Total testosterone in young men is more closely associated than free testosterone with prostate cancer disparities

Louis Calistro Alvarado

Ther Adv Urol

(2011) 3(3) 99–106

DOI: 10.1177/

1756287211405706

© The Author(s), 2011.
Reprints and permissions:
[http://www.sagepub.co.uk/
journalsPermissions.nav](http://www.sagepub.co.uk/journalsPermissions.nav)

Abstract:

Introduction: Early adulthood has been suggested as the most relevant time to determine the influence of testosterone on prostate carcinogenesis. For a more detailed assessment of this hypothesis, the present study examined whether serum total or free testosterone in young men was more closely associated with prostate cancer disparities.

Methods: A literature search was conducted for studies that reported both total and free testosterone levels for population samples of young men, along with prostate cancer incidences for the populations from which study populations were sampled. A previously developed analytical method was used to standardize the hormone levels of 19 population samples gathered from nine studies, and these standardized values were compared with disparities in prostate cancer incidence.

Results: Population differences in total testosterone levels were significantly associated with prostate cancer disparities, $r=0.833$, $p=0.001$, as were population differences in free testosterone, $r=0.661$, $p=0.027$. After controlling for age differences, total and free testosterone remained associated with prostate cancer disparities, partial $r=0.888$, $p<0.001$, and partial $r=0.657$, $p=0.039$, respectively. A marginally significant difference existed in the strength of relationships between total and free testosterone with respect to prostate cancer disparities, with total testosterone exhibiting a stronger association, $T_2=1.573$, $p=0.077$.

Conclusions: Across analyses, total testosterone demonstrated a more robust relationship than free testosterone with cancer disparities, which may suggest that total testosterone is the more sensitive biomarker for evaluating androgenic stimulation of the prostate gland.

Keywords: age-related testosterone decline, hormone-dependent disease, prostate cancer disparities, total and free testosterone

Introduction

The steroid hormone testosterone is fundamental to growth and regulation of the prostate gland [Hsing, 2001; O'Malley, 1971]. Testosterone is delivered to the prostate through circulation, where it is largely metabolized into a more potent androgenic form dihydrotestosterone, which promotes cellular proliferation of prostatic epithelium [Hsing, 2001]. Although androgens are often implicated in the development of prostate cancer, clinical evidence for this association has been mixed [Platz and Giovannucci, 2004; Grönberg, 2003; Hsing, 2001]. An extensive meta-analysis of 18 prospective studies of men with prostate carcinoma compared with similarly

aged controls found no significant relationship between levels of endogenous sex hormones and prostate cancer risk [Roddam *et al.* 2008]. However, studies included in this analysis measured sex steroid hormones in late middle-aged and elderly men when intra- and inter-population variation in testosterone levels is least detectable, such that later life would not be the most appropriate period for comparative analyses of men's testosterone profiles [Alvarado, 2010; Grönberg, 2003]. Previous research has found a relationship between testosterone levels during early adulthood and prostate cancer risk, suggesting that testosterone production in young men more appropriately captures relative differences in

Correspondence to:
Louis Calistro Alvarado
Department of
Anthropology, MSC01-
1040, 1 University of New
Mexico, Albuquerque, NM
87131, USA
lalvarad@unm.edu

cumulative hormone exposure [Alvarado, 2010]. Indeed, levels of sex steroids decline at later ages in both men and women, and ecological studies testing the relationship between young adult steroid concentrations and the risk of hormone-sensitive cancers have reported strong associations for both sexes [Alvarado, 2010; Jasińska and Thune, 2001; Jasińska *et al.* 2000].

Total and free testosterone measures are frequently reported in the epidemiological and oncological literature, but the functional significance of these different measures is rarely investigated. Free steroids are lipid-soluble molecules that diffuse across cell membranes to interact with specific endocellular receptors, but must bind to carrier proteins for transport in peripheral circulation. Most serum testosterone is bound to two primary carrier proteins, sex hormone-binding globulin (SHBG) and albumin, with only a small fraction of testosterone circulating in free form. SHBG binds testosterone with high affinity, rendering the molecule biologically inactive. In contrast, the binding affinity of albumin is several orders of magnitude less, and albumin-bound testosterone is able to dissociate from its bond for entry into target tissues [Matsumoto, 2001].

