

Chapter 2: The Role of Steroid Hormones in Prostate Carcinogenesis

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Carcinoma of the prostate is the most frequently diagnosed malignancy and the second leading cause of death as a result of cancer in men in the United States and in many other Western countries. Notwithstanding the importance of this malignancy, little is understood about its causes. The epidemiology of prostate cancer strongly suggests that environmental factors, particularly diet and nutrition, are major determinants of risk for this disease, and evidence is mounting that there are important genetic risk factors for prostate cancer. Human prostate carcinomas are often androgen sensitive and react to hormonal therapy by temporary remission, followed by relapse to an androgen-insensitive state. These well-established features of prostate cancer strongly suggest that steroid hormones, particularly androgens, play a major role in human prostatic carcinogenesis, but the precise mechanisms by which androgens affect this process are unknown. In addition, the possible involvement of estrogenic hormones is not entirely clear. The purpose of this overview is to summarize the literature about steroid hormonal factors, androgens and estrogens, and prostate carcinogenesis. From these literature observations, a multifactorial general hypothesis of prostate carcinogenesis emerges with androgens as strong tumor promoters acting via androgen receptor-mediated mechanisms to enhance the carcinogenic activity of strong endogenous genotoxic carcinogens, such as reactive estrogen metabolites and estrogen- and prostatitis-generated reactive oxygen species and possible weak environmental carcinogens of unknown nature. In this hypothesis, all of these processes are modulated by a variety of environmental factors such as diet and by genetic determinants such as hereditary susceptibility and polymorphic genes that encode for steroid hormone receptors and enzymes involved in the metabolism and action of steroid hormones. [J Natl Cancer Inst Monogr 2000;27:39–66]

Carcinoma of the prostate is the most frequently diagnosed malignancy and the second leading cause of death as a result of cancer in men in the United States and in many Western countries (not counting nonmelanoma skin cancer) (1). Notwithstanding the importance of this malignancy, little is understood about its causes. Steroid hormones, particularly androgens, are suspected to play a major role in human prostate carcinogenesis, but the precise mechanisms by which androgens affect this process and the possible involvement of estrogenic hormones are not clear. A causal relation between androgens and prostate cancer development is generally considered to be biologically very plausible because the vast majority of human prostate cancers are androgen sensitive and respond to hormonal therapy by temporary remission, later followed by relapse to a hormone-refractory state. The purpose of this overview is to summarize the literature about steroid hormonal factors and prostate carcinogenesis. Although the objective of this overview is not to be

comprehensive, an attempt is made to be complete, especially where crucial aspects of this hormonal involvement are concerned.

In contrast, the prostate is a rare site of tumor development in carcinogenesis bioassays in rodents (2,3) and in aging male laboratory rodents, with the exception of ventral prostatic neoplasms in some rat strains (4–9). Prostate cancer is also rare in male farm and companion animals, with the notable exception of the dog, which is the only species besides man that develops this malignancy. As will be discussed in this overview, steroid hormones can induce and can substantially enhance prostate carcinoma development in rodents, and this phenomenon has been exploited to further our knowledge about the involvement of hormonal factors and mechanisms in prostate cancer etiology.

In this overview, the epidemiologic evidence for a role of steroid hormonal factors in prostate carcinogenesis is summarized first, followed by review of experimental data, a discussion of the possible mechanisms whereby steroid hormones, androgens as well as estrogens, may be involved in prostate cancer causation, and overall conclusions and suggestions for future research. As will be demonstrated, there is no lack of hypotheses about the role of steroid hormones in prostate cancer etiology, but the available data are often contradictory and incomplete, and an in-depth overall mechanistic understanding of how steroid hormonal factors are involved in prostate carcinogenesis is very limited.

EPIDEMIOLOGIC EVIDENCE FOR INVOLVEMENT OF STEROID HORMONES

The epidemiology of prostatic cancer has been reviewed in depth elsewhere (10–15). Prostate cancer risk factors that are associated with hormonal factors are summarized in subsequent sections, and epidemiologic and other studies related to the metabolism and action of steroid hormones are reviewed in detail. Besides hormonal factors, there are only a few established risk factors for prostate cancer. These risk factors are briefly summarized below to put the relative importance of steroid hormonal factors in perspective.

Prostate cancer incidence and mortality rates have increased in the United States over the few decades preceding the frequent use of prostate-specific antigen (PSA) for early detection (10). Even though incidence rates have increased substantially since the mid-1980s because of the use of PSA “screening” for early detection (1), incidence has declined over the period from 1990 through 1996 by an average of 2% per year and mortality by 1.6% per year (16). Because of the increasing use of PSA for

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early detection and treatment in this period, it is too early to separately determine changes in prostate cancer rates and the impact of PSA screening on rates. In 1999, prostate cancer was the most frequent malignancy in U.S. males with 179 300 new cases expected, and it was the second most frequent cause of death as a result of cancer with 37 000 deaths expected (17).

Many studies have demonstrated that prostate cancer is more frequent in men with a family history of prostate cancer, as summarized elsewhere (10,11,15,18–20). This familial aggregation appears to be similar in African-American and in European-American men (21,22). However, inherited risk for prostate cancer can only explain a small proportion of prostate cancer cases, less than 10% (20). Besides a variety of genetic alterations identified with varying frequency in human prostate carcinomas, as summarized by Dong et al. (23), a few susceptibility loci linked to inherited prostate cancer risk have been identified on chromosome 1 (24–28) and on the X chromosome (29,30). Breast and prostate cancers cluster in some families, and there is some evidence that BRCA1 and BRCA2 mutations are involved in this clustering (18,31). However, none of these loci have thus far been associated with hormonal factors.

Evidence is limited that a history of venereal disease (11,32–34) and a history of prostatitis (34,35) are risk factors. An association between prostate cancer risk and the prior occurrence of benign prostatic hypertrophy (BPH) is biologically unlikely, even though steroid hormones are also implicated in BPH. Prostate cancer and BPH originate from different parts of the prostate (all BPH is found in the transition zone, and more than 80% of all cancers develop in the peripheral zone), and their epidemiology is dissimilar (11).

Although, in some studies (34,36,37), a relationship between smoking and risk for prostate cancer has been found, no such relationship has been observed in the vast majority of studies (37–40). In addition, smoking appears to have no effect on circulating levels of testosterone and other hormones that may be involved in prostate carcinogenesis (41,42). Most studies (11,43) addressing alcohol consumption as a potential risk factor for prostate cancer did not find evidence for an association. One notable exception is a study by Hayes et al. (44) that found a positive association in a U.S. case–control study, which was limited to heavy use of alcohol. Possible reasons for the association observed in this study are discussed by Lumey et al. (43). One of these reasons may be that prostate cancer risk is elevated in alcoholics with liver disease (11,43). This risk elevation is possibly related to the impaired clearance of estrogens described in men with liver cirrhosis (11,45,46).

Increased risk has been observed for a variety of occupations in studies of occupational factors and prostate cancer (11,14,47,48), including armed services personnel (11) and workers in the nuclear industry (11,15,48,49). Although prostate cancer risk in survivors of the atomic bomb in Japan appears not to be elevated (50), there is a rather strong international correlation between prostate cancer incidence and indoor radon levels reported (51). Thus, prostate cancer risk may be associated with exposure to ionizing radiation, but the evidence is equivocal. Associations between exposures and prostate cancer risk observed in the rubber industry are limited to one or a few plants (11). The evidence for a positive association between farming and prostate cancer risk is weak to inconclusive (11,14,47,48,52). There is only very weak, if any, evidence for an association of cadmium exposure and prostate cancer risk

(11,53,54). Hormonal factors are most likely not involved in any of these (possible) associations between risk and occupational factors.

Risk Factors Associated With Possible Hormonal Mechanisms

The results of a variety of epidemiologic studies have led to suggestions for several risk factors that may be related to a hormonal mechanism. These risks include dietary factors, vasectomy, sexual factors, the level of physical activity, and obesity.

