A population study of fasting time and serum prostate-specific antigen (PSA) level

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Prostate cancer is one of the most common cancers in men. Traditional screening and diagnostic methods include digital rectal examinations (DREs), biopsies and serum prostate-specific antigen (PSA) tests, with the latter being the more popular. PSA is a biomarker for prostate cancer; however, it is highly sensitive to external factors as well as other prostate diseases. As such, the reliability of the serum PSA level as a sole screening and diagnostic tool for prostate cancer is controversial. Recently, it has been shown that fasting extremes can affect concentrations of serum chemistry analytes, thus raising the question of whether or not fasting has an effect on the highly sensitive PSA biomarker. Patients testing for serum PSA levels are often concomitantly submitting to other tests that require fasting, subjecting certain patients to a fasting PSA level while others not. The objective of this study was to investigate whether this discrepancy in fasting state translates into an effect on serum PSA levels. Serum PSA levels and fasting time records for 157 276 men who underwent testing at Calgary Laboratory Services (CLS; Calgary, Alberta, Canada) between 01 January 2010 and 31 March 2013 were accessed. Linear regression models of mean PSA levels and fasting times revealed a statistically important relationship at certain fasting times. Applying a dynamic mathematical model to explore the clinical effect of fasting suggests minimal impact on serum PSA result interpretation. Thus, patients can be tested for serum PSA levels regardless of their fasting state.

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INTRODUCTION

Prostate cancer is the most common form of cancer diagnosed in Canadian men. The Canadian Cancer Society estimates that in the year 2013, there will be 23 569 new cases of prostate cancer. As such, discovering and developing novel biomarkers for prostate cancer screening are of utmost importance. Traditionally, digital rectal examination (DRE) has been the preferred assessment method for prostate cancer. In the 1980s, the serum prostate-specific antigen (PSA) test was introduced as an additional method for screening, diagnosing and monitoring treatment of patients with prostate cancer. PSA testing was not highly utilized in Canada until 1990. As a result of its increased utilization, the incidence rate of prostate cancer detection increased rapidly and peaked in 1993. Serum PSA testing is currently one of three common procedures: DRE, serum PSA test and transrectal ultrasound-guided biopsy, used to screen and diagnose prostate cancer.

Serum PSA testing has, however, courted controversy because of its low specificity. Despite its shortcomings, PSA is still the most common and preferred screening method within the male population due to its convenience and accessibility. North American prostate cancer screening guidelines recommend that men with a family history of prostate cancer or of African descent should begin screening at 40 years of age. For the rest of the male population, screening should commence at the age of 50 years. Currently, physicians are responsible for informing patients of the availability of the different prostate cancer screening options and their associated risks to assist the patient in making informed screening decisions. For prostate cancer screening, it is advocated that a DRE is performed in conjunction with serum PSA testing. Serum PSA levels, however, are highly sensitive and can be easily altered by prostate stimuli. PSA is a glycoprotein produced by prostate epithelial cells that line the ducts and acini of the prostate. Any type of stimuli that causes a disruption of the innate glandular architecture of the prostate can facilitate PSA to enter systemic circulation, which leads to increased levels. Apart from physical stimulation of the prostate, other conditions that elevate serum PSA concentrations from baseline include: benign prostatic hyperplasia (BPH), prostatitis, urethral instrumentation, prostate biopsy and prostate cancer. Other causes of PSA elevation are vigorous DRE and recent ejaculation.

Since the levels of serum PSA are highly responsive to its environment, we would like to investigate if diagnostic testing conditions, specifically fasting, can affect PSA results. Fasting is not required for serum PSA testing; however, serum PSA tests are often performed in combination with other diagnostic tests that do require fasting. Other common laboratory tests that have been examined for the relevance of fasting time have shown that at fasting extremes (such as less than 1 h or over 12 h) the effects of hemodilution/hemoconcentration are observed. A previous limited study by Tuncel et al. examined fasting time and serum PSA levels. In their study, 80 patients were asked to fast between 12 am and 8 am, after...
which they were provided breakfast and lunch. Serum samples were obtained periodically pre- and post-meals to monitor its effect on serum PSA levels.\textsuperscript{14} They reported that there was no difference between pre- and post-prandial serum PSA levels.\textsuperscript{15} Their findings however, are limited by a small sample size and limited fasting times examined.\textsuperscript{18} Thus, the objective of our study is to revisit this topic by examining a wider range of fasting times and a larger data set: the community population of Calgary, Alberta. We hypothesize that fasting should not affect serum PSA levels except at fasting extremes where the effects of hemodilution/hemoconcentration may play a role.