Total testosterone is a cumulative measure of circulating testosterone that includes both diffusible and protein-bound forms, whereas free testosterone is often regarded as a direct assessment of physiological availability [Rosner *et al.* 2007; Matsumoto, 2001]. Two methods widely used for determining free testosterone levels within circulation are radioimmunoassay (RIA) of an analog ligand to directly estimate free testosterone (direct RIA), and the calculation of free testosterone from total testosterone and the concentration of immunoassayed plasma proteins (calculated free testosterone) [Rosner *et al.* 2007; Vermeulen *et al.* 1999]. Experimental comparisons of laboratory methods have shown that calculated free testosterone produces reliable hormone values; however, direct RIA of free testosterone was found to be an inaccurate and inconsistent procedure [Rosner *et al.* 2007; Vermeulen *et al.* 1999; Winters *et al.* 1998], leading some researchers to question the continued use of this assay [Rosner, 2001].

In addition to free testosterone, estimations of physiologically available testosterone are often reported in the literature, most notably,

'bioavailable' testosterone and free androgen index (FAI). FAI is a unitless quotient representing the concentration of total testosterone/the concentration of SHBG [Rosner *et al.* 2007]. FAI has repeatedly shown poor correlations with free testosterone measures, especially for men [Morris *et al.* 2004; Kapoor *et al.* 1993]. In contrast, bioavailable testosterone is the combined portion of free and albumin-bound testosterone and has shown strong correlations with other validated measures of free testosterone [Rosner *et al.* 2007; Morris *et al.* 2004; Morely *et al.* 2002; Vermeulen *et al.* 1999].

A recent meta-analysis of studies reporting hormone levels for population samples of young men in relation to prostate cancer disparities found a positive, significant association between men's total testosterone levels and prostate cancer incidence [Alvarado, 2010]. It seems reasonable to expect that free testosterone would demonstrate a more robust relationship with cancer disparities than would total testosterone, given that free testosterone is able to diffuse into prostatic cells and support cell proliferation [Feldman and Feldman, 2001]. To the contrary, some studies have found significantly higher total testosterone levels in men from populations with an increased risk of prostate cancer while failing to find this relationship using free form measures of testosterone [Kehinde *et al.* 2006; Winters *et al.* 2001; Santner *et al.* 1998]. As such, the purpose of this examination was to formally test whether disparities in prostate cancer risk are more closely associated with young men's total or free testosterone measures in studies using assay procedures that are generally considered acceptable for healthy adult males.

Methods

A literature review was completed for studies reporting testosterone levels of presumably healthy men from population samples with a mean or median age of less than 39 years, so that the influence of age-related testosterone decline was reduced. The author searched PubMed for articles using the terms: androgen, epidemiology, prostate cancer, sex hormones, steroids, testosterone, ethnicity, and race. In addition, a manual search of bibliographies from the retrieved articles was completed. Some hormone datasets were reproduced within multiple publications, but only a single representation of each was used. Only studies reporting both

total and free testosterone measures were included in this analysis.

Nine studies reporting testosterone values for 19 population samples were gleaned from the published literature. Each study compared two or more groups of men. Population samples were generally apportioned according to ethnicity and geographic residence: Americans of African, Caucasian, and Mexican ancestry from several regions within the United States as well as men from China, Hong Kong, Japan, New Zealand, South Korea, and Sweden. Age-standardized rates of prostate cancer incidence were collected from the regional population in which study samples were recruited. Cancer rates were gathered from a worldwide collection of cancer registries compiled within the Cancer Incidence in Five Continents Vol. IX database [Curado *et al.* 2007]. Every effort was made to match cancer incidences with the corresponding location and ethnicity of study samples, which was particularly important for US samples considering the extensive diversity of the American populace. See Table 1 for a descriptive summary of included studies.

