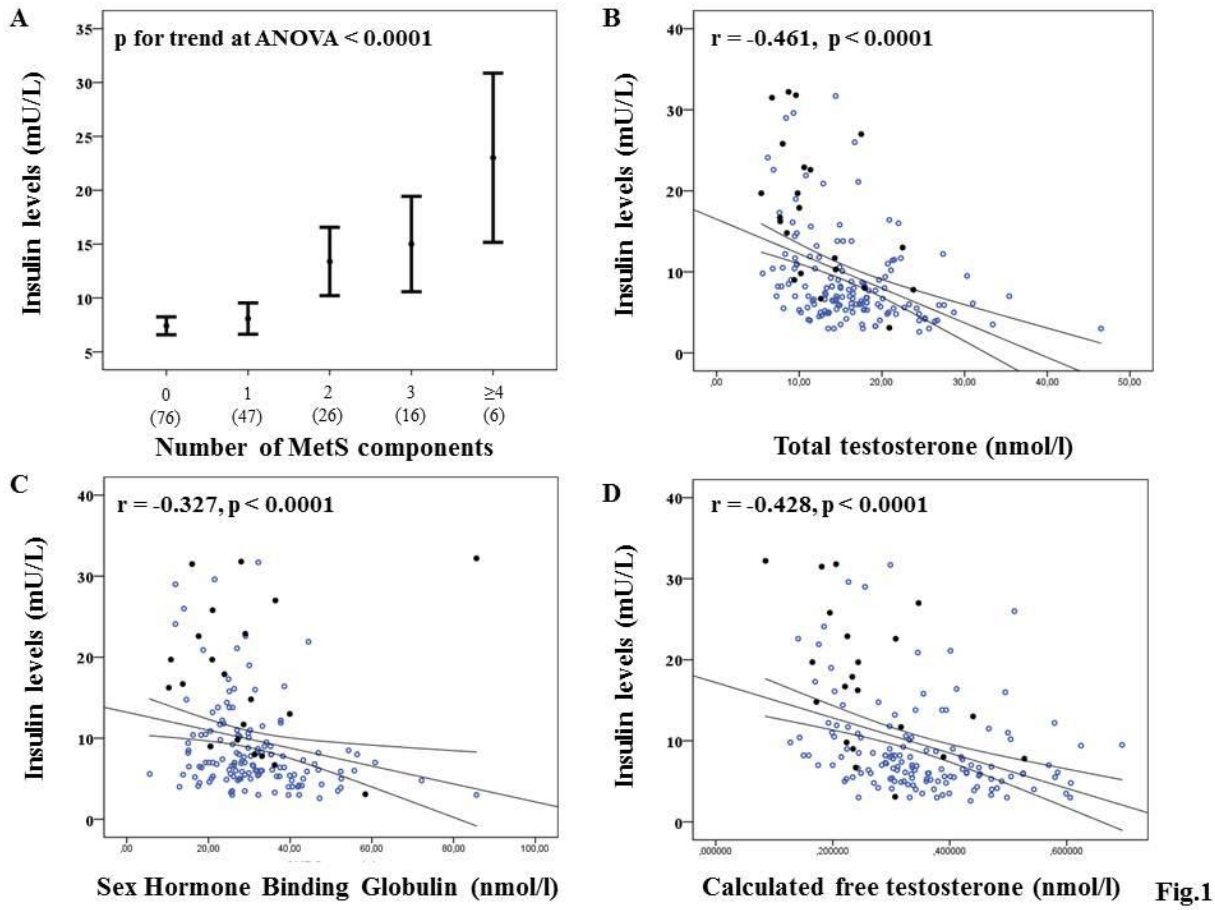

Fig.1

Figure 1. Association between insulin levels and the number of metabolic syndrome (MetS) components (NCEP-ATPIII classification) (A), total testosterone (B), sex hormone binding globulin (SHBG) (C) and calculated free testosterone levels (D). In (A), subjects with no, one or more MetS components are indicated. In (B–D), subjects with or without MetS are shown as filled or empty dots, respectively.