MATERIALS AND METHODS

Ethics statement

This study was approved by the University of Calgary Conjoint Health Review Ethics Board (ID: E-25060).

Study population and data sources

This study was conducted using secondary data from the Laboratory Information System of Calgary Laboratory Services (CLS). CLS is the sole provider of laboratory services for Calgary, Alberta and its surrounding areas (approximately 1.4 x 10\textsuperscript{6} people). The study population consisted of all men who underwent PSA testing at CLS between 01 January 2010 and 31 March 2013. All PSA tests were performed as part of routine patient care and were analyzed at CLS using a Roche Modular analyzer. Fasting time (hours) was obtained through patient self-reporting at the time of testing. In our analysis, fasting time was categorized into hourly intervals from 1 to 16. All reported fasting hours were rounded up to the next integral value. All individuals with a fasting time beyond 16 h were grouped into the 16 h category. PSA records were excluded for female patients, probable cancer patients (serum PSA level >20 µg l\textsuperscript{-1}) and if a fasting time was not recorded. As age-specific reference ranges are only commonly reported for men between the ages of 40 and 79 years, any records outside of this range were also removed. Finally, to avoid pseudoreplication we considered only the first PSA result from each patient. This resulted in a total of 157 276 PSA records and paired fasting times.

Statistical analysis

Fasting time for laboratory tests has been shown to vary as a function of age and sex.\textsuperscript{19,20} As our study only consists of males, the effects of sex on fasting time are negated. The data were fitted to a linear regression model with serum PSA measurement as the dependent variable and fasting time (in hours) and age (10-year cohorts) as independent variables. Estimated marginal means were calculated based on 95% confidence intervals at each fasting time period, with age held constant at the group mean. Statistical significance was assessed through pairwise comparison of fasting hours and its corresponding serum PSA levels using the Tukey post hoc test. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software (Version 20 for Windows, SPSS Inc., Chicago, IL, USA).

Dynamic mathematical model

To explore the impact of fasting time on serum PSA levels through a clinical perspective, a dynamic mathematical model was developed to calculate the percentage of individuals expected to experience a change in PSA diagnostic interpretation (e.g. normal vs abnormal result) based on variations in fasting time. In this model, data were first parsed into age cohorts: 40–49, 50–59, 60–69 and 70 to 79-years-old, and the number of PSA values above the normal range (2.5, 3.5, 4.5 and 6.5 µg l\textsuperscript{-1} respectively, based on age-dependent cutoffs) were calculated. In order to preclude ethnicity-related serum PSA effects, and since ethnicity data are not collected during PSA testing, only age-specific reference ranges for Caucasian males were used. PSA cutoff levels for an abnormal test were based on the Alberta Toward Optimized Practice 2010 guidelines.\textsuperscript{21} We included only paired fasting time intervals that showed a statistically significant (P < 0.05) difference in mean PSA value (see Results section). For each of these paired fasting times, we calculated the expected difference in serum PSA value if the patient had fasted for the other period of time. These calculations were performed by adding (or subtracting) the differences in estimated marginal means to each individual result. For example, results for patients with a 4 h fasting interval were modified to simulate a 9 h fasting interval by subtracting 0.367 from every value (the mean difference attributable to fasting time; Table 1). Following this operation, we recalculated the number of abnormal PSA results for each simulated change in fasting time. These are presented as a projected percent change in abnormal results due to a change in fasting time and allow us to comment on the anticipated clinical significance of variations in fasting time.