Of the retrieved articles that met the specified criteria, there were studies excluded because of methodological limitations. As reviewed earlier, some immunoassay procedures have recognized problems; direct RIA of serum free testosterone and FAI have been shown to produce unreliable values for men [Rosner *et al.* 2007; Vermeulen *et al.* 1999; Kapoor *et al.* 1993]. Studies employing either of these methods were eliminated from the dataset, resulting in the exclusion of two studies [Kehinde *et al.* 2006; Wright *et al.* 1995]. One other study was excluded due to inadequacies of hormone analysis procedures. In this study [Ross *et al.* 1992], newly acquired serum samples were compared with older specimens that were reanalyzed after storage for an extended period of time, which is methodologically problematic [Bolelli *et al.* 1995]. Also, hormone values from the older specimens in this study were reported within an earlier publication [Ross *et al.* 1986].

Studies included in the current analysis spanned more than two decades. Consequently, immunoassay procedures used to analyze hormone samples differed across nearly all of these studies, and a high degree of measurement variability among competing testosterone assays made

direct comparisons of hormone levels between studies impossible [Boots *et al.* 1998]. To standardize these data so that an aggregate dataset could be constructed, a comparison of ratios was calculated in which the proportional difference of mean testosterone levels between population samples within a study was compared with the proportional disparity in prostate cancer incidence for populations from which study populations were sampled. This analytical method was developed previously [Alvarado, 2010] and can be expressed formally as follows:

$$\frac{\text{Study} \times \text{population sample}_1 \text{ testosterone}}{\text{Study} \times \text{population sample}_2 \text{ testosterone}}$$

in relation to

$$\frac{\text{Population}_1 \text{ prostate cancer}}{\text{Population}_2 \text{ prostate cancer}}$$

Each value within this dataset represents a pairwise comparison of population samples from within a study. If a value were graphically represented, the data point would reference the proportional dissimilarity in testosterone between population samples on the *x*-axis, relative to the proportional disparity of prostate cancer on the *y*-axis. See Table 2 for calculations.

Population comparisons among 19 samples were analyzed, but employing within-study contrasts as the basic unit of analysis produced a dataset consisting of 11 values. Pearson's correlation coefficient was used to test for an association between population differences in testosterone levels and prostate cancer incidences. Population samples included in this dataset were relatively young, but it was still conceivable that age differences could exert an influence on subsequent analyses. See Table 1 for the ages of population samples. To address this concern, an average of the reported ages for each population comparison was calculated (or an average of mid-points when age ranges were reported), and population disparities of testosterone levels and prostate cancer were reanalyzed using partial correlation to control for age. All comparative population data were log transformed to better adhere to the parametric assumptions of Pearson's and partial correlation. Tests were two-tailed.

Williams T_2 statistic [Steiger, 1980; Williams, 1959] was used to determine whether a significant difference existed in the relative strength of the association between total and free

Table 1. Descriptive characteristics of population samples.