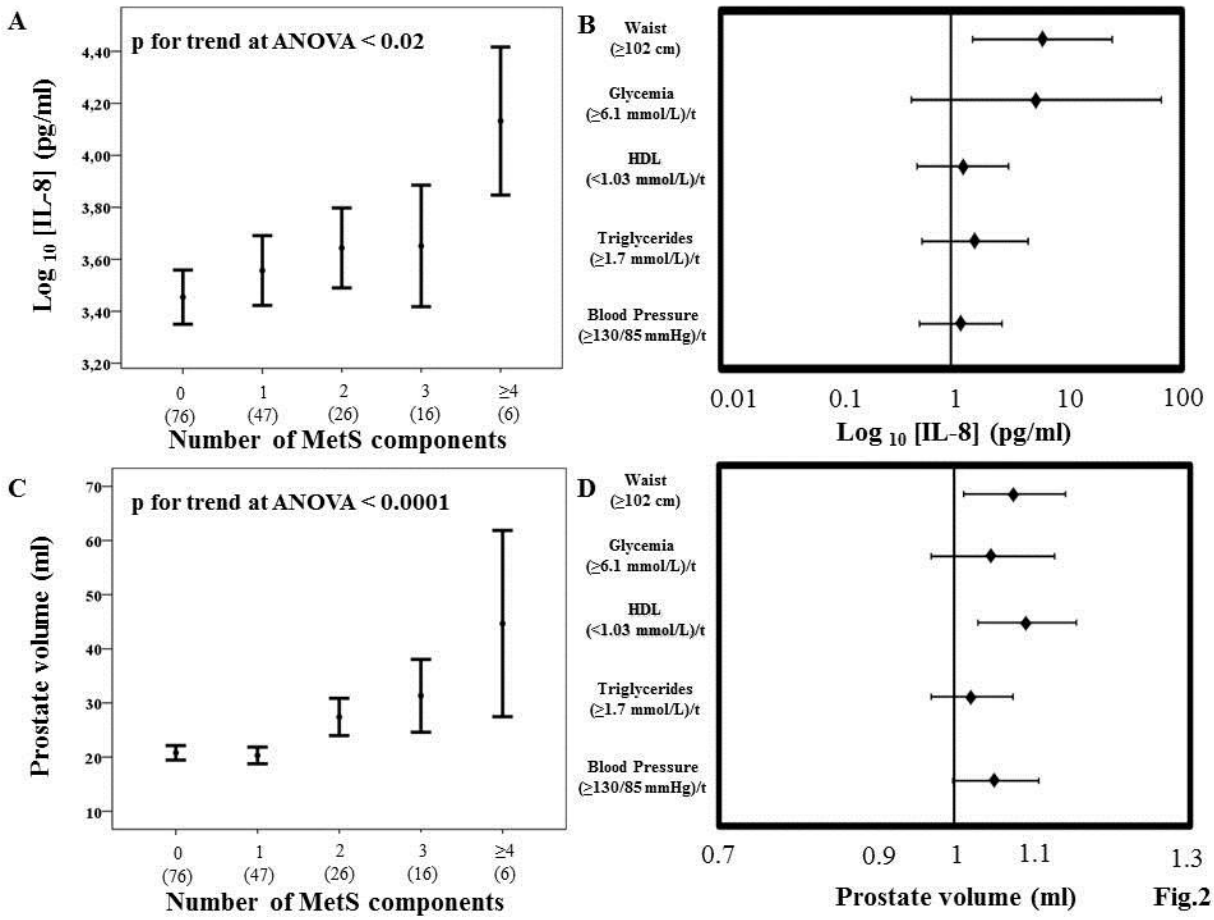

Figure 2. Association between metabolic syndrome (MetS) and seminal interleukin 8 (sIL-8) levels or prostate volume at ultrasound. Association between the number of MetS components (NCEP-ATPIII classification) and seminal interleukin 8 (sIL-8) levels (A) or prostate volume at ultrasound (C). The number of subjects with no, one or more MetS components is indicated. Hazard ratio (95% confidence interval) for sIL-8 levels (B) and prostate volume at ultrasound (D) as detected by iterative logistic regression analyses considering MetS components as putative predictors. MetS components are defined by abnormal parameters or by specific therapy (t) based on NCEP-ATPIII groupings.