RESULTS

A total of 157 276 PSA records were accessed from our Laboratory Information System. The mean PSA level for all records was 1.35 µg l\textsuperscript{-1}. The average age of patients tested was 57.0 years of age. The average fasting time of all records was 12.7 h with a minimum and maximum fasting time of 1 and 16 h, respectively. Mean PSA levels at different fasting times are shown in Table 2. Table 3 illustrates the dependence of each independent variable (fasting time and age) on the dependent variable (serum PSA levels). These results indicate that fasting time and age have a statistical effect on serum PSA levels.

Table 1: Dynamic mathematical model results showing modeled effects of changes in fasting time on abnormal PSA (>upper limit of normal) classification rates

| Starting fasting time (h) | Modeled fasting time (h) | Mean difference in abnormal serum PSA values (µg l\textsuperscript{-1}) | Total in fasting subgroup | Percent change (%) |
|---------------------------|--------------------------|---------------------------------------------------------------|--------------------------|-------------------|
| 1                         | 4                        | 0.346                                                        | 35                       | 796               | 1.63              |
| 9                         | 15                       | -0.199                                                       | 30                       | 697               | 0.57              |
| 9                         | 16                       | -0.199                                                       | 30                       | 697               | 0.57              |
| 10                        | 12                       | -0.048                                                       | 309                      | 8193              | 0.16              |
| 10                        | 13                       | 0.056                                                        | 309                      | 8193              | 0.16              |
| 10                        | 14                       | 0.098                                                        | 309                      | 8193              | 0.28              |
| 10                        | 15                       | 0.134                                                        | 309                      | 8193              | 0.39              |
| 10                        | 16                       | 0.134                                                        | 309                      | 8193              | 0.39              |
| 11                        | 12                       | 0.048                                                        | 611                      | 15483             | 0.10              |
| 11                        | 13                       | 0.056                                                        | 611                      | 15483             | 0.12              |
| 11                        | 14                       | 0.098                                                        | 611                      | 15483             | 0.26              |
| 11                        | 15                       | 0.134                                                        | 611                      | 15483             | 0.37              |
| 11                        | 16                       | 0.134                                                        | 611                      | 15483             | 0.37              |
| 12                        | 14                       | 0.050                                                        | 1841                     | 44265             | 0.18              |
| 12                        | 15                       | -0.087                                                       | 1841                     | 44265             | 0.28              |
| 13                        | 16                       | -0.086                                                       | 1841                     | 44265             | 0.28              |
| 13                        | 14                       | -0.042                                                       | 1469                     | 35040             | 0.19              |
| 13                        | 15                       | -0.079                                                       | 1469                     | 35040             | 0.25              |
| 16                        | 13                       | -0.078                                                       | 1469                     | 35040             | 0.25              |
| 4                         | 9                        | 0.367                                                        | 26                       | 374               | -1.07             |
| 4                         | 10                       | 0.347                                                        | 26                       | 374               | -0.80             |
| 4                         | 11                       | 0.302                                                        | 26                       | 374               | -0.80             |

PSA: prostate-specific antigen
Table 4 shows the specific fasting hours that have an effect on serum PSA levels. At fasting times (>8 h), serum PSA levels are generally distinct from non-fasting times (1–8 h).

Table 2: Serum PSA levels by fasting time after adjustment for the effect of age

| Fasting time (h) | Mean PSA (ng ml⁻¹) | Standard Error | 95% CI |
|-----------------|--------------------|----------------|--------|
|                 | Lower bound        | Upper bound    |        |
| 1               | 1.521              | 0.060          | 1.402  | 1.639 |
| 2               | 1.394              | 0.080          | 1.238  | 1.550 |
| 3               | 1.587              | 0.078          | 1.434  | 1.740 |
| 4               | 1.790              | 0.082          | 1.628  | 1.951 |
| 5               | 1.535              | 0.096          | 1.346  | 1.724 |
| 6               | 1.474              | 0.113          | 1.253  | 1.695 |
| 7               | 1.379              | 0.145          | 1.095  | 1.663 |
| 8               | 1.521              | 0.106          | 1.313  | 1.729 |
| 9               | 1.373              | 0.067          | 1.242  | 1.504 |
| 10              | 1.460              | 0.019          | 1.423  | 1.498 |
| 11              | 1.467              | 0.013          | 1.441  | 1.494 |
| 12              | 1.496              | 0.008          | 1.480  | 1.511 |
| 13              | 1.479              | 0.008          | 1.463  | 1.496 |
| 14              | 1.504              | 0.010          | 1.485  | 1.523 |
| 15              | 1.540              | 0.013          | 1.514  | 1.566 |
| 16              | 1.566              | 0.014          | 1.538  | 1.594 |

CI: confidence interval, PSA: prostate-specific antigen.