Study	Age ¹	Sample Size	Ethnicity	Population	Prostate cancer incidence: region/population
Ettinger <i>et al.</i> [1997] ²	30.7	109	African-American	Birmingham, Chicago,	USA (SEER): African-American
	31.3	114	Caucasian-American	Minneapolis, Oakland: USA	Caucasian-American
Jakobsson <i>et al.</i> [2006]	18.9	122	Swedish	Göteborg, Sweden	Sweden
	26.3	74	South Korean	Incheon, South Korea	Incheon, South Korea
Lewis <i>et al.</i> [2005] ³	26.6	60	Japanese	Japan	Miyagi Prefecture, Japan
Lookingbill <i>et al.</i> [1991]	25.8	60	New Zealander	New Zealand	New Zealand
	25.9	53	Hong Kong Chinese	Hong Kong	Hong Kong
	23.7	57	Caucasian-American	Hershey, Pennsylvania, USA	Pennsylvania, USA: Caucasian
Rohrmann <i>et al.</i> [2007]	28.6	239	Mexican-American	USA national sample	USA (SEER): Hispanic White
	29.5	206	African-American		African-American
	31.8	252	Caucasian-American		Non-Hispanic White
Ross <i>et al.</i> [1986]	20.6	50	African-American	Los Angeles, USA	Los Angeles County, USA:
	19.9	50	Caucasian-American		African-American Caucasian-American
Santner <i>et al.</i> [1998] ⁴	22–27	10	Caucasian-American	Pennsylvania, USA	Pennsylvania, USA: Caucasian
Tsai <i>et al.</i> [2006]	24–39	10	Chinese	Beijing, China	Shanghai, China
	34	357	African-American	San Francisco–Oakland, USA	San Francisco Bay area, USA:
	33.5	618	Caucasian-American		African-American Caucasian-American
Winters <i>et al.</i> [2001]	19.8	23	African-American	Pittsburgh, USA	Pennsylvania, USA: African-American
	21.5	23	Caucasian-American		Caucasian-American

¹Unless age range is indicated, mean age is reported for study samples, with the exception of Rohrmann *et al.* [2007] and Tsai *et al.* [2006] for which median age is reported.

²Ettinger and colleagues reported testosterone values for urban centers in a multiregional United States sample. To approximate this population, national incidences of prostate cancer were obtained from SEER registries in CI5 IX, which are also more urban than a representative sample of the US populace [SEER, 2005].

³A nation-level incident rate of prostate cancer for Japan was not available from CI5 IX, though cancer incidences were available for some Japanese prefectures. Using incident rates from different reporting prefectures, or an average value from all prefectures, had no appreciable effect on analyses.

⁴Prostate cancer incidence for Beijing was not available. Shanghai was used to approximate an urban Chinese population. Santner and colleagues also reported testosterone levels for a study sample of Pennsylvanian men of Chinese ancestry, but an incident rate of prostate cancer was not available for this population, nor was there an obvious approximation of this to use in analyses.

testosterone in relation to prostate cancer disparities. Population comparison values were age-adjusted and log transformed for this analysis, as well. A one-tailed test was performed because of the limited statistical power of the small sample size ($n=11$), along with conservative nature of the Williams T_2 statistic.

Results

Population differences in total testosterone levels of young men were associated with population

disparities in prostate cancer incidence, $r(9)=0.833$, $p=0.001$, as were population differences in free testosterone, $r(9)=0.661$, $p=0.027$. When age differences of the study samples were controlled for, total and free testosterone levels remained significantly associated with population disparities in prostate cancer incidence, partial $r=0.888$, $df=8$, $p<0.001$, and partial $r=0.657$, $df=8$, $p=0.039$, respectively. See Figure 1 for a graphical representation of these relationships.

Table 2. Data and calculations for population samples.

