phenotyping method used in the present study could conceivably give misleading information if chronic renal failure were to alter the reactivity of paraoxonase to either substrate, this seems unlikely. Nonetheless, genotyping of the paraoxonase DNA polymorphism will be required to confirm the phenotypes.

The decrease in paraoxonase activity could be the result of lower HDL concentrations in chronic renal failure, given that HDL is the main serum carrier of paraoxonase. This hypothesis is supported by the fact that paraoxonase/arylesterase activities were no longer different between the two study populations when expressed relative to HDL cholesterol concentration. A second possible explanation for the decrease of paraoxonase activity in chronic renal failure might be that paraoxonase activity could be inhibited in the uremic environment. The possibility of an endogenous circulating inhibitor of paraoxonase in blood of renal failure patients was dismissed by Schiavon et al. [20]. However, activity inhibition through posttranslational modification of paraoxonase as a result of reactions with advanced glycation end-products or urea-derived cyanate remains possible [21]. Alternatively, the expression of paraoxonase activity may be affected by HDL size and composition, which are likely to differ in patients and control groups.

PAF-AH has been shown to inhibit LDL oxidation [5] and to destroy lipid peroxides [7]. Our results suggest that the ability of PAF-AH to destroy lipid peroxides and inhibit LDL oxidation is not altered in chronic renal failure. PAF-AH is ordinarily present in LDL, HDL, and Lp(a) [3]. It therefore remains possible that PAF-AH activity associated with HDL is decreased because of the reduced concentrations of HDL cholesterol in renal failure, while increased activity in LDL or Lp(a) results in normal activity overall.

In conclusion, therefore, reduced paraoxonase activity in patients with chronic renal failure may give rise to decreased HDL antioxidant capacity in these subjects. LDL modification by lipid peroxidation might therefore be increased, contributing to the accelerated development of atherosclerosis in chronic renal failure. Paraoxonase and PAF-AH have been suggested to work in concert to inhibit LDL oxidation. Our results show that PAF-AH activity is unaltered in patients with chronic renal failure and so is unlikely to contribute to the reduced antioxidant capacity in these patients.

We are grateful to the Northern Ireland Chest, Heart and Stroke Association, DENI (Department of Education in Northern Ireland), and Deutscher Akademischer Aus- tauschdienst for their support for our work in this area.

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High Concentrations of Prostate-Specific Antigen in Urine of Women Receiving Oral Contraceptives, Ferdinando Mannello,1* Leone Condemi,2 Antonella Cardinali,1 Giuseppe Bianchi,2 and Giancarlo Gazzanelli3 (1 Ist. Istol. & Anal. Lab., Facolà Sci. MFN Università, Via E. Zeppi, 61029 Urbino, Italy; 2 Div. Ostet. & Ginecol., 3 Lab. Anal., Ospedale Civile, Urbino, Italy; *author for correspondence: fax 39-722-322370, e-mail mannello@bio.uniurb.it)

Prostate-specific antigen (PSA), first identified in 1970 [1], is a serine protease widely used for the early detection
and monitoring of prostate cancer [2]. Although previously thought to be produced exclusively by the epithelial cells of the prostate, PSA at present is considered a widespread biochemical marker present in many nonprostatic tissues and fluids [3]. Studies indicate that the PSA expression in both physiological and pathological conditions is not organ- or sex-specific, but rather a steroid hormone-mediated response [4]. In 1985 PSA immunoreactivity was demonstrated in urine, even though the source of urinary PSA appeared to be a local production rather than the renal filtering of serum PSA into urine [5, 6].

Considering the recent studies on PSA immunoreactivity in normal and tumor kidney tissues [7, 8], we undertook the present study on PSA content in urine samples (in particular on the first 5 mL of voided urine) collected from 50 healthy female volunteers, ages 25–39 years (mean 31 ± 3). All of these women did not have sexual intercourse for at least 1 or 2 weeks before the sample collection. The urine specimens were centrifuged at 800g for 10 min at 4 °C and the supernatants immediately stored at −30 °C until processed. Blood samples were also drawn and, after clotting, were centrifuged at 500g for 5 min at 4 °C and stored at −30 °C until assay. Another 50 healthy women, ages 27–41 years (mean 34 ± 4), had been taking for at least the preceding 6 months a widely prescribed estrogen-progestin oral contraceptive (Milvane®; Schering), containing both gestoden (as a progestin) and ethinylestradiol in each tablet.