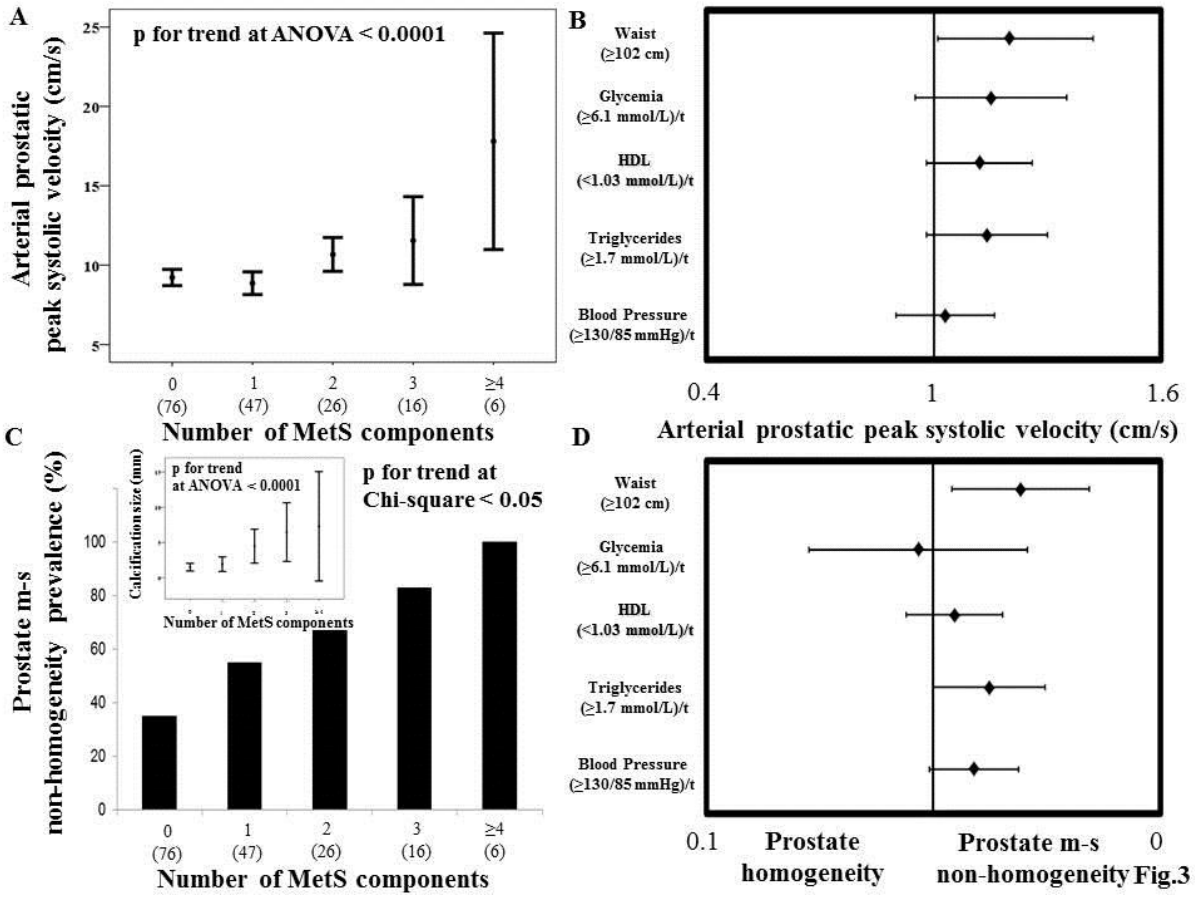

Figure 3. Association between metabolic syndrome (MetS) and arterial prostatic peak systolic velocity (APPSV), texture non-homogeneity and major calcification size of the prostate as evaluated using colour-Doppler ultrasound (CDU). Association between the number of metabolic syndrome (MetS) components (NCEP-ATPIII classification) and APPSV (A), moderate-severe non-homogeneity prevalence (C) or major calcification size (c, inset) of the prostate as evaluated using CDU. The number of subjects with no, one or more MetS components is indicated. Hazard ratio (95% confidence interval) for APPSV (B) and prostate moderate-severe non-homogeneity (D) as detected by iterative logistic regression analysis considering MetS components as putative predictors. MetS components are defined by abnormal parameters or by specific therapy (t) based on NCEP-ATPIII groupings. m-s, moderate-severe.

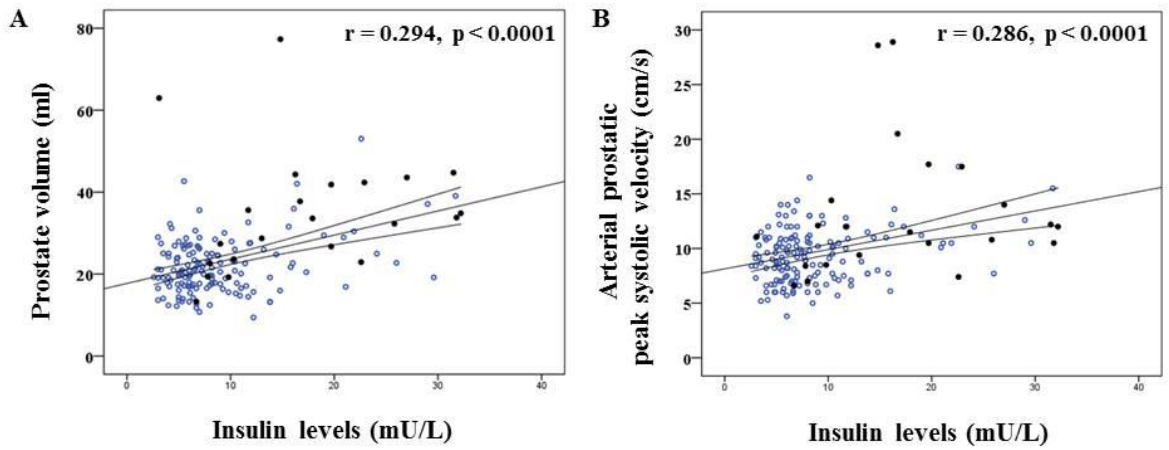

Fig.4

Figure 4. Associations between insulin levels and prostate volume (A) or arterial prostatic peak systolic velocity (B) evaluated using colour-Doppler ultrasound (CDU). Subjects with or without metabolic syndrome are shown as filled or empty dots, respectively.