Study	Proportional difference in total testosterone levels ¹	Proportional difference in free testosterone levels ²	Proportional disparity in prostate cancer incidence (per 100,000)
Ettinger <i>et al.</i> [1997]	AA/CA (ng/dl): 595/549 = 1.08	AA/CA: 152/138 (pg/ml) = 1.10	AA/CA: 190.1/116.0 = 1.64
Jakobsson <i>et al.</i> [2006]	SWE/KOR (nmol/l): 18/14.4 = 1.25	SWE/KOR: 13.4/10.8 = 1.24	SWE/KOR: 84.6/7.8 = 10.85
Lewis <i>et al.</i> [2005]	JPN/NZ (nmol/l): 30.5/29.1 = 1.04	JPN/NZ (pmol/l): 803/710 = 1.04	JPN/NZ: 22/104.4 = .21
Lookingbill <i>et al.</i> [1991]	HK/CA (umol/l): 20/19.5 = 1.03	HK/CA: 8.4/9 = .93	HK/CA: 15/110 = .14
Rohrmann <i>et al.</i> [2007] ³	MA/AA (ng/ml): 5.55/5.35 = 1.04	MA/AA (ng/ml): 1.13/1.07 = 1.06	HA/AA: 90.8/178.8 = .51
	MA/CA: 5.55/5.17 = 1.07	MA/CA: 1.13/1.04 = 1.09	HA/CA: 90.8/112.3 = .81
Ross <i>et al.</i> [1986]	AA/CA: 5.35/5.17 = 1.03	AA/CA: 107/104 = 1.03	AA/CA: 178.8/112.3 = 1.59
Santer <i>et al.</i> [1998]	AA/CA (pg/ml): 6.605/5.569 = 1.19	AA/CA (pg/ml): 166/137 = 1.21	AA/CA: 186.4/109.3 = 1.71
Tsai <i>et al.</i> [2006] ⁴	CA/CHN (ng/dl): 640/480 = 1.33	CA/CHN (ng/dl): 280/240 = 1.17	CA/CHN: 110/6.9 = 15.94
Winters <i>et al.</i> [2001]	AA/CA (ng/dl): 693.5/611.5 = 1.13	AA/CA (ng/dl): 427.75/381.75 = 1.12	AA/CA: 155.8/118 = 1.32
	AA/CA (ng/dl): 673/538 = 1.26	AA/CA (pmol/l): 215/208 = 1.03	AA/CA: 180.6/110 = 1.64

¹The original units of measurement as reported in the literature were retained so that testosterone values could be referenced.

²Jakobsson *et al.* [2006] and Lookingbill *et al.* [1991] reported bioavailable testosterone, while all other studies reported free testosterone levels.

³Rohrmann and colleagues reported testosterone values after controlling for anthropometry and lifestyle factors.

⁴Tsai and colleagues reported longitudinal data for study samples, which were divided into prostate cancer cases and controls as well as by ancestry. Testosterone values for the earliest age reported were averaged between case and control groups for each ancestral group, and these values were used in analyses.

It should be noted that the preceding analyses contained many direct population comparisons between African- and Caucasian-Americans (AA/CA). Although these comparisons were made among different regional populations within the United States, which vary greatly in their disparity in testosterone levels and prostate cancer (Table 2), there was still potential for generating a biased outcome based on an overrepresentation of AA/CA comparisons. As such, a dummy variable was created that represented whether a comparison value was an AA/CA comparison. Controlling for the influence of whether a given value in the dataset was an AA/CA comparison had little effect, and prostate cancer disparities were significantly associated with population differences in total testosterone, partial $r = 0.833$, $df = 8$, $p = 0.003$, and free testosterone, partial $r = 0.666$, $df = 8$, $p = 0.035$.

In these analyses, population differences in total testosterone consistently showed a stronger relationship than free testosterone with population disparities in prostate cancer incidence. However, a formal test was needed to conclude whether this general trend is statistically meaningful. Using Williams T_2 statistic, total testosterone demonstrated a stronger association with prostate cancer disparities than did free testosterone, though this difference was marginally significant, $T_2 = 1.573$, $p = 0.077$.

Discussion

This meta-analysis used pairwise population comparisons from within a study to account for methodological variability across hormone datasets. Population differences in young men's total and free testosterone were associated with prostate cancer disparities, and this relationship became stronger for total testosterone after controlling for age, which may reflect the influence of men's age-related decline in testosterone levels, even among samples of relatively young men. Interestingly, total testosterone demonstrated a more robust association with prostate cancer than did free testosterone. If free testosterone represents the biologically active portion of the molecule, it is puzzling why total testosterone would demonstrate a stronger relationship with prostate cancer risk. It is possible that persistent methodological difficulties in obtaining free testosterone cumulated into more measurement error than was generated by total testosterone assays. However, to the extent that assay procedures with recognized problems were identified

