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Original article

Metabolic syndrome and prostate abnormalities in male subjects of infertile couples

RUNNING TITLE: Metabolic syndrome and prostate

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Abstract

No previous study has evaluated systematically the relationship between metabolic syndrome (MetS) and prostate-related symptoms and signs in young infertile men. We studied 171 (36.5±8.3 years old) males of infertile couples. MetS was defined based on the National Cholesterol Education Program, Third Adult Treatment Panel. All men underwent hormonal (including total testosterone [TT] and insulin), seminal (including interleukin 8, seminal plasma interleukin 8 (sIL-8)) scrotal and transrectal ultrasound evaluations. Because we have previously assessed correlations between MetS and scrotal parameters in a larger cohort of infertile men, here, we focused on transrectal features. Prostate-related symptoms were assessed using the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) and the International Prostate Symptom Score (IPSS). Twenty-two subjects fulfilled MetS criteria. In an age-adjusted logistic ordinal model, insulin levels increased as a function of MetS components (Wald=29.5, $P<0.0001$) and showed an inverse correlation with TT (adjusted $r=-0.359$, $P<0.0001$). No association between MetS and NIH-CPSI or IPSS scores was observed. In an age-, TT-, insulin-adjusted logistic ordinal model, an increase in number of MetS components correlated negatively with normal sperm morphology (Wald=5.59, $P<0.02$) and positively with sIL-8 levels (Wald=4.32, $P<0.05$), which is a marker of prostate inflammation, with prostate total and transitional zone volume assessed using ultrasound (Wald=17.6 and 12.5, both

P<0.0001), with arterial peak systolic velocity (Wald=9.57, *P*=0.002), with texture non-homogeneity (HR=1.87 [1.05-3.33], *P*<0.05), with calcification size (Wald=3.11, *P*<0.05) but not with parameters of seminal vesicle size or function. In conclusion, in males of infertile couples, MetS is positively associated with prostate enlargement, biochemical (sIL8) 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.

Keywords: metabolic syndrome, prostate-related symptoms and signs, transrectal ultrasound, interleukin 8, infertile men, semen analysis

Introduction

Metabolic syndrome (MetS) is a diagnostic category based on a cluster of abnormalities (abdominal obesity, impaired glucose metabolism, hypertriglyceridaemia, reduced high-density lipoprotein (HDL) cholesterol and hypertension) that identifies subjects at high risk for diabetes and cardiovascular diseases[1-4]. Various diagnostic criteria for MetS have been proposed over recent years. Indeed, the parameters to be used for the diagnosis of MetS and their thresholds remains under debate[4,5]. Nonetheless, insulin resistance has been recognised as the common feature underlying MetS[6], with visceral adiposity being the driving force[2-5]. In addition to diabetes and cardiovascular diseases, several other pathologic conditions are associated with MetS including non-alcoholic fatty liver disease, polycystic ovarian syndrome, obstructive sleep apnoea and lipodystrophy[2]. In addition, in the male, hypogonadism, erectile dysfunction (ED) and psychological disturbances are often associated with MetS[4,7-9].

A possible association between MetS and male infertility has also been hypothesised[1]. Although several studies have evaluated the impact of being overweight and obesity on male reproductive health[10-13], only a few studies have assessed the influence of some MetS components on male fertility[1] and none have considered MetS to be a diagnostic category. We evaluated this topic specifically in a cohort of 351 males of infertile couples without known genetic abnormalities[9]. Even after adjusting for confounders, we noted that an increase in the number of MetS components associated with hypogonadism, poor sperm morphology (but not other sperm parameters), testis ultrasound non-homogeneity, ED, somatisation (expression of physical symptoms in the absence of medically explained physical illness) and depressive traits [9].

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[14-18]. In particular, we previously reported a positive correlation between

MetS and BPH-related chronic inflammation in patients undergoing surgery for BPH[19,20]. 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[21].

This study is aimed at investigating systematically the possible associations between MetS and prostate-related symptoms and signs in a cohort of young men in infertile unions and to establish whether these associations correlate with fertility.