Table 1. Clinical and transrectal colour-Doppler ultrasound characteristics of the sample (*see below, page 71*).

Data are expressed as the mean±s.d. or as the median (quartiles) where appropriate and as percentages when categorical. NIH-CPSI, National Institutes of Health Chronic Prostatitis Symptom Index; sIL-8, seminal interleukin 8; HDL, high-density lipoprotein; BP, blood pressure; tp, therapy. § Calcifications with size > 3 mm (Lotti et al., 2011a,b).

° Calculated using the "ellipsoid/prolate (d1 > d2 = d3) spheroid" formula (d1*d2*d3*4/3*π, where d1 = ½ maximum longitudinal diameter of the SV and both d2 and d3 = ½ anterior-posterior maximum diameter; based on Lotti et al., 2013a). ^ Calculated as [(pre-ejaculatory total volume – post-ejaculatory total volume)/pre-ejaculatory volume)] * 100 (based on Lotti et al., 2012a).

	All patients n = 171
Clinical and laboratory parameters	
Age (years)	36.6±8.4
Current smoker, %	27.6
Current moderate severe alcohol consumption (≥4 drinks/die), %	22.2
Diabetes mellitus (%)	1.4
Mean testis volume (Prader) (ml)	18.4±4.8
Enlarged prostate at digito-rectal examination	33.7
Current positive urine and/or seminal cultures, %	7.8
NIH-CPSI total score	4.6±6.1
NIH-CPSI pain domain	2.1±3.4
NIH-CPSI void domain	1.2±1.8
NIH-CPSI quality of life domain	1.4±2.1
IPSS total score	4.6±5.8
Waist (cm)	95.3±11.6
Glycaemia (mmol/L)	4.94±0.67
Triglycerides (mmol/L)	1.15 [0.80-1.58]
HDL cholesterol (mmol/L)	1.27±0.32
Systolic BP (mm Hg)	125.3±13.8
Diastolic BP (mm Hg)	80.1±8.1
Insulin levels (mU/L)	9.8±6.6
Total testosterone (nmol/L)	15.8±6.4
Sex hormone binding globulin (nmol/L)	30.7±12.7
Calculated free testosterone (nmol/L)	0.339±0.117
Log_{10} [sIL-8] (ng/ml)	3.55±0.45
Specific medications	
Hypoglycemic drugs	0.7
Lipid-lowering drugs	4.1
Antihypertensive drugs	8.1
Seminal parameters	
Azoospermic subjects, %	16.5
Sexual abstinence (days)	4.2±2.0
pH	7.5±0.3
Semen volume (ml)	3.5±1.8
Sperm concentration, *10⁶/ml	27.3±39.8
Spermatozoa per ejaculate, *10⁶/ml	82.2±125.9
Sperm progressive motility, %	34.8±20.8
Sperm morphology, % normal forms	5.3±5.2
Leukocytospermia, %	8.4
History of infertility	
Duration of infertility (years)	1.9±1.8
Primary infertility	82.9
Secondary infertility	17.1
Female partner age (years)	34.0±6.3
Colour-Doppler ultrasound parameters	
Prostate	
Prostate volume (ml)	23.5±9.2
Prostate transitional zone volume (TZV, ml)	4.8±5.1
Prostate calcifications	52.6
Prostate macro-calcifications *	28.1
Major calcification size (mm)	2.8±4.6
Non-homogeneous prostatic texture	51.6
Hypoechoic prostatic texture	19.2
Hyperechoic prostatic texture	36.6
Prostatic hyperaemia	21.1
Mean arterial peak systolic velocity (cm/sec)	9.9±3.4
Mean prostatic venous plexus (mm)	4.2±1.7
Dilated ejaculatory ducts	8.8
Seminal vesicles	
Total volume before ejaculation (ml) °	9.1 [5.9-14.5]
Total volume after ejaculation (ml) °	6.1 [3.7-9.2]
Ejection fraction (%) ^	31.5 [21.8-43.1]
Areas of endocapsulation before ejaculation	29.1
Areas of endocapsulation after ejaculation	16.2
Wall thickening and septa	5.8
Deferential ampullae mean diameter (mm)	4.9±1.1

Table 2: Univariate associations between metabolic syndrome (MetS)-related prostate colour-Doppler ultrasound (CDU) characteristics and semen parameters