DISCUSSION

Although a number of variations in fasting time yielded statistically important results, we were also interested in assessing the potential clinical significance of these changes. A Dynamic Mathematical Model as described earlier showed that an increased fasting time would generally result in a modest increase in the number of patients with abnormal PSA values (Table 1 and 4). The overall changes in classification (normal to abnormal or vice versa) ranges from −1.07% to 1.63% (mean = 0.19%; Table 1).

Table 3: Univariate analysis of variance showing the effect of fasting time on serum PSA value

| Source                  | Type III sum of squares | df | Mean square | F       | Significance |
|-------------------------|-------------------------|----|-------------|---------|--------------|
| Corrected model         | 28 621.3⁣               | 63 | 454.3       | 216.6   | 0.000        |
| Intercept               | 14 702.4⁣               | 1  | 14 702.4⁣   | 7099.0  | 0.000        |
| Fasting time (h)        | 139.8                   | 15 | 9.3         | 4.4     | 0.000        |
| Age group               | 1463.4                  | 3  | 487.8       | 232.5   | 0.000        |
| Fasting time age group  | 153.4                   | 45 | 3.4         | 1.6     | 0.005        |
| Error                   | 329 774.2⁣             | 157212 | 2.1     |         |              |
| Total                   | 647 006.0⁣             | 157276 |        |         |              |
| Corrected total         | 358 395.5⁣             | 157275 |        |         |              |

df: degrees of freedom; PSA: prostate-specific antigen. *R² = 0.080 (adjusted R² = 0.079)

Table 4: Multiple mean comparison between fasting hours and serum PSA values adjusting for age effects

| Fasting time (h) | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  |
|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1               | 0   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 2               | NS  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 3               | NS  | NS  | 0   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4               | −0.346 | NS | NS  | 0   |     |     |     |     |     |     |     |     |     |     |     |     |
| 5               | NS  | NS  | NS  | NS  | 0   |     |     |     |     |     |     |     |     |     |     |     |
| 6               | NS  | NS  | NS  | NS  | NS  | 0   |     |     |     |     |     |     |     |     |     |     |
| 7               | NS  | NS  | NS  | NS  | NS  | NS  | 0   |     |     |     |     |     |     |     |     |     |
| 8               | NS  | NS  | NS  | NS  | NS  | NS  | NS  | 0   |     |     |     |     |     |     |     |     |
| 9               | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | 0   |     |     |     |     |     |     |     |
| 10              | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | 0   |     |     |     |     |     |     |
| 11              | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | 0   |     |     |     |     |     |
| 12              | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | 0   |     |     |     |     |
| 13              | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | 0   |     |     |     |
| 14              | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | 0   |     |     |
| 15              | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | 0   |     |
| 16              | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | NS  | 0   |     |

NS: not significant; PSA: prostate-specific antigen. Only mean differences which were statistically significant at P < 0.05 are shown.
is a general increasing trend. A notable feature is the peak in serum PSA level observed at 4 h of fasting (Figure 1). We considered collection time (as opposed to fasting interval) as a possible confounding factor which could have biased the observed increase in PSA levels at 4 h of fasting. To remove this possible bias we reperformed the analysis with collection time as an additional variable. The overall trend, however, was unchanged (data not shown). A potential reason for this increase may instead be related to diurnal variation in serum PSA levels. It has been suggested that PSA expression is controlled by hormones and in particular, in cases of patients with BPH, testosterone has been shown to directly influence serum PSA levels and exhibits a well-established circadian rhythm. Thus, it is of interest to examine if serum PSA follows the same diurnal variation as testosterone. Diurnal variation of serum PSA has been previously examined and is controversial as some studies suggest its existence, while others disagree. A major potential shortcoming of these studies is their small sample size; the study with the largest sample contained 44 men.