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[22]. Subjects with karyotypic 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), [23] as the presence of three or more of the following five factors:

central obesity (waist circumference >102 cm), elevated triglycerides (≥1.7 mmol l^{-1} 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^{-1} or treated for diabetes) and reduced high-density lipoprotein (HDL) cholesterol (<1.03 mmol l^{-1} 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). [24]

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[25]. In addition, routine urine and seminal cultures were assessed in all men. Furthermore, sIL-8, a reliable surrogate marker of prostatitis, [26] 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[26]. Each seminal plasma sample was diluted from 1:5 to 1:625. Assay sensitivity for sIL-8 was <1 pg ml^{-1}.

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)[27], 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), 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. [28]

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[9]. 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[29-33] 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 π/6) based on previous publications[31, 34, 35]. During this exam, similar methods were also used to determine the transitional zone volume (TZV) based on a previous publication[35]. Prostate echogenicity was defined based on previous studies[29-32]. 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[31,32,36,37] and evaluated prior to ejaculation to avoid post-ejaculatory changes in vascular flow pattern as has been previously reported[38]. Seminal vesicle ultrasound features and abnormalities were defined based on previous studies[30,31,33,39,40]. Ejaculatory duct CDU characteristics were evaluated after ejaculation to better emphasise indirect CDU signs of subobstruction[31,33,39].

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[29-31]. Testicular and epididymal CDU features were examined based on previous studies[29-31].

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^{-1}), smoking habit (current/non-smoker) and moderate-severe alcohol consumption (current/no consumption of ≥4 drinks per day based on a previous publication[41]). 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 01.31], $P<0.05$). 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^{-1} 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^{-1} increment), hyperaemia (RR=1.05 [1.00–1.11], P<0.05 for each insulin mU l^{-1} 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^{-1} 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[20,42-49]. Thus far, only a few studies have examined relatively young adults, and they offer conflicting results[50,51]. 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 [14,52], but not all previous studies[20]. Moreover, low HDL cholesterol has been previously reported as a risk factor for the development of BPH [20,42-45]. 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[26,53-55]. 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[56-58]. 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)[59-61]. Because IL-8 in seminal plasma is considerably higher when compared with serum levels[62,63], local (within the male genital tract) production has been suggested[55,59,63]. Of different cytokines and chemokines, sIL-8 appears to be the most reliable and predictive surrogate marker of prostatitis[26,54], and it is associated with CDU features suggestive of prostate inflammation in patients with male accessory gland infection (MAGI)[31]. IL-8 is actively involved in BPH-associated chronic inflammation and mediates epithelial and stromal cell proliferation[54]. In BPH tissues, epithelial and stromal cells secrete IL- 8 actively[26,64] in response to varying stimuli including the proinflammatory cytokines IFNγ and IL-17 that are produced by prostate-infiltrating Th1 and Th17 cells, respectively[53,65-67]. 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[68]. 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[26]. Finally, IL-8 stimulates overgrowth of prostate stromal and epithelial cells by directly promoting the proliferation of senescent epithelial cells[69], stromal trans-differentiation of myofibroblasts[70] and by increasing secretion of fibroblast growth factor 2[71]. Hence, IL-8 appears to be the link between T-cell-mediated inflammatory responses and cell proliferation in the pathogenesis of BPH[54,67].

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[29] and has been previously associated with elevated sIL-8 levels in infertile subjects with MAGI[31]. Moreover, prostatic hyperechogenicity, which is associated with areas of calcification, has been previously proposed to be a CDU feature suggestive of MAGI[30]. 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[72]. 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[73]. 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[74,75]. A positive correlation between sIL-8 levels and calcification size has also been previously reported by our group[31]. Finally, we noted that APPSV correlated with MetS severity. Arterial PSV reflects tissue inflammation at various sites including the thyroid[76,77], exocrine glands[78,79] and synovial membrane/joints[80,81]. Thus far, APPSV has been studied for varying purposes including evaluation of BPH[82], prostate cancer[83], varicocele-related prostate CDU changes[84] and premature ejaculation[37]. More recently, elevated APPSV has been proposed as a CDU parameter that correlates with prostate inflammation[31,32,37], and in the prostate, APPSV is closely related to sIL-8[31].

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[20] and inflammation[19,85] in cohorts of aged subjects. In the

study of Gacci et al.[20], 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[19,85]. 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[19]. In addition, TNF-α sensitised BPH cells to oxLDL by inducing its receptor (LOX-1)[19]. 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[86]. 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[86]. 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[3,6], 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[19]. However, in that study, the effect of insulin was negligible when compared with oxLDL (six-fold lower)[19]. The growth-promoting activity of insulin on the prostate gland is well-documented in several experimental and epidemiological studies[87].