Semen parameters	MetS-related prostate CDU characteristics			
	Prostate volume (ml)	Arterial prostatic peak systolic velocity (cm/s)	Prostate moderate-severe inhomogeneity	Major calcification size (mm)
pH	r = -0.112, p=0.146	r = -0.024, p=0.760	r = 0.032, p=0.760	**r = 0.155, p=0.044**
Semen volume (ml)	r = 0.109, p=0.155	r = 0.001, p=0.988	r = -0.024, p=0.822	r = -0.024, p=0.759
Sperm concentration, $*10^6$/ml	r = -0.012, p=0.879	r = 0.013, p=0.865	r = -0.035, p=0.743	r = -0.017, p=0.828
Spermatozoa per ejaculate, $*10^6$/ml	r = -0.003, p=0.973	r = -0.016, p=0.840	r = -0.079, p=0.454	r = -0.048, p=0.533
Sperm progressive motility, %	r = -0.106, p=0.216	r = -0.096, p=0.260	r = -0.016, p=0.898	r = -0.110, p=0.201
Sperm morphology, % normal forms	r = -0.052, p=0.538	r = -0.001, p=0.994	r = -0.172, p=0.149	r = -0.009, p=0.918

Table 3. Comparisons between subjects with metabolic syndrome (MetS) and 1:2 ratio-matched controls (matched for age, total testosterone, smoking habits and moderate-severe alcohol consumption). The data are expressed as the mean ± standard deviation and as percentages when categorical. sIL8, seminal interleukin 8; CDU, colour-Doppler ultrasound.

	Case patients (≥ 3 MetS factors, n=22)	Controls (matched 1:2, n=44)	p
Age	43.8±10.6	41.0±6.6	0.269
Total testosterone (nmol/L)	12.2±5.3	13.0±4.5	0.523
Current smoker, %	22.8	23.0	0.532
Current moderate-severe alcohol consumption (≥4 drinks/die), %	18.2	16.1	0.574
Semen volume (ml)	3.3±2.5	3.5±1.7	0.733
Sperm concentration,*10^6/ml	29.6±40.6	32.0±45.7	0.839
Spermatozoa per ejaculate,*10^6/ml	89.6±134.2	89.6±134.2	0.628
Sperm progressive motility, %	34.3±19.3	34.8±22.5	0.929
Sperm morphology, % normal forms	3.1±2.3	5.8±6.1	**0.025**
Leukocytospermia, %	4.6	4.6	0.674
Current positive urine and/or seminal culture, %	4.6	6.9	0.625
Log.$_{10}$ [sIL-8] (ng/ml)	3.8±0.4	3.5±0.4	**0.035**
Prostate volume (ml) at CDU	35.0±14.6	24.9±8.4	**0.006**
Prostate transitional zone volume (ml) at CDU	10.7±10.8	5.1±4.3	**0.004**
Arterial prostatic peak systolic velocity (cm/s) at CDU	13.3±2.7	9.6±2.7	**0.014**
Prostate moderate-severe inhomogeneity (%) at CDU	95.6	46.0	**0.009**
Prostate calcification size (mm) at CDU	6.7±3.5	2.5±4.1	**0.022**

Appendices

Appendix 1. Comparisons of definitions of metabolic syndrome: National Cholesterol Education Program-Third Adult Treatment Panel (NCEP-ATPIII), International Diabetes Federation (IDF), American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) and common definition by IDF and AHA/NHLBI. In blue are shared factors among different definitions (see **Corona et al., 2011a**) .

NCEP-ATPIII	IDF
3 or more of the following • Central obesity (waist circumference >102 cm) • Hypertriglyceridaemia: triglycerides ≥150 mg/dl (1.7 mmol/L) or treatment • Low HDL-cholesterol: <40 mg/dl (1.03 mmol/L) or treatment • Hypertension: blood pressure ≥130/85 mmHg or treatment • Fasting plasma glucose: ≥110 mg/dl (6.1 mmol/L) or diabetes	Central obesity (waist circumference ≥94 cm) and 2 or more of the following • Hypertriglyceridaemia: triglycerides ≥150 mg/dl (1.7 mmol/L) or treatment • Low HDL-cholesterol: <40 mg/dl (1.03 mmol/L) or treatment • Hypertension: blood pressure ≥130/85 mmHg or treatment • Fasting plasma glucose: ≥100 mg/dl (5.6 mmol/L) or diabetes

AHA/NHLBI	IDF&AHA/NHLBI
3 or more of the following • Central obesity (waist circumference >102 cm) • Hypertriglyceridaemia: triglycerides ≥150 mg/dl (1.7 mmol/L) or treatment • Low HDL-cholesterol: <40 mg/dl (1.03 mmol/L) or treatment • Hypertension: blood pressure ≥130/85 or treatment • Fasting plasma glucose: ≥100 mg/dl (5.6 mmol/L) or treatment	3 or more of the following • Central obesity (population- and country-specific definitions) • Hypertriglyceridaemia: triglycerides ≥150 mg/dl (1.7 mmol/L) or treatment • Low HDL-cholesterol: <40 mg/dl (1.03 mmol/L) or treatment • Hypertension: blood pressure ≥130/85 mmHg or treatment • Fasting plasma glucose: ≥100 mg/dl (5.6 mmol/L) or treatment