Diet and Nutrition

The associations between dietary factors and prostate cancer risk have been extensively summarized elsewhere (10,11,15,55–57). Considerable consistency across studies indicates that a high intake of fat, particularly total fat and saturated fat, is a risk factor for prostate cancer, but the strength of the associations is modest at best (57,58) and may be greater for African-Americans than for European-Americans (59). Results from Hawaiian case–control studies suggest that as much as 25% of prostate cancer in the United States may be attributable to a high saturated-fat intake (60). However, Whittemore et al. (22) estimated that dietary fat intake may account for only 10%–15% of the difference in prostate cancer occurrence between European-Americans and African-Americans or Asians. The mechanism that could underlie an enhancing effect of fat on prostate carcinogenesis is not understood, but several hypotheses, including hormonal mediation, have been discussed elsewhere (11,15,57,61). In addition, a high intake of protein and energy and a low intake of dietary fiber and complex carbohydrates have been found to be associated with the increased risk for prostate cancer in some studies (10,11,15,55).

Associations with prostate cancer risk reported for individual nutrients or foods are not very strong. However, migration from low-risk areas, such as Japan, to high-risk countries, such as the United States, increases risk considerably (10,11). These changes in risk are thought to be due to differences in environment, including lifestyle and particularly dietary habits (10,11). It is, therefore, conceivable that the combined effects of dietary factors on prostate carcinogenesis are more important than the separate effects of any individual dietary factor (62). This idea is supported by the lack of any effect of dietary fat per se on the induction of prostate cancer in animal models, whereas epidemiologic studies (11,15,62) rather consistently show a positive association between prostate cancer risk and intake of dietary fat.

Older studies (63–67) of the effects on hormonal status of dietary changes and of the consumption of vegetarian or health food diets, which have been summarized previously (11), did not separately address the effects of dietary fat. However, they clearly indicate that diet can influence circulating hormone levels by changing androgen production rates and/or the metabolism and clearance of androgens and estrogens. In a study reported by Dorgan et al. (68), controlled changes in fat and fiber were applied to healthy men. The combination of a high-fat, low-fiber diet increased both total testosterone (by 13%) and testosterone bound to sex hormone-binding globulin (SHBG; by 15%) in the plasma as well as urinary testosterone excretion (13%), compared with a low-fat, high-fiber diet. However, urinary excretion of estrone, estradiol, and the 2-hydroxy metabo-

lites of these estrogens was lower. All of these studies indicate that diet can affect steroid hormone status, but no studies have addressed the separate effects of single dietary factors, such as fat intake.

Complete consistency is lacking among epidemiologic studies of prostate cancer risk and intake of dietary vitamin A and β -carotene (10,11,15,69). It is possible that retinoids and/or carotenoids enhance rather than inhibit prostatic carcinogenesis under certain circumstances or in certain populations (69), although animal and *in vitro* studies suggest a protective effect of retinoids (11). In two more recent experiments on prostate cancer chemoprevention in a rat model, 9-*cis*-retinoic acid, a major retinol metabolite in mammalian species, strongly inhibited the induction of prostate cancer (70), but *N*-(4-hydroxyphenyl)retinamide (4-HPR), a synthetic retinoid, did not have any effect (71). 9-*cis*-Retinoic acid is unique in that it is a pan-agonist for retinoic acid receptors, binding both retinoic acid receptor (RAR) and retinoid X receptor (RXR) receptors. *In vitro*, however, both 9-*cis*-retinoic acid and 4-HPR inhibit the growth and induce apoptosis of the androgen-sensitive human prostate cancer LNCaP cell line, and so does all-*trans*-retinoic acid, which only binds to RAR (72–74). There are indications that 4-HPR acts via a nonreceptor mechanism (74). The specific mechanism is not known by which retinoids and/or carotenoids may inhibit or enhance prostate carcinogenesis, but inhibition seems biologically more plausible than enhancement, as discussed previously (11). The retinoic acid and androgen receptors both belong to the steroid receptor superfamily (75). This circumstance raises the intriguing possibility that retinoids may be able to bind to and activate mutated forms of the androgen receptor or that the retinoic acid RAR and/or RXR may activate transcription of androgen-regulated genes. Studies on the regulation by sex steroids and retinoic acid of glutathione *S*-transferase in hamster smooth muscle tumor cells (76) and on androgen-receptor gene expression in human breast cancer cells (77) suggest that such mechanisms may exist.

Vasectomy

Vasectomy has been identified as a possible risk factor for prostate cancer in seven case-control studies (34,78–83) and in three cohort studies (84–86). The range of risk ratios in the case-control studies was 1.4 to 5.3. No elevation of risk for prostate cancer following vasectomy was found in six other case-control studies (87–92) and in two retrospective cohort studies (93–95). Although a meta-analysis (96) of 14 studies indicated that there is no causal relation between vasectomy and prostate cancer, further studies, particularly cohort studies, will be required to definitively establish whether or not vasectomy is a true risk factor for prostate cancer (58,97–99).

Three mechanisms by which vasectomy could enhance risk have been proposed: elevation of circulating androgens, immunologic mechanisms involving antisperm antibodies, and reduction of seminal fluid production (34,78,79,85,90,98,100). Most studies (101–105) that investigated the effect of vasectomy on pituitary-gonadal function did not find any effect, but some studies (90,100,106–110) found slight, but statistically significant, changes in circulating levels of certain hormones. Four groups (34,100,108,111) reported slightly elevated circulating testosterone levels, but only in two of these groups (100,108) was the increase statistically significant. Mo et al. (100) also found elevated levels of 5 α -dihydrotestosterone (DHT), the ac-

tive metabolite of testosterone in the prostate, in vasectomized men. John et al. (90) reported a decrease in SHBG, and Honda et al. (34) observed an increase in the ratio of testosterone to SHBG. These results suggest an elevation of circulating free testosterone following vasectomy, which may be a critical factor associated with risk for prostate cancer. A possible specific mechanism whereby vasectomy could influence the hypothalamic-pituitary-gonadal axis is not known.

Sexual Factors

Attempts have been made in several case-control studies (11,32–34,112–114) to investigate the possibility that sexual factors play a role in prostate cancer etiology. The results of these studies suggest that prostate cancer risk may be associated with the level of sexual activity, but no conclusive evidence exists for such a relation (11). Tsitouras et al. (115) reported a significant positive association between the level of sexual activity (intercourse and masturbation) and circulating total testosterone levels in men between the ages of 60 and 79 years as well as an absence of a decrease in testosterone levels with aging in sexually active men. These findings suggest that a hormonal mechanism may underlie a possible association between prostate cancer risk and sexual activity suggested by the aforementioned case-control studies.

Physical Activity and Anthropometric Correlates of Risk

Contradictory indications are found that the level of physical activity may be a possible risk factor for prostate cancer, but the evidence for such an association is inconclusive at present (15,62,116). Sports exercise may decrease, as well as increase, circulating androgen concentrations or have no effect, depending on such factors as the time of blood sampling in relation to the exercise, the level of exercise, and the training protocol followed (117,118). Therefore, it is possible that the type and extent of physical activity influence circulating androgen concentrations and, thereby, perhaps prostate cancer risk. At present evidence is contradictory that obesity or an increased body mass index is a risk factor for prostate cancer (15,62,119). Severson et al. (120) observed a significant increase in prostate cancer risk with increasing upper-arm circumference and upper-arm muscle area but not fat area. A positive association between prostate cancer risk and muscle mass, but not fat mass, may suggest exposure to endogenous or exogenous androgenic hormones or other anabolic factors (120,121). Indeed, evidence is available that body mass index is inversely associated with plasma testosterone and SHBG levels and positively associated with estradiol levels (119,122,123), as discussed elsewhere (11,42).

Epidemiologic Studies of Endogenous Hormones and Hormone Metabolism

As indicated earlier, a causal relation between androgens and prostate cancer development is generally considered biologically plausible because this malignancy develops in an androgen-dependent epithelium and is usually androgen sensitive. In addition, a few case reports (124–129) are available of prostate cancer in men who used androgenic steroids as anabolic agents or for medical purposes, suggestive of a causal relationship.