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[15, 16, 18]. 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[9]. 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[30,88-90], a specific association between prostate CDU features and sperm parameters alterations has not been demonstrated. Here, we here report that a 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.

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.

Author contributions: FL, GC and MM conceived and designed the study, performed the statistical analysis and drafted the manuscript. LV, MG and GF participated in the design of the study. FL, EM, MR and SC participated in data collection and management. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests

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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.

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.

	All patients *n*=171
Clinical and laboratory parameters	
Age (year)	36.6 ±8.4
Current smoker (%)	27.6
Current moderate/severe alcohol consumption (≥4 drinks per day, %)	22.2
Diabetes mellitus (%)	1.4
Mean testis volume (Prader) (ml)	18.4 ±4.8
Enlarged prostate upon 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^{-1})	4.94 ±0.67
Triglycerides (mmol l^{-1})	1.15 (0.80-1.58)
HDL cholesterol (mmol l^{-1})	1.27 ±0.32
Systolic BP (mm Hg)	125.3 ±13.8
Diastolic BP (mm Hg)	80.1 ±8.1
Insulin levels (mU l^{-1})	9.8 ±6.6
Total testosterone (nmol l^{-1})	15.8 ±6.4
Sex hormone binding globulin (nmol l^{-1})	30.7 ±12.7
Calculated free testosterone (nmol l^{-1})	0.339 ±0.117
$Log._{10}$ (sIL-8) (ng ml^{-1})	3.55 ±0.45
Specific medications	
Hypoglycaemic drugs	0.7
Lipid-lowering drugs	4.1
Antihypertensive drugs	8.1
Seminal parameters	
Azoospermic subjects (%)	16.5
Sexual abstinence (day)	4.2 ±2.0
pH	7.5 ±0.3
Semen volume (ml)	3.5 ±1.8
Sperm concentration (10^6 ml^{-1})	27.3 ±39.8
Spermatozoa per ejaculate (10^6 ml^{-1})	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 (year)	1.9 ±1.8
Primary infertility	82.9
Secondary infertility	17.1
Female partner age (year)	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[a]	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 s^{-1})	9.9 ±3.4
Mean prostatic venous plexus (mm)	4.2 ±1.7
Dilated ejaculatory ducts	8.8
Seminal vesicles	
Total volume before ejaculation[b] (ml)	9.1 (5.9-14.5)
Total volume after ejaculation[b] (ml)	6.1 (3.7-9.2)
Ejection fraction[c] (%)	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

Abbreviations: NIH-CPSI, National Institutes of Health Chronic Prostatitis Symptom Index; sIL-8, seminal interleukin 8; HDL, high-density lipoprotein; BP, blood pressure; tp, therapy. [a]Calcifications with size > 3 mm (based on [31, 32]). [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).[33] [c]Calculated as [(pre-ejaculatory total volume − post-ejaculatory total volume)/pre-ejaculatory volume)] H 100.[33]
Data are expressed as the mean ±s.d. or as the median (quartiles) where appropriate and as percentages when categorical.

Table 2: Univariate associations between MetS-related prostate (CDU) characteristics and semen parameters.

Semen parameters	MetS-related prostate CDU characteristics			
	Prostate volume (ml)	Arterial prostatic peak systolic velocity (cm s^{-1})	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^{-1})	*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^{-1})	*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