Figure 1. Proportional disparity in prostate cancer incidence with respect to proportional disparity in (a) total testosterone, $y = 12.96x - 0.56$, $R^2 = 0.69$, and (b) free testosterone, $y = 11.68x - 0.36$, $R^2 = 0.44$. After adjustment for age, proportional disparity in prostate cancer incidence with respect to proportional disparity in (c) total testosterone $y = 15.37x + 0.00$, $R^2 = 0.79$, and (d) free testosterone, $y = 11.78x + 0.00$, $R^2 = 0.43$. Population comparison values were log transformed. Abbreviations for population samples: AA, African-American; CA, Caucasian-American; MA, Mexican-American; CHN, Chinese; JPN, Japanese; KOR, South Korean; NZ, New Zealander; SWE, Swedish. Data points were labeled according to the ethnicity and region of population samples.

and eliminated from analyses, then paying closer attention to the underlying reproductive physiology may provide further insight.

Testosterone is synthesized by the Leydig cells within the testes, and is secreted from the testes primarily into the spermatic vein where testosterone binds to plasma proteins for transport to target tissues [Matsumoto, 2001]. The precise circulatory pathways by which testosterone is delivered throughout the body are not understood completely, but studies on circulatory processes of the male reproductive tract in humans and other mammalian species have demonstrated that: the spermatic vein has the highest concentration of androgens compared with all other points in circulation; the testicular artery has a higher androgen concentration than is found in peripheral circulation because of venous–arterial

transfer with spermatic vein blood; and reproductive organs in close anatomical proximity to the testes, including the prostate, have a higher concentration of androgens compared to other body tissues [Nishihara and Suzuki, 1980; Damber *et al.* 1979; Free, 1977]. Taken together, blood throughout the male reproductive tract is rich in androgenic hormones and accessory sex organs surrounding the testes experience increased hormone exposure.

Recently, a previously unrecognized route of venous flow from the testes to the prostate that bypasses systemic circulation was identified in men with impaired testicular venous drainage (varicocele), and selective occlusion of this circulatory pathway with microsurgery or sclerotherapy substantially reduced prostate exposure to free testosterone [Gat *et al.* 2009, 2008].

This pathway has not been examined in men with uncompromised reproductive vasculature, but if direct venous flow between the testes and prostate exists to some degree in healthy men (although certainly to a lesser extent than men diagnosed with varicocele), androgenic stimulation of the prostate would be much greater than is indicated by free testosterone levels collected from peripheral circulation. Because total testosterone is a cumulative measure of free and bound forms, it may serve as a closer approximation of free testosterone concentration within reproductive tract circulation given that a considerable fraction of protein-bound testosterone collected at periphery would have circulated in free form near the testes.

Acknowledgements

I thank Grażyna Jasieńska, Jane Lancaster, Martin Muller, and Melissa Emery Thompson along with two anonymous reviewers for their helpful comments and suggestions regarding this manuscript.

Funding

This work was supported by the Graduate Research Fellowship Program from the National Science Foundation, 2008–2011.

Conflict of interest statement

The author declares that there are no conflicts of interest.