Studies (11,15,130) comparing the endocrine status of human prostate cancer patients with that of control subjects are probably not very informative about the endocrine status prior to the

onset of the disease and are, therefore, not meaningful to explore this relationship; in addition, the presence of the malignancy may by itself alter hormonal status. Indeed, the results of such studies do not provide a consistent pattern as summarized by Andersson et al. (130), which is confirmed in other case-control studies (119,131–133). These types of studies will, therefore, not be discussed here.

Nested case-control studies of steroid hormonal factors in ongoing cohort studies, as well as studies comparing healthy males in populations that are at high risk for prostate cancer with populations at lower risk, are likely to be more meaningful. These studies are summarized in the following sections. The two major hypotheses for these studies were that increased risk for prostate cancer would be associated with either an increased testicular production of testosterone or an increased conversion of testosterone to DHT because of an increased 5 α -reductase activity (134–136). Studies have focused on the notion that functional genetic polymorphisms in the 5 α -reductase gene or in genes involved in testosterone biosynthesis (the CYP17 gene) or DHT catabolism (the 3 β -hydroxysteroid dehydrogenase gene) could be responsible for increased testosterone production or increased DHT levels (136). In addition, polymorphisms have been discovered in the androgen receptor gene that can have functional significance for androgen receptor activity (137–139). Such polymorphisms have been postulated to be critical determinants of prostate cancer risk at the population or individual level by affecting intraprostatic DHT concentrations and androgen receptor transactivation (18,136).

Steroid Hormonal Factors in Populations That Differ in Risk For Prostate Cancer

Circulating of steroid hormone levels. A summary of the results of studies that compared circulating levels of steroid hormones in very high-risk African-Americans with those in high-risk European-Americans, lower-risk Asian-Americans, and very low-risk Asians living in Asia or African black men is provided in Table 1. The details of each study are summarized in the following paragraphs.

Ahluwalia et al. (140) studied 170 African-Americans and 55 black-Nigerian men who were matched control subjects in a case-control study of prostate cancer and were older than 50 years. Plasma levels of testosterone and estrone were significantly higher in the African-American men than in the Nigerian men, whereas levels of DHT and estradiol were not different. Similar differences were found for the prostate cancer case patients.

Hill et al. (63–65) compared the hormonal status of small groups ($n = 11$ –20) of 40- to 55-year-old African-American, European-American, and black (rural) South African men consuming their customary diets (the effects of diet changes were also studied in these men; see earlier section on “Diet and Nutrition”). In a separate study (141), African-American, European-American, and black South African boys (ages 12–15 years) and young African-American and black South African men (ages 18–21 years) were compared. In the older men, plasma levels of the testosterone processor dehydroepiandrosterone (DHEA) were significantly lower in the two groups of black men than in the white men, whereas estrone levels were higher. Plasma levels of the testosterone processor androstenedione and estradiol were significantly higher in the African men than in the two American groups, whereas no differences were

noted among these groups in testosterone levels. In the study with the 12- to 15-year-old boys and young men, similar findings were obtained for testosterone and DHEA. However, androstenedione levels were significantly lower (not higher) in the African than in the American subjects, and estradiol was lower in young black boys (12–14 years old) than in white boys but higher in older black boys (12–14 years old) and young black men than in white boys and men. These data suggest a complex interaction between ethnic background and environmental differences that change over the years of sexual maturation. In these studies by Hill and colleagues among South African black men, the 18- to 21-year-old men were different from those in 40- to 55-year-old men for androstenedione and DHEA. This divergence suggests that it is probably important for the interpretation of hormonal profiles to separately consider younger and older men.

Ross et al. (134) compared 50 healthy young African-American men (at very high risk for prostate cancer) and 50 young European-American males (at half the risk of the black men). Total circulating testosterone was 19% higher, and free testosterone was 21% higher in the group of black subjects than in the group of white subjects. Serum estrone concentrations were also significantly higher (16%) in the black than in the white group. No significant differences were seen between the groups in circulating estradiol and SHBG levels. The authors estimated that the 19%–21% difference in circulating testosterone is sufficiently large to explain the twofold difference in prostate cancer risk between white and black men in the United States. This study suggests an association between prostate cancer risk and high concentrations of circulating androgens and, possibly, estrogens.

Henderson et al. (142) compared circulating hormone levels in 20 pregnant African-American with 20 European-American women in their first trimester. Serum testosterone levels were 47% higher in black women than in white women, and estradiol levels were 37% higher. No significant differences were observed in circulating SHBG and human chorionic gonadotrophin, or in relevant pregnancy characteristics, such as the sex ratio of the offspring. These findings suggest that African-American males are exposed to higher androgen concentrations than European-American males even before birth.

The U.S. black and white men from the study by Ross et al. (134) were compared with 54 Japanese men of the same age (mean age, 19–23 years) in a follow-up study (135). The serum testosterone levels of Japanese men were not lower than those of the U.S. whites and blacks but were intermediate between these two groups, whereas their SHBG levels were significantly lower. This finding may suggest a higher percentage of free testosterone in the Japanese (at very low risk) than in the U.S. men (at high risk), but free testosterone was not measured. Compared with the Japanese men, the two U.S. groups had significantly higher circulating levels of the conjugated androgen metabolites androsterone glucuronide (41%–50% higher) and 3 α ,17 β -androstenediol glucuronide (25%–31% higher). This finding suggests that, in comparison with the high-risk U.S. groups, the low-risk Japanese population has a lower testosterone metabolism, most likely a lower activity of the enzyme 5 α -reductase that converts testosterone to DHT and the testosterone precursor androstenedione to androsterone. However, the markedly higher levels of androsterone glucuronide in U.S. men could also be indicative of a higher testosterone production in comparison with Japanese men.

Table 1. Summary of 11 studies of circulating steroid hormone levels (given as percentage of a referent group) in men from different ethnic groups*

Hormone	Study†	Mean age‡ (range, y)	African-American	European (American)	Asian-American		Asian		(South) African black men
					Japanese	Chinese	Japanese	Chinese	
Testosterone	1	≥50 y	100						Lower§
	2	43–49 (40–55)	108	100					108
	3	12–15	Same	100					Same
		18–21		100					Same
	4	20 (18–22)	118.6§	100					
	5	20, 19, 23¶	111.3	100	104.7				
	6	25 (18–47)		100					
	7	63, 70 (50–79)		100			85.7§	Same	
	8	38 (30–50)	103.5	100		101.6			
	9	70 (35–89)	105.2	100	110.5§	108.8§			
	10	20–39		100				Lower#	
Free testosterone	4	20 (18–22)	121.2§	100					
	6	25 (18–47)		100					~100
	9	70 (35–89)	104.0	100	106.7	112.0§			
	10	20–39		100		Same			Same
% Free testosterone	4	20 (18–22)	103.2	100					
Bioavailable testosterone	6	25 (18–47)		100					Same
	9	70 (35–89)	103.3	100	105.1	111.4§			
SHBG	4	20 (18–22)	104.8	100					
	5	20, 19, 23¶	109.2	100	71.4§				
	7	63, 70 (50–79)		100			115.7§		
	9	70 (35–89)	106.0	100	107.8§	96.6			
	10	20–39		100		Higher			Same#
Dihydrotestosterone (DHT)	1	≥50 y	100						Same
	6	25 (18–47)		100					
	7	63, 70 (50–79)		100			89.0	112.6	
	9	70 (35–89)	107.0§	100	107.2§	99.1			
DHT/testosterone ratio	7	63, 70 (50–79)		100			107.0**		
	9	70 (35–89)	102.0	100	96.0	89.9§			
Androsterone–glucuronide	5	20, 19, 23¶	93.8	100	66.6§				
	6	25 (18–47)		100					59.8§
Androstanediol–glucuronide	5	20, 19, 23¶	95.4	100	76.4§				
	6	25 (18–47)		100					56.8§
DHEA	2	43–49 (40–55)	76.9§	100					67.6§
	3	12–15	Same to lower§	100					Much lower§
		18–21		100					Same
DHEA–sulfate	9	25 (18–47)		100					68.4§
	11	≥50¶	83.8–99.4	100					
Androstenedione	2	43–49 (40–55)	96.8	100					128.6§
	3	12–15	Same to higher§	100					Much lower§
		18–21		100					Lower§
	6	25 (18–47)		100					75.6§
Estrone	1	≥50 y	100						Same
	2	43–49 (40–55)	117.4§	100					150.8§
	4	20 (18–22)	116.1§	100					
Estradiol-17β	1	≥50 y	100						Same
	2	43–49 (40–55)	103.1	100					128.3§
	3	12–14	Lower§	100					Same to lower§
		15	Higher§	100					Higher§
		18–21		100					Higher§
	4	20 (18–22)	109.8	100					
	7	63, 70 (50–79)		100			87.0§		

*Values are presented as the percentage of the value (set at 100%) in a referent group [European(-American) group, except in study 1, in which African-Americans are used as the reference group]. A two-sided *P* value of less than .05 was considered significant. Unless designated, none of the other differences are statistically significant.