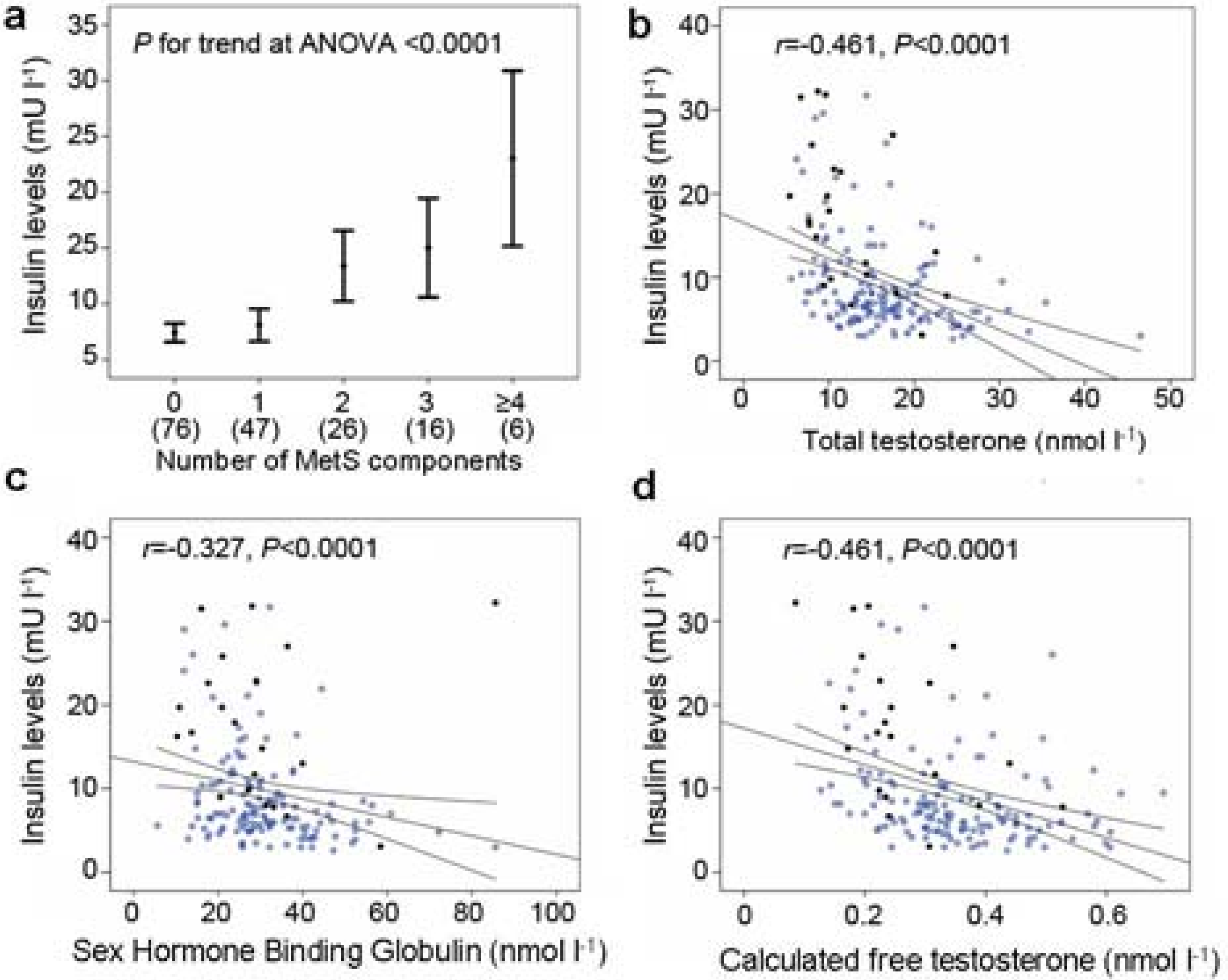
Abbreviations: MetS, metabolic syndrome; CDU, colour-Doppler ultrasound.

