Fat boosts, while androgen receptor activation counteracts, BPH-associated prostate inflammation

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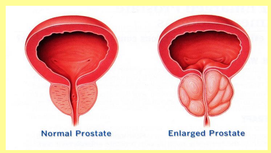
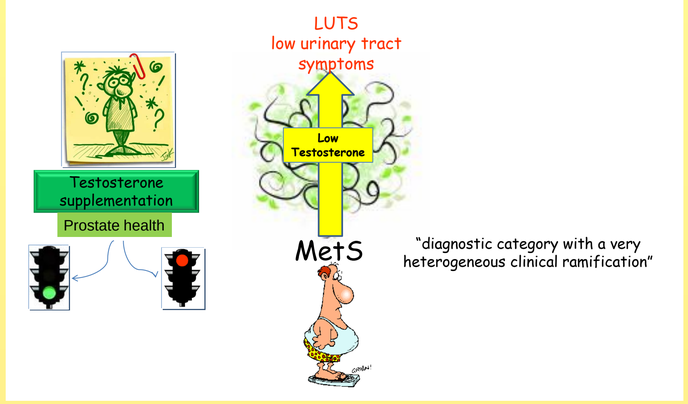
Background



The condition known as benign prostatic hyperplasia (BPH) is the most frequent benign neoplasm in ageing men. Although primarily characterized by increased proliferation of both myofibroblast and epithelial cells, more recent epidemiological and histopatological studies have suggested that chronic inflammation is the crucial event in BPH pathogenesis. The nature of this immune dysregulation is almost completely unknown.
Epidemiological studies have also demonstrated that BPH is a common clinical finding in male patients affected by metabolic syndrome (MetS), suggesting that a metabolic insult could contribute to the pathogenesis and progression of BHP.



AIM:

1. The aim of this study is to evaluate whether MetS is associated with BPH-related inflammation in BPH patients undergoing prostatectomy for BPH

and

2. To investigate the in vitro inflammatory effects of different metabolic insults on human prostatic myofibroblast cells isolated from BPH patients (hBPH).

We have therefore examined the histological characteristics of inflammatory infiltrates (Inflammatory score, IS) in prostatectomy specimens from a cohort of BPH patients and their correlation with pre-operatory MetS features, including hypogonadism.
A series of in vitro experiments stimulating myofibroblast hBPH cells with different metabolic stimuli was also performed.

Study Population
Between January 2010 and September 2011, 244 consecutive patients undergoing prostate surgery for BPH were selected for this study in two tertiary referral centres for LUTS/BPH. Ethical permission for this study was obtained from the Careggi Hospital Ethics Committee (Ethics Committee Approval # 679/11), with standard informed consent from the patients for use of their anonymous data. The inclusion criteria were: prostate surgery for severe LUTS due to BPH not responding to conventional medical treatment (a-blockers, 5 a-reductase inhibitors), ability to communicate, understand and comply with study requirements, and written informed consent. Exclusion criteria were: history of previous prostate surgery, chronic medication for prostatitis and/or urinary infection or bladder stone, known malignant disease including prostate cancer, and chronic renal failure. Men with chronic use of anti-inflammatory medications were excluded from the study, while sporadic use of these drugs was admitted. The main characteristics of the sample are summarized in Table 1.

Definition of Metabolic Syndrome parameters and hypogonadism
Metabolic syndrome (MetS) was diagnosed using the AHA/NHLBI criteria (23-24). According to AHA/NHLBI, MetS was defined as the presence of three or more of the following five factors: (1) waist circumference (WC) >102 cm; (2) triglycerides ≥1.7mmol/L (150 mg/dL), (3) HDL-C < 1.03mmol/L (40 mg/dL), (4) blood pressure ≥130/85 mm Hg, and (5) fasting blood glucose ≥ 5.6mmol/L (100mg/dL). Moreover, all individuals receiving pharmacological treatment for hypertension, hypertriglyceridaemia, or low HDL cholesterol, and all individuals previously diagnosed with type 2 diabetes was considered as afflicted with these factors. Hypogonadism was defined when total testosterone (TT) was lower than 10.4 nmol/L (300 ng/dL) according to the Endocrine Society recommendations (25).