The PSA determinations were carried out with two commercially available kits: a solid-phase two-site IRMA (PSA-RIACT™; CIS Bio International) and a microparticle enzyme immunoassay (MEIA; IMX® from Abbott Labs.). The procedures of the PSA determinations, performed according to manufacturers' recommendations, were described in detail elsewhere [9]. Results, expressed as mean ± SE, were considered statistically significant when P < 0.01. All statistical analyses were performed by using the StatView v.4.1 package (Abacus Concepts) on a Macintosh Power PC (Apple Computers). The present work was carried out in accordance to the ethical standard of the Helsinki Declaration of 1975, as revised in 1983.

Because the pH seemed to influence the measured PSA concentration as well as the ionic strength [2], we excluded urines with a pH <6.0 and >7.0; occasionally, we diluted the urine samples to optimize the conditions for the immunological reaction. To exclude possible matrix effects in the PSA assays (from the presence of divalent cations, proteins, and bilirubin), we performed dilutions of urines having high PSA content. The relation between PSA content and dilution showed good linearity (y = −0.139 + 78.6x; r = 0.962), confirming that other urinary constituents do not affect the assays' performance. Comparing the urinary PSA values obtained with PSA-RIACT (y) and IMx-PSA (x) gave a regression equation of y = 1.11x − 0.29 (r = 0.983, P < 0.001).

In agreement with recent literature [10], all serum samples examined contained PSA concentrations <0.06 μg/L and showed no statistically significant difference between the control women and the group who had been taking hormonal contraceptives. Of the 50 patients examined for each group, 80% of healthy control women contained detectable amounts of urinary PSA, with a median value of 0.035 μg/L and a mean ± SE of 0.038 ± 0.005 μg/L (range 0.02–0.15 μg/L), whereas 92% of the women taking hormonal contraceptives were positive for urinary PSA (median 0.451 μg/L, mean 0.521 ± 0.05 μg/L, range 0.09–1.239 μg/L)—a difference that was significant (t = −8.685, P < 0.0001). In the contraceptive takers, the mean PSA concentration in urine samples was higher in first voided urine than in serum (t = −9.924, P < 0.0001); no statistically significant difference was found between serum and urine samples in healthy control women (t = −2.296, P = 0.0467), even though 8% (n = 4) of the voided urines from the contraceptive takers and 20% (n = 10) of the control women’s urines were PSA-negative. No statistically significant correlation between PSA concentrations and the women’s ages was found, even after log-transformation. Moreover, we found no significant correlation with serum PSA values in any of our urine tests.

Western blot analysis (with an anti-human PSA monoclonal antibody supplied by Dako) of the urines detected a major 33-kDa band corresponding to free PSA and occasionally a minor band of 100 kDa for α1-antichymotrypsin-bound PSA, with no additional spurrious bands (data not shown).

Although previous reports demonstrated that PSA is present in undetectable or very low concentrations in nephrostomy urine specimens [6], recent data have revealed immunoreactive PSA in kidney tumor tissue extracts and sera of patients with renal cell carcinoma [7, 11]. Current opinion, however, is that the human kidney does not play a significant role in eliminating (by filtration or secretion) PSA from serum into the urine, as has been confirmed by the absence of renal PSA clearance in normal and hemodialysis conditions [6, 14–16]. The source of urinary PSA is probably a local production (through the periurethral Skene’s glands, the kidney and urachal structures, and the urothelium lining the bladder neck and trigone) [8, 12, 13]. Moreover, the androgen control of extraprostatic production/secretion of female PSA into the urine [17] may represent another possible molecular mechanism of enhanced urinary PSA expression, related to the reported modulation of this serine protease by several steroid hormones [4, 9, 18, 19].

To our knowledge (after a careful review of literature), this is the first report concerning the oral contraceptive-induced expression of PSA in female urine at quantities measurable by commercial methods. The detectable amounts of PSA in first-voided urine samples give further evidence of the distinct presence of this serine protease in nonprostatic sources, even though its biological effects and physiological role are currently unknown.
References


Normal and mutant DNA stock solution concentrations were equalized after spectrophotometric quantitation, and four dilutions of mutant: normal DNA were tested: 1:V:FT, 1:10, 1:100V:FT, and 1:100. PCR constituents were as described previously [3], except that dimethyl sulfoxide (BDH), 10 mL/L, replaced 0.05% W1. The sense oligonucleotide used to force a TaqI restriction site in the normal sequence. Following an established principle [4], the addition of TaqI to the PCR reaction during the early cycles of amplification destroys the normal-strand templates containing the forced TaqI site of the CpG-PCR and leaves the mutant strands unrestricted for selective amplification in subsequent cycles. We have demonstrated the technique by using a CpG mutation in the LDL receptor gene, E80K [5]. E80K heterozygotes were identified in a large-scale scan of FH probands for LDL receptor mutations previously undertaken in this laboratory [6].