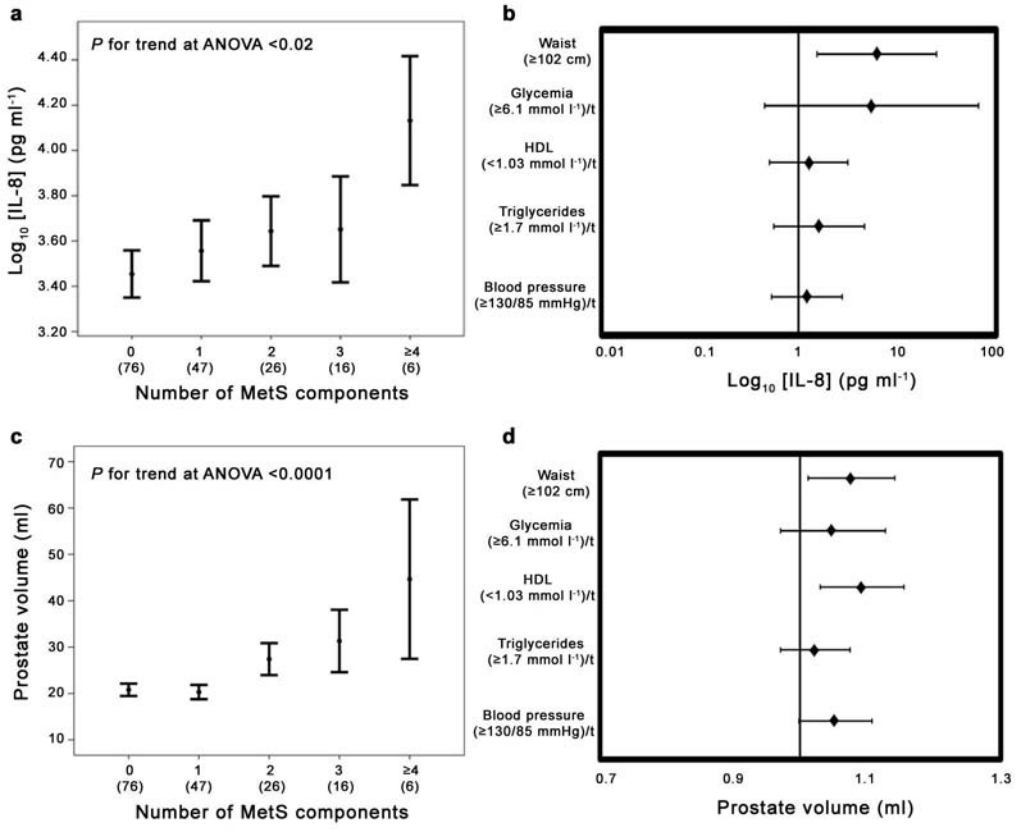
Table 3. Comparisons between subjects with metabolic syndrome (MetS) and 1:2 ratio-matched controls (matched for age, total testosterone, smoking habits and moderate-severe alcohol consumption).

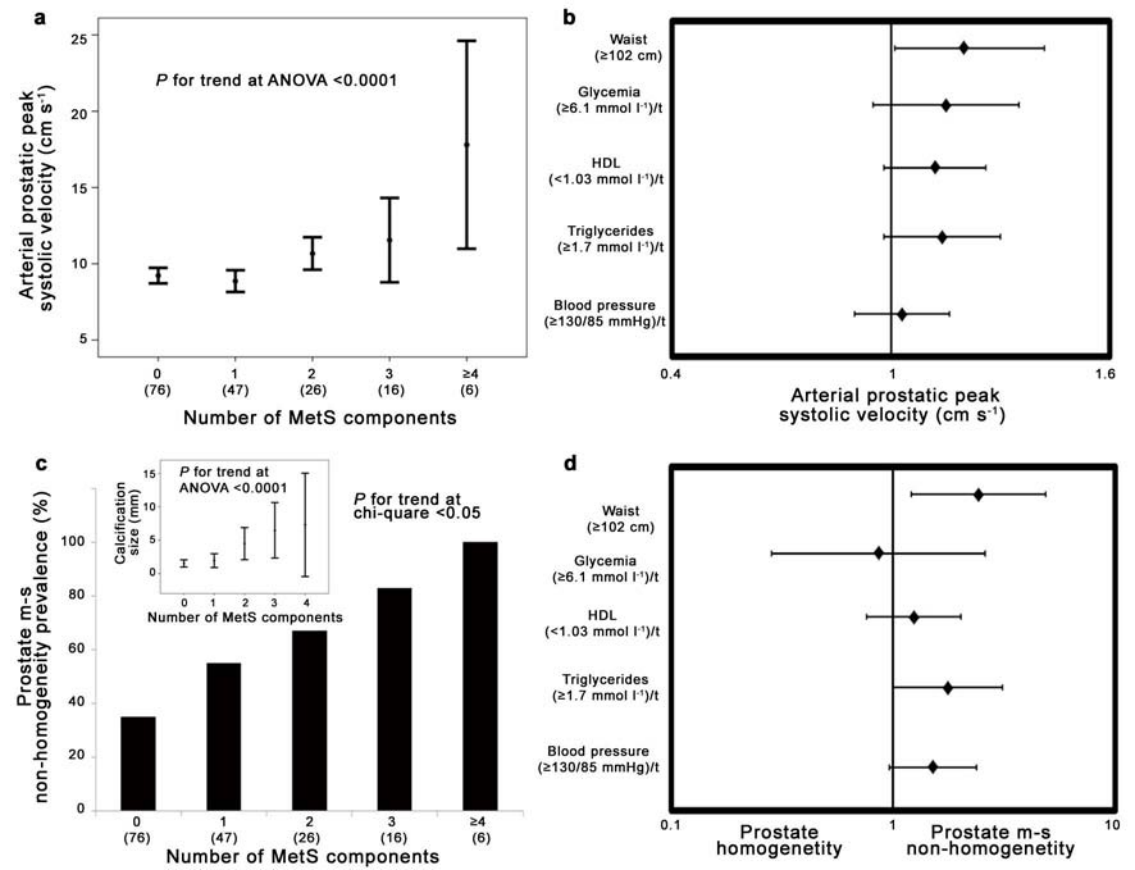
	Case patients (≥3 MetS factors, *n*=22)	**Controls (matched 1:2, *n*=44)**	***P***
Age	43.8±10.6	41.0±6.6	0.269
Total testosterone (nmol l^{-1})	12.2±5.3	13.0±4.5	0.523
Current smoker (%)	22.8	23.0	0.532
Current moderate-severe alcohol consumption (≥4 drinks per day, %)	18.2	16.1	0.574
Semen volume (ml)	3.3±2.5	3.5±1.7	0.733
Sperm concentration (10^6 ml^{-1})	29.6±40.6	32.0±45.7	0.839
Spermatozoa per ejaculate (10^6 ml^{-1})	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^{-1})	3.8±0.4	3.5±0.4	0.035
Prostate volume at CDU (ml)	35.0±14.6	24.9±8.4	0.006
Prostate transitional zone volume at CDU (ml)	10.7±10.8	5.1±4.3	0.004
Arterial prostatic peak systolic velocity at CDU (cm s^{-1})	13.3±2.7	9.6±2.7	0.014