References

- Alvarado, L.C. (2010) Population differences in the testosterone levels of young men are associated with prostate cancer disparities in older men. *Am J Hum Biol* 22: 449–455.
- Bolelli, G., Muti, P., Micheli, A., Sciajno, R., Franceschetti, F., Krogh, V. *et al.* (1995) Validity for epidemiological studies of long-term cryoconservation of steroid and protein hormones in serum and plasma. *Cancer Epidemiol Biomarkers Prev* 4: 509–513.
- Boots, L.R., Potter, S., Potter, D. and Azziz, R. (1998) Measurement of total serum testosterone levels using commercially available kits: high degree of between-kit variability. *Fertil Steril* 69: 286–292.
- Curado, M.P., Edwards, B., Shin, H.R., Storm, H., Ferlay, J., Heanue, M. *et al.* (eds) (2007). *Cancer Incidence in Five Continents, Vol. IX*, IARC Scientific Publications No. 160, Lyon, IARC.
- Damber, J.-E., Tomic, R., Bergman, B. and Bergh, A. (1979) Increased concentration of testosterone in testis artery blood as compared to peripheral venous blood in man. *Int J Androl* 2: 315–318.
- Ettinger, B., Sidney, S., Cummings, S.R., Libanati, C., Bikle, D.D., Tekawa, I.S. *et al.* (1997) Racial differences in bone density between young adult black and white subjects persist after adjustment for anthropometric, lifestyle, and biochemical differences. *J Clin Endocrinol Metab* 82: 429–434.
- Feldman, B.J. and Feldman, D. (2001) The development of androgen-independent prostate cancer. *Nat Rev Cancer* 1: 34–45.
- Free, M.J. (1977) Blood supply to the testis and its role in local exchange and transport of hormones in the male reproductive tract., In: Johnson, A.D. and Gomes, W.R. (eds). *The Testis, Vol. 4*, Academic Press: New York, pp. 39–84.
- Gat, Y., Gornish, M., Heiblum, M. and Joshua, S. (2008) Reversal of benign prostate hyperplasia by selective occlusion of impaired venous drainage in the male reproductive system: novel mechanism, new treatment. *Andrologia* 40: 273–281.
- Gat, Y., Joshua, S. and Gornish, M.G. (2009) Prostate cancer: a newly discovered route for testosterone to reach the prostate: Treatment by super-selective intraprostatic androgen deprivation. *Andrologia* 5: 305–315.
- Grönberg, H. (2003) Prostate cancer epidemiology. *Lancet* 361: 859–864.
- Hsing, A.W. (2001) Hormones and prostate cancer: what's next? *Epidemiol Rev* 23: 42–58.
- Jakobsson, J., Ekström, L., Inotsume, N., Garle, M., Lorentzon, M., Ohlsson, C. *et al.* (2006) Large differences in testosterone excretion in Korean and Swedish men are strongly associated with a UDP-glucuronosyl transferase 2B17 polymorphism. *J Clin Endocrinol Metab* 91: 687–693.
- Jasieńska, G. and Thune, I. (2001) Lifestyle, hormones, and risk of breast cancer. *BMJ* 322: 586–587.
- Jasieńska, G., Thune, I. and Ellison, P.T. (2000) Energetic factors, ovarian steroids and the risk of breast cancer. *Eur J Cancer Prev* 9: 231–239.
- Kapoor, P., Luttrell, B.M. and Williams, D. (1993) The free androgen index is not valid for adult males. *J Steroid Biochem Mol Biol* 45: 325–326.
- Kehinde, E.O., Akanji, A.O., Memon, A., Bashir, A.A., Daar, A.S., Al-Awadi, K.A. *et al.* (2006) Prostate cancer risk: The significance of differences in age related changes in serum conjugated and unconjugated steroid hormone concentrations between Arab and Caucasian men. *Int Urol Nephrol* 38: 33–44.
- Lewis, J.G., Nakajin, S., Ohno, S., Warnock, A., Florkowski, C.M. and Elder, P.A. (2005) Circulating levels of isoflavones and markers of 5 α -reductase activity are higher in Japanese compared with New Zealand males: what is the role of circulating steroids in prostate disease? *Steroids* 70: 974–979.