Appendix 2. International Index of Sexual Function-15

(IIEF-15) (Rosen et al., 1997)

"IIEF-15-erectile function domain": questions #1-2-3-4-5-15

(Cappelleri et al., 1997)

APPENDIX

Individual items of International Index of Erectile Function Questionnaire and response options (US version)

Question*	Response Options
Q1: How often were you able to get an erection during sexual activity? **Q2**: When you had erections with sexual stimulation, how often were your erections hard enough for penetration?	0 = No sexual activity 1 = Almost never/never 2 = A few times (much less than half the time) 3 = Sometimes (about half the time) 4 = Most times (much more than half the time) 5 = Almost always/always
Q3: When you attempted sexual intercourse, how often were you able to penetrate (enter) your partner? **Q4**: During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?	0 = Did not attempt intercourse 1 = Almost never/never 2 = A few times (much less than half the time) 3 = Sometimes (about half the time) 4 = Most times (much more than half the time) 5 = Almost always/always
Q5: During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	0 = Did not attempt intercourse 1 = Extremely difficult 2 = Very difficult 3 = Difficult 4 = Slightly difficult 5 = Not difficult
Q6: How many times have you attempted sexual intercourse?	0 = No attempts 1 = One to two attempts 2 = Three to four attempts 3 = Five to six attempts 4 = Seven to ten attempts 5 = Eleven + attempts
Q7: When you attempted sexual intercourse, how often was it satisfactory for you?	0 = Did not attempt intercourse 1 = Almost never/never 2 = A few times (much less than half the time) 3 = Sometimes (about half the time) 4 = Most times (much more than half the time) 5 = Almost always/always

Q8: How much have you enjoyed sexual intercourse?	0 = No intercourse 1 = No enjoyment 2 = Not very enjoyable 3 = Fairly enjoyable 4 = Highly enjoyable 5 = Very highly enjoyable
Q9: When you had sexual stimulation or intercourse, how often did you ejaculate? **Q10**: When you had sexual stimulation or intercourse, how often did you have the feeling of orgasm or climax?	0 = No sexual stimulation/intercourse 1 = Almost never/never 2 = A few times (much less than half the time) 3 = Sometimes (about half the time) 4 = Most times (much more than half the time) 5 = Almost always/always
Q11: How often have you felt sexual desire?	1 = Almost never/never 2 = A few times (much less than half the time) 3 = Sometimes (about half the time) 4 = Most times (much more than half the time) 5 = Almost always/always
Q12: How would you rate your level of sexual desire?	1 = Very low/none at all 2 = Low 3 = Moderate 4 = High 5 = Very high
Q13: How satisfied have you been with your overall sex life? **Q14**: How satisfied have you been with your sexual relationship with your partner?	1 = Very dissatisfied 2 = Moderately dissatisfied 3 = About equally satisfied and dissatisfied 4 = Moderately satisfied 5 = Very satisfied
Q15: How do you rate your confidence that you could get and keep an erection?	1 = Very low 2 = Low 3 = Moderate 4 = High 5 = Very high

** All questions are preceded by the phrase "Over the past 4 weeks."*

Appendix 3. Premature Ejaculation Diagnostic Tool (PEDT)

(Symonds et al., 2007)

Definition:
Ejaculation here refers to ejaculation (release of semen) after penetration (when your penis enters your partner)

	Not difficult at all	Somewhat difficult	Moderately difficult	Very difficult	Extremely difficult
1. How difficult is it for you to delay ejaculation?	❑ 0	❑ 1	❑ 2	❑ 3	❑ 4

	Almost never or never 0%	Less than half the time 25%	About half the time 50%	More than half the time 75%	Almost always or always 100%
2. Do you ejaculate before you want to?	❑ 0	❑ 1	❑ 2	❑ 3	❑ 4
3. Do you ejaculate with very little stimulation?	❑ 0	❑ 1	❑ 2	❑ 3	❑ 4

	Not at all	Slightly	Moderately	Very	Extremely
4. Do you feel frustrated because of ejaculating before you want to?	❑ 0	❑ 1	❑ 2	❑ 3	❑ 4
5. How concerned are you that your time to ejaculation leaves your partner sexually unfulfilled?	❑ 0	❑ 1	❑ 2	❑ 3	❑ 4

Appendix 4. Middlesex Hospital Questionnaire (MHQ)

(Crown & Crisp, 1966)

INSTRUCTIONS

The following questions are concerned with the way you feel or act. They are all simple. Please *tick* the answer that applies to you. Don't spend long on any one question.