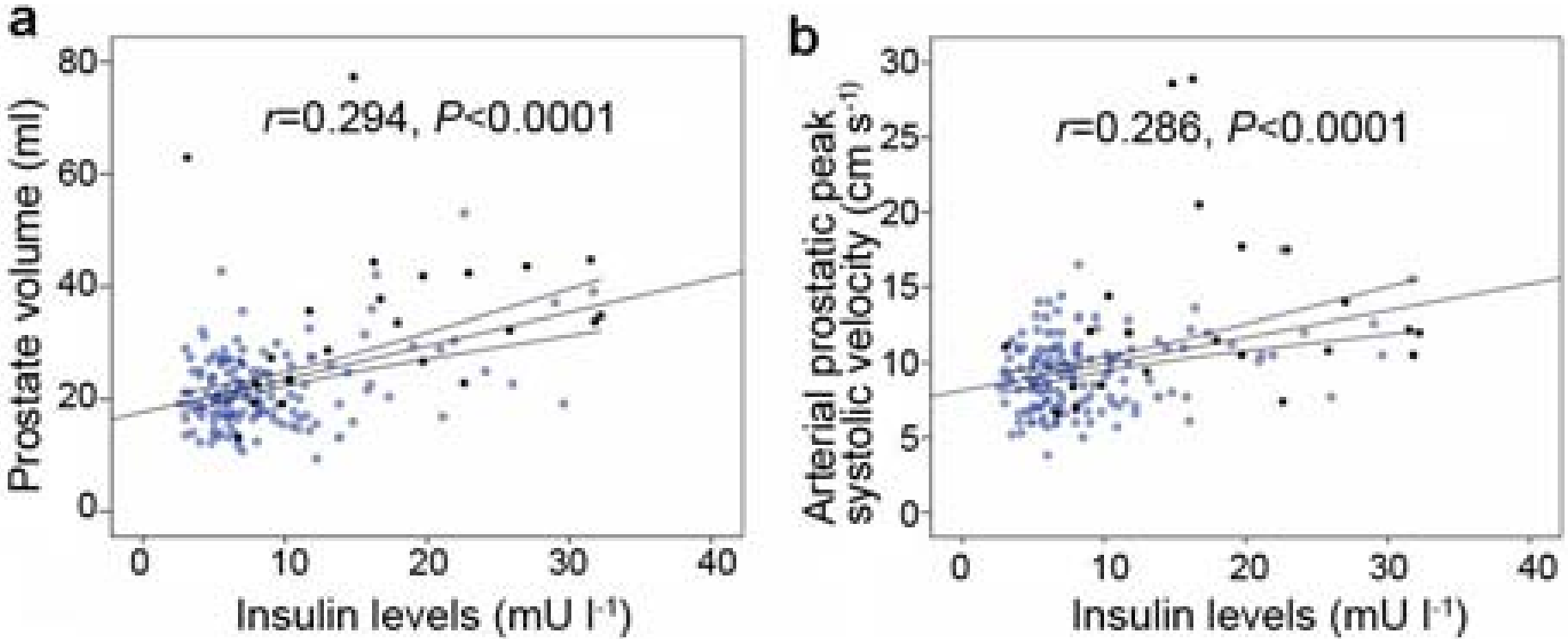
Prostate moderate-severe inhomogeneity at CDU (%)	95.6	46.0	0.009
Prostate calcification size at CDU (mm)	6.7 ±3.5	2.5 ±4.1	0.022