The second region encompassing fasting hours of 10–16 shows a gradual increase in mean PSA values (Figure 1). Analyzing the mean differences in PSA values between the different fasting times shows that at fasting extremes (1 h or less or 9 or more hours), PSA values are significantly different (Table 4). This is similar to observations for other chemistry analytes. Since the trend past 9 h is an increasing one (Figure 1), it should be noted that the longer the fasting time the greater the deviation in detected PSA values. Our statistical analysis suggests that the optimal time to obtain an accurate PSA level is fasting for 7–9 h, which avoids the increased PSA levels observed with fasting extremes as well as possible diurnal rhythm variations that may exist in shorter fasting times. It should be noted that the larger standard deviations observed in serum PSA levels for 1–9 h of fasting compared to 10 or more hours (Figure 1) are likely the result of smaller sample sizes for these time periods (160–800 men).

To explore the clinical effect of fasting time on serum PSA levels, a dynamic mathematical model was developed. Simulating the changes that would occur in PSA values if fasting times were altered revealed that the percentage change in the number of abnormal PSA values can range from −1.07% to 1.63% (Table 1). Therefore, despite the statistical significance of PSA variations with fasting time, our model predicts that this would translate into minimal clinical effect.

There were several limitations in this study that were unaccounted for in our analysis. The first is the possibility of patient recall error regarding fasting time. Another limitation that should be noted is our use of secondary data on individuals and not a random sample of individuals taken from the general population. As such, these findings can only be interpreted as representative of individuals presenting for testing and may be a biased representation of the overall population.

In conclusion, certain fasting states (1 or less hours or 4 h or 9 or more hours) have a statistically significant effect on serum PSA levels which substantiates both our hypothesis and other previous studies that have observed deviations in concentrations of serum analytes at fasting extremes. However, when translating this to an anticipated clinical effect, we suggest this would have a minimal impact on overall classification rate of normal versus abnormal results. It appears that patients can be tested for serum PSA levels regardless of their fasting state. Future research should focus on identifying additional biomarkers and/or chemical analytes for prostate cancer that are robust and less sensitive to external stimuli. This will help reduce the ambiguity and increase the reliability associated with prostate cancer testing.

AUTHOR CONTRIBUTIONS
CKL, MG, JAZ and CTN participated in the conception and design of the study. JAZ acquired all relevant data. CKL, MG and CTN critically interpreted and analyzed the data. MG performed all statistical analyses. CKL and CTN drafted the manuscript. CKL, MG, JAZ and CTN all participated in the critical revision of the manuscript for intellectual content. CTN supervised the study. All authors read and approved the manuscript.

COMPETING INTERESTS
All authors declare no competing interests.