†Studies: 1) Ahluwalia et al. (140); 2) Hill and colleagues (63–65); 3) Hill et al. (141); 4) Ross et al. (134); 5) Ross et al. (135); 6) Lookingbill et al. (143); de Jong et al. (144); 8) Ellis and Nyborg (145); 9) Wu et al. (146); 10) Santner et al. (148); 11) Corder et al. (147).

‡Mean (occasionally median) age (in years) is given with the range (if available) in parentheses. For studies 5 and 7, the mean age is given for each ethnic group consecutively. If mean age is not available, only the age range is given.

§Statistically significantly different from the European(-American) group (referent).

||If exact numerical information is not available, significant higher or lower values are indicated as such and absence of significant differences are indicated by “same.”

¶Exact age range is not available.

#Significantly different from Chinese-Americans.

**Significant only after age adjustment.

Lookingbill et al. (143) reported a similar observation, comparing 53 normal healthy U.S. Caucasians and 57 Chinese males in Hong Kong between the ages of 24 and 26 years. The Caucasian men had 67% higher serum levels of androsterone glucuronide and 76% higher levels of 3 α ,17 β -androstanediol glucuronide than the Chinese men did. Circulating levels of testosterone, free testosterone, or DHT were not significantly different, but Caucasian men had 46% higher serum levels of the androgen precursor DHEA sulfate and 32% higher levels of androstenedione. These data are also suggestive of a higher 5 α -reductase activity in high-risk Caucasians than in low-risk Chinese men, and they suggest an increased production of androgen precursors in the Caucasians.

In contrast to the observations of Ross et al. (134,135) and those of Lookingbill et al. (143), De Jong et al. (144) found 71% higher circulating total testosterone levels in 123 Caucasian-Dutch men (high risk) than in 91 Japanese men (low risk). The men in these studies were considerably older (50–79 years) than those studied by the previous two other groups. DHT levels were not different, but the ratio of DHT to testosterone was 10% lower in Dutch men than in Japanese men, possibly reflecting lower 5 α -reductase activity; no data were presented on androgen metabolites. Serum levels of estradiol were 15% higher (significant) in the Dutch men than in the Japanese men. SHBG levels were not different, but the ratio of testosterone to SHBG concentrations was 34% higher in Dutch men than in Japanese men, which suggests higher amounts of free testosterone in Dutch men, but this parameter was not measured separately.

Ellis and Nyborg (145) studied 4462 U.S. Army Vietnam veterans, ages 31–50 years, and compared serum testosterone levels in 3654 non-Hispanic white men (mean 6.37 ng/mL) with those in 525 African-Americans (6.58), 200 Hispanics (6.33), 34 Asian/Pacific Islanders (6.89), and 49 Native Americans (6.31). The serum testosterone levels in the African-American men were significantly higher than those in the non-Hispanic white men, but the differences among the other groups were not significant. The serum testosterone difference between black and white men was larger in men between 31 and 35 years of age (6.6%) than for men ages 35–40 years (3.7%) or ages 40–50 years (0.5%). No other hormones were measured in this study.

Wu et al. (146) conducted a population-based study, comparing circulating hormone levels in 1127 healthy men: 325 African-American men, 411 European-American men, 126 Chinese-Americans, and 275 Japanese-Americans with a median age of 69.6 years (range, 35–89 years), 8.2% of whom were 60 years or younger. Serum levels of total testosterone were slightly, but significantly, higher (9%–11%) in Asian-Americans than in European-American men, whereas they were intermediate and not significantly different from the two other groups in African-Americans. The same pattern was found for serum levels of bioavailable testosterone (not bound to SHBG) and the percentage of free testosterone (not bound to either SHBG or albumin), but only the 11%–12% difference between Chinese-American and European-American men was significant. SHBG levels were not different among the four groups. In comparison with European-American men, DHT levels were 7% higher (significant) in high-risk African-Americans and low-risk Japanese-Americans, but similar in Chinese-Americans. The ratio of DHT to testosterone was 10% lower (significant) in Chinese-Americans than in European-Americans, but not significantly different in African-Americans and Japanese-Americans who had slightly higher

and lower ratios, respectively, than European-Americans. These data do not appear to provide clear support for the notions of a relation between increased 5 α -reductase activity or testosterone production and prostate cancer risk, but this study did not include more direct indicators, such as androsterone glucuronide and 3 α ,17 β -androstanediol glucuronide.

DHEA sulfate was measured by Corder et al. (147) in stored serum samples of 90 African-American and 91 European-American men with prostate cancer and equal numbers of matched controls who were identified in a nested case-control study in a cohort of men in the Kaiser Permanente Medical Care Program in Northern California. Regardless of age, no significant differences were found between the two groups in DHEA sulfate levels, which were lower in men 57 years and older than in younger men.

Santner et al. (148) conducted the only study to date in which androgen production and metabolism by 5 α -reductase were determined in a direct fashion in populations with different risk for prostate cancer. A radioisotope method involving intravenous administration of tritiated testosterone was used to measure the conversion of testosterone to DHT in healthy European-Americans (ages 22–27 years), Chinese-Americans (ages 20–37 years), and Chinese men living in Beijing, China (ages 24–39 years). No differences in conversion of testosterone to DHT were found among these three groups. Circulating testosterone and SHBG levels were lower in the Beijing Chinese than in the two U.S. groups, and the differences with the U.S. Chinese subjects were significant, whereas no differences were found in free testosterone. There was a nonsignificant trend toward lower calculated metabolic conversion rates of testosterone comparing European-Americans with the Chinese groups and U.S. Chinese with Beijing Chinese. Calculated testosterone production rates were lower in Beijing Chinese than in the two U.S. groups, the difference with American Chinese being significant. The ratios of urinary 5 β - to 5 α -reduced steroids, which are an indicator of overall 5 α -reductase activity, were also not different in 20 European-American male students compared with 20 Chinese students living in Hong Kong. Urinary excretion of androsterone, etiocholanolone, and total ketosteroids was lower in the Chinese than in the U.S. students, which was significant when the data were combined with those of 20 female students from each of the two populations. Taken together, these data indicate that 5 α -reductase activity is not different in Asian and Caucasian men and is not affected by the environment in which Asian men live. However, these results suggest that the living environment influences testosterone production in Asian men.

Polymorphisms in genes involved in steroid hormone metabolism and action. Studies have addressed the hypothesis (137–139) that functional polymorphisms in the 5 α -reductase gene, in genes involved in testosterone biosynthesis or DHT catabolism, and in the androgen receptor gene could be associated with the differences in prostate cancer risk among various populations. These studies are summarized in the following paragraphs.