Pathological characterization of prostatic inflammatory infiltrates
All specimens were examined twice on hematoxylineosin-stained sections by two independent pathologists in each centre, blinded to clinical findings.
All surgical specimens were investigated for the presence of an inflammatory infiltrate, according to the standardized classification system of chronic prostatitis (CP-CPPS) of the National Institutes of Health (29). As previously described (30), for the purposes of statistical analysis, an "inflammatory score" (IS) calculated as the sum of the three different histological inflammatory parameters (anatomical location; grade; extent) was used. These inflammatory parameters were defined and scored as follows: prevalent anatomical location (stromal: infammatory cells lie within prostatic stroma but not centred on prostatic glands/ducts [1]; periglandular: if infammatory infltrates lie within stroma, are centred around ducts/glands, and approach ducts/glands [2]; glandular: if infammatory infltrates lie within duct/gland epithelium and/or lumens [3]), grade (mild: Light scattering of individual inflammatory cells or small, patchy inflammatory aggregates [1]; moderate: presence of larger, multifocal aggregates, which were detectable at low magnification [2]; severe: large, multifocal and confluent sheets of inflammatory cells, occasionally in association with gland destruction, [3]) and extent (focal: if tissue area involved by infammatory cell infltrates was <10% [1]; multifocal: if tissue area involved by infammatory cell infltrates was 10-50% [2]; diffuse: if tissue area involved by infammatory cell infltrates was >50%[3]) of inflammatory infiltrates. Hence, IS ranges from 3 to 9. In each patient, two independent pathologists examined and scored these inflammatory parameters considering all the available glass slides. The number of glass slides per patient varies depending on the type of surgical procedure and the total amount of resected prostatic tissue.

CLINICAL STUDY

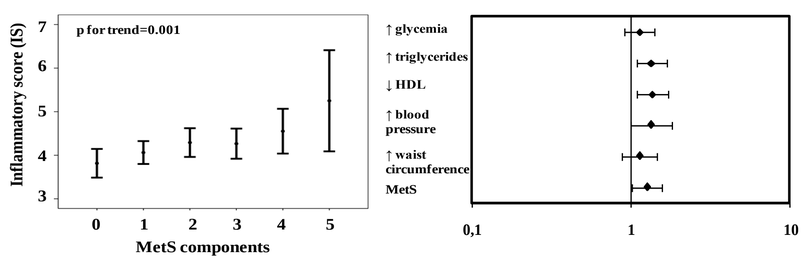
ble 1. Physical, metabolic, and hormonal parameters .

Clinical Features	Age (years)	68.1±7.6
	BMI	26.5±3.2
Metabolic Syndrome Features	Waist circumference (cm)	97.9±10.5
	DBP (mmHg)	80 [70-80]
	SBP (mmHg)	130 [120-140]
	HDL (mmol/L)	1.16±0.22
	Triglycerides (mmol/L)	1.38± [0.99-1.9]
	Fasting glycemia (mmol/L)	5.33 [4.83-5.88]
	MetS (%)	37.2
	• Waist circumference >102 cm (%)	21.6
	• BP ≥130/85 mmHg or treatment (%)	77.6
	• HDL <1.03mmol/L or treatment (%)	29.1
	• Triglycerides ≥1.7mmol/L or treatment (%)	39.5
	• Fasting glycemia ≥5.6mmol/L or treatment (%)	41.8
Hormonal Features	Testosterone (nM)	13.9±5.7

ta are expressed as mean±SD or median [quartiles] when normally or not normally distributed, and as percentage when categorical. BMI= body mass index, SBP= systolic blood pressure; DBP= diastolic blood pressure, HDL= high density lipoprotein.