1 Abbreviations: sIL8, seminal interleukin 8; CDU, colour-Doppler ultrasound.
2 The data are expressed as the mean ± s.d. and as percentages when categorical.


a
P for trend at ANOVA <0.0001
Insulin levels (mU l-1)
35
30
25
20
25
10
5
0 (76)
1 (47)
2 (26)
3 (16)
≥4 (6)
Number of MetS components
b
r=-0.461, P<0.0001
Insulin levels (mU l-1)
40
30
20
10
0
0
10
20
30
40
50
Total testosterone (nmol l-1)
c
r=-0.327, P<0.0001
Insulin levels (mU l-1)
40
30
20
10
0
0
20
40
60
80
100
Sex Hormone Binding Globulin (nmol l-1)
d
r=-0.461, P<0.0001
Insulin levels (mU l-1)
40
30
20
10
0
0
0.2
0.4
0.6
Calculated free testosterone (nmol l-1)


a
P for trend at ANOVA <0.02
Log10 [IL-8] (pg ml-1)
4.40
4.20
4.00
3.80
3.60
3.40
3.20
0 (76)
1 (47)
2 (26)
3 (16)
≥4 (6)
Number of MetS components
b
Waist (≥102 cm)
Glycemia (≥6.1 mmol l-1)/t
HDL (<1.03 mmol l-1)/t
Triglycerides (≥1.7 mmol l-1)/t
Blood pressure (≥130/85 mmHg)/t
0.01
0.1
1
10
100
Log10 [IL-8] (pg ml-1)
c
P for trend at ANOVA <0.0001
Prostate volume (ml)
70
60
50
40
30
20
10
0 (76)
1 (47)
2 (26)
3 (16)
≥4 (6)
Number of MetS components
d
Waist (≥102 cm)
Glycemia (≥6.1 mmol l-1)/t
HDL (<1.03 mmol l-1)/t
Triglycerides (≥1.7 mmol l-1)/t
Blood pressure (≥130/85 mmHg)/t
0.7
0.9
1.1
1.3
Prostate volume (ml)


a
Arterial prostatic peak systolic velocity (cm s-1)
P for trend at ANOVA <0.0001
25
20
15
10
5
0 (76)
1 (47)
2 (26)
3 (16)
≥4 (6)
Number of MetS components
b
Waist (≥102 cm)
Glycemia (≥6.1 mmol l-1)/t
HDL (<1.03 mmol l-1)/t
Triglycerides (≥1.7 mmol l-1)/t
Blood pressure (≥130/85 mmHg)/t
0.4
1
1.6
Arterial prostatic peak systolic velocity (cm s-1)
c
Prostate m-s non-homogeneity prevalence (%)
P for trend at ANOVA <0.0001
Calcification size (mm)
Number of MetS components
P for trend at chi-quare <0.05
100
80
60
40
20
0
0 (76)
1 (47)
2 (26)
3 (16)
≥4 (6)
Number of MetS components
d
Waist (≥102 cm)
Glycemia (≥6.1 mmol l-1)/t
HDL (<1.03 mmol l-1)/t
Triglycerides (≥1.7 mmol l-1)/t
Blood pressure (≥130/85 mmHg)/t
0.1
1
10
Prostate homogenetity
Prostate m-s non-homogenetity


a
Prostate volume (ml)
r=0.294, P<0.0001
Insulin levels (mU l-1)
b
Arterial prostatic peak systolic velocity (cm s-1)
r=0.286, P<0.0001
Insulin levels (mU l-1)