The SRD5A2 gene, which encodes for human type II 5 α -reductase enzyme, is expressed in the prostate and is located on chromosome 2p23 (149,150) and contains polymorphic TA dinucleotide repeats in its transcribed 3' untranslated region (151). Reichardt et al. (152) demonstrated that TA(0) [87 base pairs (bp)] is the most common allele and was homozygous in 81% of non-Hispanic, white Americans (n = 68), 78% of Asian-

Americans (n = 37), and 67% of African-Americans (n = 94). The next most common allele TA(9) (103–105 bp) is heterozygous with the TA(0) allele and occurred in 19% of the non-Hispanic, white American men, 22% of the Asian-Americans, and 15% of the African-Americans. The TA(18) allele (212–131 bp) was only found in African-Americans (18%) as heterozygous with the TA(0) allele in all except one who was homozygous. Thus, the longer alleles are unique to African-Americans and may be related to their extremely high risk for prostate cancer. However, the functional significance of these polymorphisms is not yet known.

Makridakis et al. (153) identified another polymorphism in the SRD5A2 gene, the presence of a valine to leucine mutation at codon 89. If this mutation occurs in a homozygous state, it confers 28% lower 5 α -reductase activity as measured in Asian men with this genotype compared with heterozygous men and men without the mutation. These researchers observed that the frequency of the 89 valine–valine genotype was 59% in African-American men (n = 95), 57% in non-Hispanic white Americans (n = 49), 48% in Latino Americans (n = 40), and 29% in Asian-Americans (n = 102). The 89 valine–leucine genotype occurred in 37%–39% of African-Americans, non-Hispanic white Americans, and Latino Americans, and in 49% of Asian-Americans. The frequency of the 89 leucine–leucine genotype (lower 5 α -reductase activity) was 3%–4% in African-American and non-Hispanic white Americans, 15% in Latino Americans, and 22% in Asian-Americans. A recent report from another, larger study by Lunn et al. (154) is essentially consistent with these findings. In this study, the frequency of the 89 valine–valine genotype was 65% in African-American men (n = 118), 41% in European-Americans (n = 176), and 15% in Asians (Taiwanese) (n = 108). The 89 valine–leucine genotype occurred in 32% of African-Americans, 50% of European-Americans, and in 57% of Asians. The frequency of the 89 leucine–leucine genotype (lower 5 α -reductase activity) was 2.5% in African-Americans, 8.5% in European-Americans, and 28% in Asian-Americans. The higher frequency of the 89 leucine–leucine genotype in Latino American men and particularly Asians may be related with the lower risk for prostate cancer found in these two ethnic groups, and the low frequency of 86 leucine alleles in African-Americans may be related to their extremely high risk. However, there appears not to be a relation between plasma concentrations of 3 α -androstane-diol-glucuronide as an indicator of 5 α -reductase activity and the three different SRD5A2 gene codon 89 genotypes (155).

Makridakis et al. (156) also identified another polymorphism in the SRD5A2 gene, a mis-sense alanine to threonine mutation at codon 89. An *in vitro* construction of the mutant enzyme displayed a substantial increase in activity (V_{max}). The frequency of the mutation was very low, 1.0% and 2.3%, in healthy, high-risk African-Americans and lower-risk Hispanic men, respectively. Although no data were presented on other ethnic/racial groups, it seems unlikely that this mutation is responsible for the large ethnic/racial variations in prostate cancer risk.

The CYP17 gene, which encodes for the cytochrome P450C17 α enzyme that has both 17 α -hydroxylase and 17,20-lyase activity in the adrenal and testicular biosynthesis of androgens, is located on chromosome 10q24.3 (157). This gene is polymorphic with two common alleles, the wild-type CYP17A1 allele and the CYP17A2 allele containing a single base pair

mutation in the untranscribed 5' region of exon 1 (157). This mutation creates an additional Sp1 site in the promoter region, suggestive of increased expression potential (157). The functional significance of this polymorphism in men is not known, but premenopausal and postmenopausal women with the A2 allele have been reported to have higher circulating estradiol and progesterone levels than women homozygous for the A1 allele (158,159). Circulating levels of DHEA and androstenedione, but not testosterone, were increased in postmenopausal women (159). Lunn et al. (154) recently reported that the frequencies of the A1/A1 and A1/A2 genotype were between 40% and 44%, and the frequency of the A2/A2 genotype was 16%–17% in both African-American men (n = 115) and European-Americans (n = 115), accounting for an A2 allele frequency of 0.36–0.38. In Asians (Taiwanese; n = 110), however, the A1/A1 genotype occurred in 24%, the A1/A2 genotype in 49%, and the A2/A2 genotype in 27%, with an A2 allele frequency of 0.52. The frequency differences between the Asians and the two American groups were statistically significant and are perhaps related to the low risk for prostate cancer in Asian men.

Verreault et al. (160) reported complex dinucleotide polymorphisms in the 3rd intron of the human HSD3B2 gene, located on chromosome 1p13, which encodes type II 3 β -hydroxysteroid dehydrogenase, which is expressed in the adrenals and testes, and catabolizes DHT (161). Devgan et al. (162) reported that the frequency of HSD3B2 alleles differs between African-American, European-American, and Asian men. One minor allele is unique for African-American men (6% allele frequency), whereas the most common allele is more frequent in European-Americans (52%) than among African-American or Asian men (34%–37%). The second most common allele is more frequent in African-Americans (25%) than in either Asians (15%) or European-American men (11%). As with the TA dinucleotide polymorphisms in the SRD5A2 gene, the functional significance of these HSD3B2 gene polymorphisms is not known.

The human androgen receptor gene, which is located on the X chromosome, also contains polymorphisms that are found as 8–31 CAG and 8–17 GGC (or GGN) microsatellite repeats in exon 1 encoding for the N-terminal domain of the protein where transactivation activity resides (139). The CAG repeat length has been demonstrated to determine transactivation activity of the androgen receptor, with 40 or more repeats being associated with human androgen insensitivity syndromes, such as spinal and bulbar muscular atrophy, and reduction of repeat length leading to increased transactivation activity *in vitro* (137–139). The functional significance of the GGC repeat length is not clear. Irvine et al. (163) reported that 75% of African-Americans (n = 44) had CAG repeat lengths of less than 22, whereas 62% of European-Americans (n = 39) and 49% of Asian-Americans (n = 39) had such shorter alleles. Very short alleles (<17 CAG repeats) occurred almost exclusively in African-Americans. The most common GGC allele (16 repeats) was found in 70% of Asian-Americans, 57% of European-Americans, and only 20% of African-Americans. The frequency of short GGC repeats (<16) was 61% in African-Americans, 27% in Asian-Americans, and 11% in European-Americans. GGC repeats longer than 16 were rare in the Asian-American men (3%) but more frequent in African-Americans (20%) and European-Americans (32%). Sartor et al. (164) essentially confirmed the findings on CAG repeats in a sample of African-Americans (n = 65) and European-American men (n = 130). Mean and median number of CAG

repeats was 19 in African-Americans and 21 in European-Americans, and 57% of the African-American men had less than 20 repeats, whereas only 28% of European-American men had such short repeats. Ekman et al. (165), however, did not find significant differences in the distribution of CAG repeats comparing Swedish and Japanese men with BPH but without cancer (n = 38 and 33, respectively), but Swedish men with prostate cancer (n = 118) had somewhat shorter CAG repeats (mean, 15.9; median, 15) than Japanese prostate cancer patients (n = 34; mean, 17.5; median, 17). In conclusion, in two studies short CAG repeat alleles in the androgen receptor gene, which are probably associated with greater androgen receptor transactivation activity, were most frequent in the highest-risk population (African-Americans) and least frequent in the lowest-risk group (Asian-Americans), whereas the frequency was intermediate in intermediate-risk European-Americans. The high frequency of short GGC repeats found in African-Americans may also be related with their extremely high risk for prostate cancer, but the functional significance of this polymorphism is not yet known.