The inflammatory score (IS) significantly increased as a function of MetS components. Among MetS components, in a age-adjusted model, reduced HDL cholesterol and elevated triglycerides - but not high waist circumference, glycaemia, or blood pressure - were significantly associated with elevated IS

Figure 1



In the subset of patients in whom testosterone evaluation was available (n=92), the prevalence of hypogonadism (TT <10.4nM) was detected in 24.2% of subjects. In this sample, increased IS was significantly associated with hypogonadism (age-adjusted O.R.= 1.433 [1.05-1.940] for each IS increment; p= 0.02). Furthermore, after adjustment for hypogonadism and age, higher triglycerides and lower HDL cholesterol levels were still associated with increased IS (Adj.r= 0.331; p= 0.027 and 0.174; p= 0.007; for triglycerides and HDL respectively).

PRE-CLINICAL STUDY

We first measured cytokines, chemokines, and growth factors detected in culture supernatants of myofibroblast hBPH cells

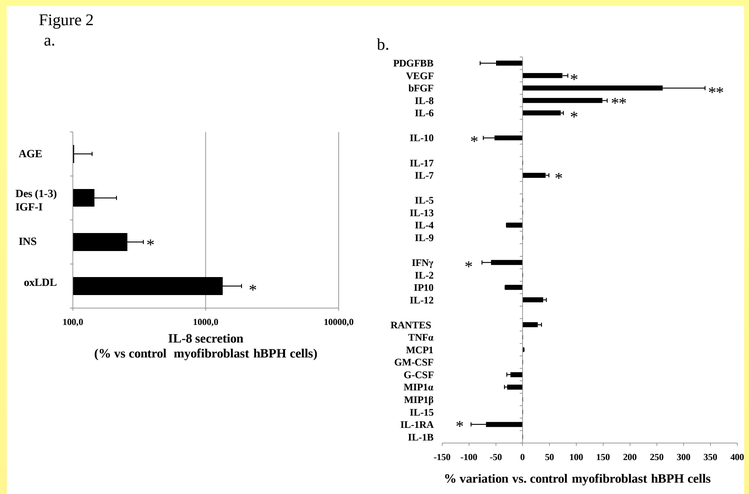
Table 2. Cytokines, chemokines and growth factor levels in myofibroblast hBPH cell cultures.

	pg/10⁴ cells		pg/10⁴ cells
PDGFBB	28.6±5.9	IL-2	*Undetectable*
VEGF	2958.5±855	IP10	86.7±16
bFGF	10.9±2.3	IL-12	16.6±1.8
IL-8	70.3±6.85	RANTES	15.8±3.8
IL-6	88.0±11.4	TNFα	4±0.3
IL-10	3.7±1.1	MCP1	2400±616.7
IL-17	*Undetectable*	GM-CSF	*Undetectable*
IL-7	22±1.9	G-CSF	10±2.5
IL-5	*Undetectable*	MIP1α	2.4±1
IL-13	*Undetectable*	MIP1β	*Undetectable*
IL-4	4.5±1.3	IL-15	*Undetectable*
IL-9	3.2±0.2	IL-1RA	9.5±2.5
IFNγ	20±1.7	IL-1B	*Undetectable*

Levels (pg/10⁴ cells) of the indicated cytokines, chemokines and growth factors were determined in culture supernatants by bead-based multiplex immunoassay. Data are expressed as mean ± SEM of basal secretion in three different preparations of myofibroblast hBPH cells cultured in triplicate. Undetectable (below the limit of detection).

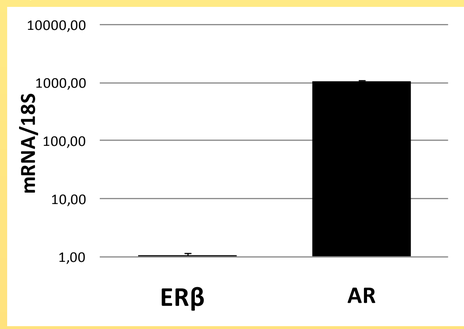
#To investigate whether metabolic factors could directly trigger prostate inflammation, we performed preliminary in vitro studies in human prostatic myofibroblast hBPH, using as a readout IL-8 secretion.