- Lookingbill, D.P., Demers, L.M., Wang, C., Leung, A., Rittmaster, R.S. and Santen, R.J. (1991) Clinical and biochemical parameters of androgen action in normal healthy Caucasian versus Chinese subjects. *J Clin Endocrinol Metab* 72: 1242–1248.
- Matsumoto, A.M. (2001) The testis, In: Felig, F. and Frohman, L.A. (eds). *Endocrinology and Metabolism*, 4th edn, McGraw-Hill Professional: New York, pp. 635–706.
- Morley, J.E., Patrick, P. and Perry III, H.M. (2002) Evaluation of assays available to measure free testosterone. *Metabolism* 51: 554–559.
- Morris, P.D., Malkin, C.J., Channer, K.S. and Jones, T.H. (2004) A mathematical comparison of techniques to predict biologically available testosterone in a cohort of 1072 men. *Eur J Endocrinol* 151: 241–249.
- Nishihara, M. and Suzuki, Y. (1980) Androgen distribution in male rats. *Endocrinol Jpn* 27: 637–642.
- O'Malley, B.W. (1971) Mechanism of action of steroid hormones. *N Engl J Med* 284:370–377.
- Platz, E.A. and Giovannucci, E. (2004) The epidemiology of sex steroid hormones and their signaling and metabolic pathways in the etiology of prostate cancer. *J Steroid Biochem Mol Biol* 92: 237–253.
- Roddam, A.W., Allen, N.E., Appleby, P. and Key, T.J. for the Endogenous Hormones and Prostate Cancer Collaborative Group. (2008) Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. *J Natl Cancer Inst* 100: 170–183.
- Rohrmann, S., Nelson, W.G., Rifai, N., Brown, T.R., Dobs, A., Kanarek, N. *et al.* (2007) Serum estrogen, but not testosterone, levels differ between black and white men in a nationally representative sample of Americans. *J Clin Endocrinol Metab* 92: 2519–2525.
- Rosner, W. (2001) An extraordinarily inaccurate assay for free testosterone is still with us. *J Clin Endocrinol Metab* 86: 2903.
- Rosner, W., Auchus, R.J., Azziz, R., Sluss, P.M. and Raff, H. (2007) Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. *J Clin Endocrinol Metab* 92: 405–413.
- Ross, R.K., Bernstein, L., Judd, H., Hanisch, R., Pike, M. and Henderson, B. (1986) Serum testosterone levels in healthy young black and white men. *J Natl Cancer Inst* 76: 45–48.
- Ross, R.K., Bernstein, L., Lobo, R.A., Shimizu, H., Stanczyk, F.Z., Pike, M.C. *et al.* (1992) 5-alpha-reductase activity and risk of prostate cancer among Japanese and US white and black males. *Lancet* 339: 887–889.
- Santner, S.J., Albertson, B., Zhang, G.Y., Zhang, G.H., Santulli, M., Wang, C. *et al.* (1998) Comparative rates of androgen production and metabolism in Caucasian and Chinese subjects. *J Clin Endocrinol Metab* 83: 2104–2109.
- SEER (2005) Surveillance, Epidemiology, and End Results Program. NIH Publication No. 05-4772. Bethesda, MD: National Institutes of Health.
- Steiger, J.H. (1980) Tests for comparing elements of a correlation matrix. *Psychol Bull* 87: 245–251.
- Tsai, C.J., Cohn, B.A., Cirillo, P.M., Feldman, D., Stanczyk, F.Z. and Whittemore, A.S. (2006) Sex steroid hormones in young manhood and the risk of subsequent prostate cancer: a longitudinal study in African-Americans and Caucasians (United States). *Cancer Causes Control* 17: 1237–1244.
- Vermeulen, A., Verdonck, L. and Kaufman, J.M. (1999) A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 84: 3666–3672.
- Williams, E.J. (1959) The comparison of regression variables. *J R Statist Soc B* 21: 396–399.
- Winters, S.J., Brufsky, A., Weissfeld, J., Trump, D.L., Dyky, M.A. and Hadeed, V. (2001) Testosterone, sex hormone-binding globulin, and body composition in young adult African American and Caucasian men. *Metabolism* 50(10): 1242–1247.
- Winters, S.J., Kelley, D.E. and Goodpaster, B. (1998) The analog free testosterone assay: are the results in men clinically useful? *Clin Chem* 44: 2178–2182.
- Wright, N.M., Renault, J., Willi, S., Veldhuis, J.D., Pandey, J.P., Gordon, L. *et al.* (1995) Greater secretion of growth hormone in black than in white men: possible factor in greater bone mineral density—a clinical research center study. *J Clin Endocrinol Metab* 80: 2291–2297.