REFERENCES
1. Prostate Cancer. Available from: http://www.phac-aspc.gc.ca/cd-mc/cancer/prostate_cancer-cancer prostate-eng [Last accessed on 2013 Aug 22].
2. Canadian Cancer Statistics 2013. Available from: http://www.cancer.ca/en/cancer-information/cancer-101/canadian-cancer-statistics-publication/?region=ab [Last accessed on 2013 Aug 22].
3. Amling CL. Prostate-specific antigen and detection of prostate cancer: what have we learned and what should we recommend for screening? Curr Treat Options Oncol 2006; 7: 337–45.
4. Parekh DJ, Ankerst DP, Toyer D, Srinavasta S, Thompson IM. Biomarkers for prostate cancer detection. J Urol 2007; 178: 2525–9.
5. Thompson IM, Ankerst DP. Prostate-specific antigen levels in the early detection of prostate cancer. CMAJ 2007; 176: 1853–8.
6. Kramer BS, Brown ML, Prorok PC, Potosky AL, Gohagan JK. Prostate cancer screening: what we know and what we need to know. Ann Intern Med 1993; 119: 914–23.
7. Bunting PS, Goel V, Williams JJ, Iscoe NA. Prostate-specific antigen testing in Ontario: reasons for testing patients without diagnosed prostate cancer. CMAJ 1999; 160: 70–5.
8. Levy I. Trends in incidence and mortality of prostate cancer in Canada. Chronic Dis Can 1995; 16: S2–6.
9. McGregor SE, Bryant HE, Brant RF, Corbett PJ. Prevalence of PSA testing and effect of clinical indications on patterns of PSA testing in a population-based sample of Alberta men. Chronic Dis Can 2002; 23: 111–9.
10. Ilic D, Neuberger MM, Djulbegovic M, Dahm P. Screening for prostate cancer. Cochrane Database Syst Rev 2013; 1: CD004720.
11. Screening and Early Diagnosis of Prostate Cancer. Available from: http://www.topalbertadepctors.org/cpgs/?sid=2&cpg_cats=26 [Last accessed on 2013 Aug 22].
12. Pearce A, Newcomb C, Huisain S. Recommendations by Canadian urologists and radiation oncologists for the treatment of clinically localized prostate cancer. Can Urol Assoc J 2008; 2: 197–203.
13. Iwaza JI, Klotz L, Siemens DR, Kassouf W, So A, et al. Prostate cancer screening: Canadian guidelines 2011. Can Urol Assoc J 2011; 5: 235–40.
14. Bastani B, Behzadi AH, Abbasi MA, Hassanloo J, Ansari K, et al. The effect of cystoscopy on PSA levels in patients with urologic diseases. Int J Neptrol Urol 2010; 2: 251–4.
15. Tchetgen MB, Song JT, Strangerman M, Jacobsen SJ, Oesterling JE. Ejaculation increases the serum prostate-specific antigen concentration. Urology 1996; 47: 511–6.
of a light meal on routine haematological tests. *Blood Transfus* 2010; 8: 94–9.

17 Siddhu D, Naugler C. Fasting time and lipid levels in a community-based population: a cross-sectional study. *Arch Intern Med* 2012; 172: 1707–10.

18 Tuncel A, Aksut H, Agras K, Tekdogan U, Ener K, et al. Is serum prostate-specific antigen level affected by fasting and nonfasting? *Urology* 2005; 66: 105–7.

19 Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. *Circulation* 2008; 118: 2047–56.

20 Lima-Oliveira G, Salvagno GL, Lippi G, Gelati M, Montagnana M, et al. Influence of a regular, standardized meal on clinical chemistry analytes. *Ann Lab Med* 2012; 32: 250–6.

21 De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, et al. European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force of European and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. *Eur Heart J* 2003; 24: 1601–10.

22 National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; 106: 3143–421.

23 Csapo Z, Brand K, Walther R, Fokas K. Comparative experimental study of the serum prostate specific antigen and prostatic acid phosphatase in serially transplantable human prostatic carcinoma lines in nude mice. *J Urol* 1988; 140: 1032–8.

24 Weber JP, Oesterling JE, Peters CA, Partin AW, Chan DW, et al. The influence of reversible androgen deprivation on serum prostate-specific antigen levels in men with benign prostatic hyperplasia. *J Urol* 1989; 141: 987–92.

25 Rowe PH, Lincoln GA, Racey PA, Lehane J, Stephenson MJ, et al. Temporal variations of testosterone levels in the peripheral blood plasma of men. *J Endocrinol* 1974; 61: 63–73.

26 Akimoto S, Masai M, Shimazaki J. Relationship between diurnal rhythm of serum testosterone and two prostate markers (PSA and PAP) in untreated prostate cancer. *Urology* 1994; 43: 337–41.

27 Dejter SW Jr, Martin JS, McPherson RA, Lynch JH. Daily variability in human serum prostate-specific antigen and prostatic acid phosphatase: a comparative evaluation. *Urology* 1988; 32: 286–92.

28 el-Shirbiny AM, Nilson T, Pawar HN. Serum prostate-specific antigen: hourly change/24 hours compared with prostatic acid phosphatase. *Urology* 1990; 35: 88–92.

29 Mannini D, Mauer P, Aiello E, Corrado G, Vecchi F, et al. Spontaneous circadian fluctuations of prostate specific antigen and prostatic acid phosphatase serum activities in patients with prostatic cancer. *Urol Res* 1988; 16: 9–12.

30 Tekin A, Atsu N, Ozen H. Daily variability of serum prostate-specific antigen in men over 50 years of age. *Int Urol Nephrol* 2001; 33: 641–4.