... 1. Do you often feel upset for no obvious reason? Yes..2.. No..0..
... 2. Do you have an unreasonable fear of being in enclosed spaces such as shops, lifts, etc.? Often..2.. Sometimes..1.. Never..0..
... 3. Do people ever say you are too conscientious? No..0.. Yes..2..
... 4. Are you troubled by dizziness or shortness of breath? Never..0.. Often..2.. Sometimes..1..
... 5. Can you think as quickly as you used to? Yes..0.. No..2..
.... 6. Are your opinions easily influenced? Yes..2.. No..0..
... 7. Have you felt as though you might faint? Frequently..2.. Occasionally..1.. Never..0..
... 8. Do you find yourself worrying about getting some incurable illness? Never..0.. Sometimes..1.. Often..2..
... 9. Do you think that "cleanliness is next to godliness"? No..0.. Yes..2..
... 10. Do you often feel sick or have indigestion? Yes..2.. No..0..
... 11. Do you feel that life is too much effort? At times..1.. Often..2.. Never..0..
.... 12. Have you, at any time in your life, enjoyed acting? Yes..2.. No..0..
... 13. Do you feel uneasy and restless? Frequently..2.. Sometimes..1.. Never..0..
... 14. Do you feel more relaxed indoors? Definitely..2.. Sometimes..1.. Not particularly..0..
... 15. Do you find that silly or unreasonable thoughts keep recurring in your mind? Frequently..2.. Sometimes..1.. Never..0..
... 16. Do you sometimes feel tingling or pricking sensations in your body, arms or legs? Rarely..1.. Frequently..2.. Never..0..
... 17. Do you regret much of your past behaviour? Yes..2.. No..0..
.... 18. Are you normally an excessively emotional person? Yes..2.. No..0..
... 19. Do you sometimes feel really panicky? No..0.. Yes..2..
... 20. Do you feel uneasy travelling on buses or the Underground even if they are not crowded? Very..2.. A little..1.. Not at all..0..
... 21. Are you happiest when you are working? Yes..2.. No..0..
... 22. Has your appetite got less recently? No..0.. Yes..2..
... 23. Do you wake unusually early in the morning? Yes..2.. No..0..
.... 24. Do you enjoy being the centre of attention? No..0.. Yes..2..
... 25. Would you say you were a worrying person? Very..2.. Fairly..1.. Not at all..0..
... 26. Do you dislike going out alone? Yes..2.. No..0..
... 27. Are you a perfectionist? No..0.. Yes..2..
... 28. Do you feel unduly tired and exhausted? Often..2.. Sometimes..1.. Never..0..
... 29. Do you experience long periods of sadness? Never..0.. Often..2.. Sometimes..1..
.... 30. Do you find that you take advantage of circumstances for your own ends? Never..0.. Sometimes..1.. Often..2..
... 31. Do you often feel "strung-up" inside? Yes..2.. No..0..
... 32. Do you worry unduly when relatives are late coming home? No..0.. Yes..2..
... 33. Do you have to check things you do to an unnecessary extent? Yes..2.. No..0..
... 34. Can you get off to sleep alright at the moment? No..2.. Yes..0..
... 35. Do you have to make a special effort to face up to a crisis or difficulty? Very much so..2.. Sometimes..1.. Not more than anyone else..0..
.... 36. Do you often spend a lot of money on clothes? Yes..2.. No..0..

... 37. Have you ever had the feeling you are "going to pieces"? Yes..2.. No..0..
... 38. Are you scared of heights? Very..2.. Fairly..1.. Not at all..0..
... 39. Does it irritate you if your normal routine is disturbed? Greatly..2.. A little..1.. Not at all..0..
... 40. Do you often suffer from excessive sweating or fluttering of the heart? No..0.. Yes..2..
... 41. Do you find yourself needing to cry? Frequently..2.. Sometimes..1.. Never..0..
.... 42. Do you enjoy dramatic situations? Yes..2.. No..0..
... 43. Do you have bad dreams which upset you when you wake up? Never..0.. Sometimes..1.. Frequently..2..
... 44. Do you feel panicky in crowds? Always..2.. Sometimes..1.. Never..0..
... 45. Do you find yourself worrying unreasonably about things that do not really matter? Never..0.. Frequently..2.. Sometimes..1..
... 46. Has your sexual interest altered? Less..2.. The same or greater..0..
... 47. Have you lost your ability to feel sympathy for other people? No..0.. Yes..2..
.... 48. Do you sometimes find yourself posing or pretending? Yes..2.. No..0..

1. *Duplicating*

The test is designed for duplication on a foolscap sheet, questions 1–24 on the front, 25–48 on the back, with space for scoring each sub-test (A, P, O, etc.) and for diagnostic notes at the end. We would welcome use of the test, but it is important that it is duplicated *exactly* as arranged, otherwise the standardization figures may not apply. We should be pleased to send copies to anyone interested. The scores for each answer are included, but these should *not* be duplicated.