Summary and conclusions. When examining Table 1, few clear or convincing patterns emerge about associations between circulating hormone concentrations and prostate cancer risk at the population level. Two studies examined levels of androstenedione glucuronide and $3\alpha,17\beta$ -androstenediol glucuronide, which are considered (166–168) indicators of 5α -reductase activity, particularly $3\alpha,17\beta$ -androstenediol glucuronide, which is a direct metabolite of DHT. In both studies, the levels of these 5α -reduced androgen metabolites were lower in low-risk Asian populations than in high-risk European-Americans (135,143). These findings suggest lower 5α -reductase activity in the Asians and consequently reduced formation of DHT and androgenic stimulation of the prostate. This notion is supported by the reported higher frequency in Asians than in European-Americans or African-Americans of a polymorphism in the 5α -reductase (SRD5A2) gene that appears to be associated with lower 5α -reductase activity (a valine to leucine mutation at codon 89) (136,153). However, no differences were found between Asians and European-Americans in a study in which overall conversion of testosterone into DHT was directly measured (148). Furthermore, androstenedione glucuronide and $3\alpha,17\alpha$ -androstenediol glucuronide levels were not higher in very high-risk African-Americans than in intermediate-risk European-Americans, and circulating levels of DHT and the ratio of DHT to testosterone were not different in ethnic populations (Asian, black, and white) that differ in prostate cancer risk (143,144,146). Thus, the relation between 5α -reductase activity and prostate cancer risk at the population level remains unclear at present.

Circulating levels of testosterone and/or free testosterone were slightly higher in African-Americans than in European-Americans in five of six studies that examined this question, but this finding is statistically significant in only one study (134). Furthermore, lower as well as higher testosterone concentrations have been found in lower-risk Asian or African men compared with European-Americans or African-Americans, although testosterone levels were lower in Asians living in Asia than in American populations regardless of ethnicity in two of three studies. Thus, these studies in ethnic/racial groups provide at present no substantive evidence in support of the hypothesis of a causal positive relation between elevated 5α -reductase activity and prostate cancer risk at the population level and only very

limited evidence for elevated (free) testosterone levels being associated with prostate cancer risk.

The only other patterns appearing in the data in Table 1 are that levels of estrogens are slightly higher (in five of five studies) and those of DHEA (sulfate) lower (in three of three studies) in black Africans and African-Americans than in men of European descent—hardly any data are available on Asians in this regard (63–65,134,140,141,144). The biologic significance of these observations is unclear, but they may be related to the high susceptibility of black men to prostate cancer when they live in the American environment. However, the above summarized endocrine differences between very high-risk African-Americans and high-risk European-Americans were not consistent in younger and older men, and they were not similar to the differences observed between the high-risk U.S. populations and the low-risk African black men (63–65,140,141). These inconsistencies raise the possibility that the factors and endocrine mechanisms that determine the difference in risk between African black men and African-Americans are dissimilar from those that determine the risk difference between African-Americans and European-Americans (11).

Finally, CAG repeat length polymorphism in the androgen receptor gene was found to be associated with prostate cancer risk in two studies. Short CAG repeat alleles are probably associated with greater androgen receptor transactivation activity. Such short CAG repeat alleles were most frequent in African-Americans (very high-risk), least frequent in Asian-Americans (low risk), and intermediate in European-Americans (intermediate risk). Another androgen receptor polymorphism in GGC repeats may also be related with risk for prostate cancer, but the functional significance of this polymorphism is unknown.

Association of Steroid Hormonal Factors With Prostate Cancer Risk in Population-Based Case–Control Studies

Circulating of steroid hormone levels in nested case–control studies. A summary of the results of population-based, nested case–control studies that examined the association between circulating levels of steroid hormones and risk for prostate cancer is provided in Table 2. The details of each study are summarized in the following paragraphs. One study by Carter et al. (169) concerned only 16 case subjects and contained considerable bias because of storage effects on hormone measurements, which were recognized but not controlled for. This study is, therefore, not further discussed here.

Nomura et al. (170) compared 98 prostate cancer case patients with matched control subjects from a cohort of 6860 Hawaiian-Japanese men, which were followed for an average of 14 years. No significant differences were found between case patients and control subjects or associations with risk for serum levels of testosterone, DHT, estrone, estradiol, and SHBG, measured once at the start of the cohort study (free testosterone levels were not determined). An elevation in risk was only observed for an increasing ratio of testosterone to DHT, which was borderline significant. This latter observation perhaps suggests an inverse relation between (peripheral) 5α -reductase activity and prostate cancer risk.

Barrett-Connor et al. (171) followed a Californian cohort of 1008 white, upper-middle class men between the ages of 40 and 79 years for a period of 14 years, during which time 57 cases of prostate cancer occurred (26 deaths and 31 incident cases). No significant relation was found between the risk for prostate can-

cer and baseline serum concentrations of testosterone, estrone, and SHBG. However, RR increased linearly with an increasing serum level of androstenedione, a testosterone precursor. RR also increased linearly with an increasing serum level of estradiol, but this finding was not statistically significant.

Hsing and Comstock (172) and Comstock et al. (173) reported results of a population-based, nested case-control study in a cohort of 25 620 men (98% European-American) in Maryland. Blood samples were obtained in 1974, and 98 cases of prostate cancer were identified in the first 13 years of follow-up (81 cases in 12 years of follow-up for DHEA and DHEA sulfate). Men 70 years and older as well as men younger than 70 years were studied separately (except for DHEA and DHEA sulfate). No significant differences were found between case patients and control subjects or associations with risk for baseline serum testosterone, DHT, DHEA, DHEA-sulfate, estrone, or estradiol. The ratio of testosterone to DHT was higher in case patients than in control subjects of all ages, and, for men younger than 70 years but not for older men, risk for prostate cancer increased with an increasing testosterone/DHT ratio; both findings were borderline significant ($0.05 < P < 0.1$). This latter observation could suggest an inverse relation between (peripheral) 5α -reductase activity and prostate cancer risk.

Nomura et al. (174) reported a follow-up of their 1988 study, including 141 case patients and 141 matched control subjects from their cohort of 6860 Hawaiian-Japanese men followed for an average of more than 20 years. In this population-based, nested case-control study, there were no significant differences between case patients and control subjects or associations with risk for baseline serum testosterone, free testosterone, DHT, ratio of testosterone to DHT, androsterone-glucuronide, 3α -androstanediol-glucuronide, and androstenedione.

DHEA sulfate was measured by Corder et al. (147) in stored serum samples of 181 men with prostate cancer (90 African-Americans and 91 European-Americans) and equal numbers of matched control subjects who were identified in a nested case-control study in a cohort of men in the Kaiser Permanente Medical Care Program in Northern California. For men younger than 57 years or for older men there were no significant differences between case subjects and control subjects in DHEA sulfate levels, which were also not associated with risk.

Gann et al. (175) conducted a prospective, nested case-control study on 222 case patients with prostate cancer and 390 matched control subjects obtained from the Physician's Health Study (a randomized intervention trial with aspirin and β -carotene in 22 071 U.S. male physicians, probably largely white), with a mean follow-up of approximately 6 years. There were no significant differences between case patients and control subjects for plasma testosterone, SHBG, DHT, ratio of testosterone to DHT, 3α -androstanediol-glucuronide, or estradiol. Several highly significant associations were found between plasma levels of SHBG and the steroid hormones studied. Therefore, odd ratios were calculated after simultaneous adjustment for all these endocrine factors for 222 matched case-control sets. This approach resulted in significant positive associations with risk for testosterone and the ratio of testosterone to DHT and inverse associations with risk for SHBG and estradiol. A positive association was also found with risk for 3α -androstanediol-glucuronide, which was borderline significant, but there was no association with risk for DHT. These observations support a relationship between elevated testosterone and

prostate cancer risk, but they are contradictory regarding a relation between (peripheral) 5α -reductase activity and prostate cancer risk.

Guess et al. (176) reported on a population-based, case-control study from a cohort of more than 125 000 European-American men in the Kaiser Permanente Medical Care Program. They compared 106 case patients and matched control subjects selected from men, with a median follow-up of 14 years (range, 5–23 years). No significant differences were found between case patients and control subjects or associations with risk for baseline serum testosterone, free testosterone, DHT, androsterone-glucuronide, or 3α -androstanediol-glucuronide.