Figure 2



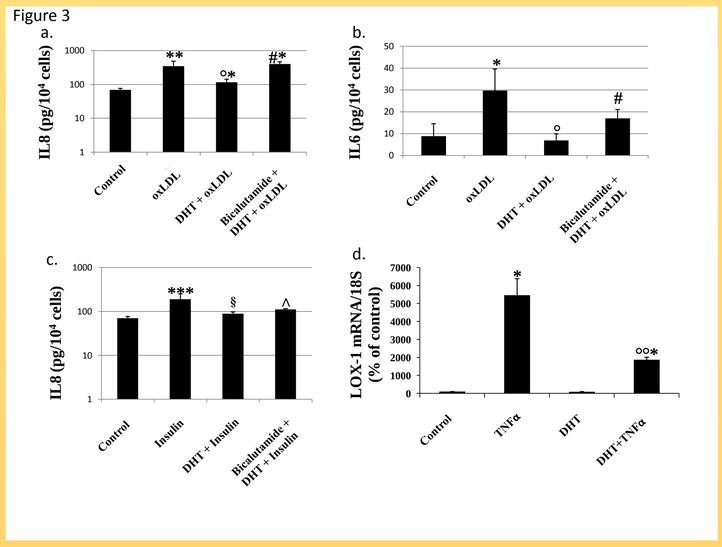
Among the different factors tested (insulin, AGE, [Des (1-3) IGF-I], oxLDL), only insulin (10nM) and oxLDL (25 μg/ml) significantly (2 and 13 folds, respectively) increased IL-8 secretion (Fig. 2 panel a). We therefore focused our further experiments on oxLDL, being the most potent stimulus. The capacity of oxLDL to modulate the in vitro release of inflammatory factors in conditioned medium, using a bead-based multiplex immunoassay, was evaluated (Fig. 2 panel b). oxLDL (25 μg/ml, for 24 hours) significantly increased levels of a series of proinflammatory factors promoting BPH cell growth, such as IL-8, IL-6, bFGF, and VEGF. Secretion of the T cell growth factor IL-7 also significantly increased. Conversely, no significant modification of the Th1 inducer (IL-12) and Th1-type IP-10 was observed, although IFN-γ was significantly reduced. Th2-type cytokines, as well as Th17-specific cytokine IL-17, were not significantly modified. In keeping with the overall proinflammatory effects of oxLDL on myofibroblast hBPH cells, secretion of the anti-inflammatory cytokines IL-10 and IL-1RA were significantly inhibited

#In preliminary experiments we found that AR was abundantly expressed by all hBPH cultures, its mRNA level resulting as at least three-log unit higher than ERβ, which is known to be activated - besides its natural ligands (estrogens)- by some DHT metabolites



#To further validate results from multiplex immunoassay, we repeated the experiments with oxLDL (25 μg/ml, 24hours) on IL-8, IL-6 and IP-10 secretion, by using a specific ELISA.

Figure 3



OxLDL (25 μg/ml, 24 hours) significantly up-regulated IL-8 and IL-6 secretion (Fig. 3 panels a-b), whilst IP10 secretion was not affected (not shown). Co-treatment with DHT (30nM, for 24 hours) significantly inhibited oxLDL-induced secretion of both IL-8 and IL-6. The effect of DHT was completely reversed by bicalutamide (1μM, for 24 hours).
Similar results were obtained after insulin (10nM, 24 hours) or TNFα priming ((10 ng/ml for 6 hours).

Conclusions

We we demonstrated that MetS, and in particular dyslipidaemia, is associated with prostate inflammation. Fats could have, therefore, a detrimental effect on prostate cells, boosting prostate inflammation, a key factor in the development and progression of BPH/LUTS. DHT ability in counteracting lipid- and insulin-induced prostatic alterations, suggest that testosterone - via its conversion into DHT- may have unexpected beneficial effects on prostate health. Clinical studies specifically addressing this point are urgently needed.