2. *Scoring*

i. Between each group of 6 questions, (6, 12, 18, etc.) four dots will be found on the left hand side. Join these up.

ii. Enter the score for each answer from 1–48 on the dotted lines on the left.

iii. Add up the scores on each sub-test separately and enter in the appropriate place at the end of the test. *Free-floating anxiety* consists of questions 1, 7, 13, 19, 25, 31, 37, 43; *phobic* consists of questions 2, 8, 14, 20, 26, 32, 38, 44; *obsessional* consists of questions 3, 9, 15, 21, 27, 33, 39, 45; *somatic* questions 4, 10, 16, 22, 28, 34, 40, 46; *depressive* of questions 5, 11, 17, 23, 29, 35, 41, 47; *hysteric* of questions 6, 12, 18, 24, 30, 36, 42, 48.

iv. Check that there are no arithmetical mistakes by adding the scores of all questions 1–48 and seeing that this agrees with the total of the separate sub-test scores.

v. Standardization figures are given in Table III so that individual, or group mean, scores can be compared.

Appendix 5. National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) (Litwin et al., 1999)

Pain or Discomfort

1. In the last week, have you experienced any pain or discomfort in the following areas?

	Yes	No
a. Area between rectum and testicles (perineum)	❑1	❑0
b. Testicles	❑1	❑0
c. Tip of the penis (not related to urination)	❑1	❑0
d. Below your waist, in your pubic or bladder area	❑1	❑0

2. In the last week, have you experienced:

	Yes	No
a. Pain or burning during urination?	❑1	❑0
b. Pain or discomfort during or after sexual climax (ejaculation)?	❑1	❑0

3. How often have you had pain or discomfort in any of these areas over the last week?
   - ❑0 Never
   - ❑1 Rarely
   - ❑2 Sometimes
   - ❑3 Often
   - ❑4 Usually
   - ❑5 Always

4. Which number best describes your AVERAGE pain or discomfort on the days that you had it, over the last week?

❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
0	1	2	3	4	5	6	7	8	9	10

NO PAIN — PAIN AS BAD AS YOU CAN IMAGINE

Urination

5. How often have you had a sensation of not emptying your bladder completely after you finished urinating, over the last week?
   - ❑0 Not at all
   - ❑1 Less than 1 time in 5
   - ❑2 Less than half the time
   - ❑3 About half the time
   - ❑4 More than half the time
   - ❑5 Almost always

6. How often have you had to urinate again less than two hours after you finished urinating, over the last week?
   - ❑0 Not at all
   - ❑1 Less than 1 time in 5
   - ❑2 Less than half the time
   - ❑3 About half the time
   - ❑4 More than half the time
   - ❑5 Almost always

Impact of Symptoms

7. How much have your symptoms kept you from doing the kinds of things you would usually do, over the last week?
   - ❑0 None
   - ❑1 Only a little
   - ❑2 Some
   - ❑3 A lot

8. How much did you think about your symptoms, over the last week?
   - ❑0 None
   - ❑1 Only a little
   - ❑2 Some
   - ❑3 A lot

Quality of Life

9. If you were to spend the rest of your life with your symptoms just the way they have been during the last week, how would you feel about that?
   - ❑0 Delighted
   - ❑1 Pleased
   - ❑2 Mostly satisfied
   - ❑3 Mixed (about equally satisfied and dissatisfied)
   - ❑4 Mostly dissatisfied
   - ❑5 Unhappy
   - ❑6 Terrible

Scoring the NIH-Chronic Prostatitis Symptom Index Domains

Pain: Total of items 1a, 1b, 1c, 1d, 2a, 2b, 3, and 4 = _____

Urinary Symptoms: Total of items 5 and 6 = _____

Quality of Life Impact: Total of items 7, 8 and 9 = _____

Appendix 6. (Barry et al., 1992)

International prostate symptom score (IPSS)

Name: Date:

	Not at all	Less than 1 time in 5	Less than half the	About half the time	More than half the	Almost always	**Your score**
Incomplete emptying Over the past month, how often have you had a sensation of not emptying your bladder completely after you finish urinating?	0	1	2	3	4	5	
Frequency Over the past month, how often have you had to urinate again less than two hours after you finished urinating?	0	1	2	3	4	5	
Intermittency Over the past month, how often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
Urgency Over the last month, how difficult have you found it to postpone urination?	0	1	2	3	4	5	
Weak stream Over the past month, how often have you had a weak urinary stream?	0	1	2	3	4	5	
Straining Over the past month, how often have you had to push or strain to begin urination?	0	1	2	3	4	5	

	None	1 time	2 times	3 times	4 times	5 times or more	**Your score**
Nocturia Over the past month, many times did you most typically get up to urinate from the time you went to bed until the time you got up in the morning?	0	1	2	3	4	5	

Total IPSS score	

Quality of life due to urinary symptoms	Delighted	Pleased	Mostly satisfied	Mixed – about equally satisfied and dissatisfied	Mostly dissatisfied	Unhappy	Terrible
If you were to spend the rest of your life with your urinary condition the way it is now, how would you feel about that?	0	1	2	3	4	5	6

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