Vatten et al. (177) conducted a population-based, nested case-control study of 59 case patients with prostate cancer and 180 matched control subjects from a cohort of approximately 28 000 Norwegian men, with a mean follow-up of 10 years (range, 1–19 years). There were no significant differences between case patients and control subjects or associations with risk for baseline serum testosterone, DHT, ratio of testosterone to DHT, or 3α -androstanediol-glucuronide.

Dorgan et al. (178) reported results from a population-based, nested case-control study of 116 case patients with prostate cancer and 231 matched control subjects from a cohort of 29 133 Finnish men from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study of cigarette smokers with a follow-up of 5–8 years. No significant differences were found between case patients and control subjects or associations with risk for baseline serum testosterone, free testosterone, SHBG, DHT, DHEA-sulfate, 3α -androstanediol-glucuronide, androstenedione, estrone, or estradiol. There was a nonsignificant trend toward a higher ratio of testosterone to DHT in case patients than in control subjects and a positive association with risk for this ratio. This finding may suggest an inverse relation between (peripheral) 5α -reductase activity and prostate cancer risk.

Heikkilä et al. (179) reported on the results of a population-based, nested case-control study in a Finnish cohort study in which serum was collected and stored, and a cohort of 16 481 men was followed for up to 24 years. During this period, 166 case patients with prostate cancer were identified and were matched to 300 control subjects. Serum levels of testosterone, SHBG, and androstenedione were similar in case patients and control subjects, and they were not associated with prostate cancer risk. When case patients identified in the first 8 years of follow-up were excluded, there was a borderline significant ($P = .06$) trend for increasing risk with higher levels of testosterone but not with SHBG or androstenedione. This finding supports the notion of a relationship between elevated androgen levels and prostate cancer risk.

Polymorphisms in genes involved in steroid hormone metabolism and action in population-based case-control studies. Several case-control studies have addressed the hypothesis (137–139) that functional polymorphisms in the 5α -reductase gene, in genes involved in testosterone biosynthesis or DHT catabolism, and in the androgen receptor gene could be associated with differences in prostate cancer risk. These studies are summarized in the following paragraphs.

Kantoff et al. (180) studied the association between prostate cancer risk and the polymorphisms in TA dinucleotide repeats in the transcribed 3' untranslated region of the human SRD5A2 gene encoding for type II 5α -reductase enzyme; the functional significance of these polymorphisms is not known, as indicated

Table 2. Summary of 10 nested case-control studies of circulating steroid hormone levels in men and their relation to risk for prostate cancer*

	Nomura et al. (170)	Barrett-Connor et al. (171)†	Hsing and colleagues (172,173)	Corder et al. (147)
No. of case patients/control subjects	98/98	57/NA†	98/98 (DHEA: 81/81)	181/181
Follow-up, y	14, approx.	14, mean	13 (DHEA: 12)	<17–24, maximum
Adjustment	—	BMI, age	—	—
Presentation values*	Mean	Median	Mean	Mean
Testosterone	97.6 OR = 0.99	105.2 RR = 1.00	102.0 OR = 1.5	
Free testosterone				
SHBG	97.5 OR = 0.85	101.4 RR = 1.04		
Dihydrotestosterone (DHT)	92.6 OR = 0.66		99.6 OR = 1.0	
Testosterone/DHT ratio	Increased** OR = 2.69§¶		94.1†† OR = 0.7 (3.0 for <70 y)	
Androsterone-glucuronide				
Androstanediol-glucuronide				
DHEA-sulfate (DHEA)			88.4 (DHEA: 89.0) OR = 0.82 (DHEA: 0.94)	96.5 OR = 1.00 (in men ≥57 y at baseline)
Androstenedione		105.2 RR = 1.26§		
Estrone (E ₁)	94.2 OR = 0.89	105.9 RR = 1.09	99.2 OR = 0.8	
Estradiol (E ₂)	95.2 OR = 0.57	107.7 RR = 1.10	105.3 OR = 1.0	

earlier. These investigators conducted a nested case-control study with the use of the Physician's Health Study cohort with 590 men with prostate cancer and 802 age-matched control subjects. They observed that the frequency of the most common genotype, TA(0)/TA(0), was 76.4% in case patients and 75.4% in control subjects, the frequency of the next most common genotypes, TA(0)/TA(9) and TA(0)/TA(18), was 22.4% in case patients and 22.1% in control subjects, and the frequency of genotypes TA(9)/TA(9) and TA(18)/TA(18) was 1.2% in case patients and 2.5% in control subjects; only two control subjects had a TA(9)/TA(18) genotype. Men that were homozygous for long repeats, TA(9)/TA(9) or TA(18)/TA(18), were at lower risk for prostate cancer than men with the predominant TA(0)/TA(0) genotype, with a borderline significant ($P = .08$) odds ratio (OR) of 0.47. These findings sharply contrast with the aforementioned observation that the long TA(18) alleles are unique to African-Americans, who are at very high risk for prostate cancer (152).

As indicated earlier, Makridakis et al. (153) identified another polymorphism in the SRD5A2 gene, a mis-sense alanine to threonine mutation at codon 89, apparently associated with an increase in 5 α -reductase activity. In a nested case-control study conducted in the Hawaii-Los Angeles Multiethnic Cohort Study of Diet and Cancer, the frequency of the mutation appeared to be low but was responsible for 8%–9% of cases in African-

American (203 case patients and 257 unmatched control subjects) and Hispanic men (160 case patients and 193 control subjects); no data were presented on other ethnic/racial groups (153). The RR (age-adjusted) of prostate cancer for possessing a mutated allele was 3.28 (95% confidence interval [CI] = 1.09–11.87) in African-American men and 2.50 (95% CI = 0.90–7.40) in Hispanics. For advanced prostate cancer, the RRs for possessing a mutated allele were more significant: 7.22 (95% CI = 2.17–27.91) in African-American men and 3.60 (95% CI = 1.09–12.27) in Hispanics. Although the results of this study support the notion that increased 5 α -reductase activity may be related to prostate cancer risk, it seems unlikely that the alanine to threonine mutation at codon 89 in the SRD5A2 gene is involved in a substantial proportion of prostate cancer cases.

The relation between prostate cancer risk and the occurrence of the aforementioned valine to leucine mutation at codon 89 in the SRD5A2 gene, a polymorphism that is associated with reduced 5 α -reductase activity, was examined by Febbo et al. (155) and Lunn et al. (154). Febbo et al. (155) conducted a nested case-control study with the use of the Physician's Health Study cohort with 584 men with prostate cancer and 799 matched control subjects. The valine-leucine and leucine-leucine genotypes were found in 50% of case patients and 51% of control subjects and were not associated with elevated prostate cancer

Table 2 (continued). Summary of 10 nested case-control studies of circulating steroid hormone levels in men and their relation to risk for prostate cancer*

Nomura et al. (174)	Gann et al. (175)‡	Guess et al. (176)	Vatten et al. (177)	Dorgan et al. (178)	Heikkilä et al. (179)‡
141/141	222/390	106/106	59/180	116/231	166/300
>20	6.3, mean	14 (5–23)	10, mean	4.1, median	<8–24
Age at entry, date/time of blood sampling	BMI, age, smoking, alcohol, exercise, all other hormones	BMI, smoking, alcohol, diabetes	—	—	Smoking, BMI, other hormones
Mean	Median	Median	Mean	Mean	Mean
100.2 OR = 1.03	102.8 OR = 2.60§	104.7 OR = 1.00	97.1 OR = 0.83	98.2 RR = 0.8	101.6 RR = 1.23¶#
103.6 OR = 1.09		100.0 OR: 1.14		102.6 RR: 1.1	
	91.7 OR = 0.46§			96.9 RR = 0.8	100.9 RR = 1.12
103.4 OR = 0.82	94.4 OR = 0.71 0.32 for ≥62 y)		98.0 OR = 0.83	95.7 RR = 0.7	
99.0 OR = no data	102.7 OR = 2.35§		99.2 OR = 1.31	Increased** RR: 1.7	
97.4 (OR = 1.37)		101.2 (OR = 1.13)			
109.2 OR = 0.85	104.6 OR - 1.60¶	106.4 OR = 1.16	102.1 OR = 1.10	102.3 RR = 1.2 106.3 RR = 1.2	
104.7 OR = 1.24				99.4 RR = 1.0 100.0 RR = 0.8 100.0 RR = 1.1	92.0 RR = 0.92
	97.7 OR = 0.56§				

*Values presented are hormone values (means or medians) of cases as percentage of the values in controls (set at 100%) and risk estimates [as either odds ratios (OR) or relative risks (RR)] of highest tertile or quartile relative to the lowest tertile, quartile, or quintile (set at 1.00). The indicated values are for cases presented as the percentages of the values in controls (set at 100%) or risk estimates indicating the relation with prostate cancer risk. Results of statistical analysis are indicated only when significant at a (two-sided) *P* value of less than .05, considering tests for differences between the lowest (referent) and highest tertile or quartile as well as for trend.

‡This study calculated prostate cancer rates for case patients and compared them with population data, which were also used to present median hormone levels for case patients and control subjects. RRs were calculated for an increase in hormone concentration equal to 1 standard deviation.

‡The risk estimates by hormone level were adjusted for the concentrations of all other hormones considered; this adjustment was not done in any of the other studies.

§Statistically significant difference between lowest (referent) and highest tertile or quartile different from controls.

||Statistically significant trend.

¶Borderline significant trend (.05 < *P* < .1).

#This trend was borderline significant (*P* = .06) only for a follow-up of longer than 8 years.

**No exact value is available, indicates increased value compared with control subjects.

‡‡Borderline significant (.05 < *P* < .1).

risk as compared with the valine–valine genotype (OR = 0.84–0.96). Lunn et al. (154) confirmed these findings in a case-control study that employed 108 prostate cancer patients from urology clinics at the University of North Carolina and nearby Duke University. Control subjects (*n* = 156) were drawn from BPH and impotence patients at the same clinics and not matched to case patients. The groups were predominantly European-American (5%–11% were African-American). The valine–leucine and leucine–leucine genotypes were found in 56% of case patients and 49% of control subjects and were not associated with prostate cancer risk as compared with the valine–valine genotype (OR = 1.3; 95% CI = 0.8–2.2). These obser-

vations are consistent with the absence of a relation between plasma concentrations of 3 α ,17 β -androstanoediol glucuronide and the three different SRD5A2 gene codon 89 genotypes reported by Febbo et al. (155).

In the same case-control study, Lunn et al. (154) also examined the association between prostate cancer risk and the aforementioned single base-pair mutation polymorphism in CYP17 gene (152c), encoding for the 17 α -hydroxylase and 17,20-lyase activity. The CYP17A2 allele containing a single base-pair mutation was found in 69% of case patients and 57% of control subjects and was associated with prostate cancer risk with an OR of 1.7 (95% CI = 1.0–3.0). The association appeared to be

limited to men younger than 65 years, with an increased OR of 2.30 (95% CI = 1.0–4.8). Contradictory findings of this association between prostate cancer risk and the presence of the CYP17A2 allele were reported from a Swedish case-control study by Wadelius et al. (181). The frequency of the A1/A2 or A2/A2 genotype was 61% in prostate cancer cases (n = 178) and 71% in population controls (n = 160). The OR of having the A1/A1 genotype versus the A1/A2 or A2/A2 genotype was 1.61 (95% CI = 1.02–2.53). This latter finding is consistent with a preliminary report of higher circulating testosterone levels found in men homozygous for the A1 allele than in men with an A2 allele of the CYP17 gene (181).

Six population-based, case-control studies with substantive numbers of cases examine the association between the aforementioned CAG and GGC (or GGN) repeat polymorphisms in the human androgen receptor gene (163,182–186). The results of these studies are summarized in Tables 3–5. As shown in Table 3, these studies indicate that CAG repeat lengths shorter than 22 may be associated with slightly increased risk for prostate cancer with elevated ORs or RRs found for <22 repeats (versus ≥ 22) in four studies (163,182–184), and for 20–21 repeats (versus <20 or ≥ 22) in one study (185). However, only in the study by Giovannucci et al. (182), but not in four other studies (163,183–185), the tendency of increased risk with decreasing repeat length was statistically significant. In addition to these six population-based, case-control studies, a study by Hakimi et al. (186) compared the CAG repeat lengths in the androgen receptor of 59 prostate cancer patients with published data of the general population (n = 370). Short repeats (≤ 17) were more frequent in cases than in the general population (OR = 3.7; 95% CI = 1.3–10.5; $P = .02$). Thus, these findings consistently indicate the possibility that prostate cancer risk is slightly increased with shorter CAG repeat alleles. This possibility may be related to a greater androgen receptor transactivation activity associated with shorter CAG repeat alleles, as indicated earlier.

Two of these six case-control studies and a seventh population-based, case-control study examined GGN or GGC repeats in the androgen receptor gene, the functional significance of which is not known at present (163,184,187). As shown in Table 4, these studies indicate that GGN or GGC repeat lengths may influence risk, but the results do not agree with one another. Hakimi et al. (186) also compared androgen receptor GGC repeat lengths in 54 prostate cancer patients with published population data (n = 110). Short repeats (≤ 14) were more frequent in case patients than in the general population (OR = 4.6; 95% CI = 1.3–16.1; $P = .02$). However, Correa-Cerro et al. (185) did not find such an association in a French-German population. Thus, even though two studies consisted of substantial numbers of case patients and control subjects (184,187), the influence of GGN or GGC repeat length on prostate cancer risk is presently not clear. The combined effects of CAG and GGN repeat length were also examined in the three case-control studies, and they were greater than those either polymorphism separately in all three, as indicated in Table 5. CAG repeats of less than 22 or 21 appeared to be the consistent factor in this interaction in all three studies and were associated with (borderline) significantly increased risk. However, the functional significance of this combined effect is not clear.

Summary and conclusions. When examining Table 2, no clear or convincing patterns emerge about associations between circulating hormone concentrations and prostate cancer risk, with few exceptions. In most studies, an association was found between increased risk and increased ratios of testosterone to DHT, which reached statistical significance in three of six studies. Although this finding suggests a relation between reduced 5α -reductase activity and prostate cancer risk, no associations were found between risk and the levels of androsterone glucuronide and $3\alpha,17\beta$ -androstenediol glucuronide, indicators of 5α -reductase activity (166–168)—a borderline significant trend for such an association was found in only one (175) of five studies (174–178). Significant associations between prostate

Table 3. Summary of five case-control studies of CAG repeat length polymorphisms in the androgen receptor circulating levels in relation to risk for prostate cancer

Study (reference No.)	No. of case subjects	No. of control subjects	CAG repeat comparison	OR/RR	95% CI	N (case subjects/control subjects)
Irvine et al. (163)	57	39	≥ 22	1.00	Referent	19/15
			<22	1.25	0.88–1.73	38/24
Ingles et al. (183)	57	169	≥ 22	1.00	Referent	19/68
			20–21	0.89	0.41–1.94	14/56
			<20	1.91	0.94–3.88	24/45
				Trend: not significant		
Stanford et al. (184)	281	266	≥ 22	1.00	Referent	136/140
			<22	1.23	0.88–1.73	145/126
Giovannucci et al. (182)	587	588	≥ 26	1.00	Referent	60/72
			24–25	1.02	0.66–1.58	98/115
			22–23	1.17	0.76–1.80	116/119
			21	1.35	0.87–2.09	113/101
			20	1.28	0.79–2.08	69/65
			19	1.22	0.75–2.00	62/61
≥ 18	1.52	0.92–2.49	69/55			
				Trend: $P = .04$		
Correa-Cerro et al. (185)	132	105	≥ 24	1.00	Referent	39/28
			22–23	1.02	0.49–2.13	30/22
			20–21	1.43	0.73–2.82	34/35
			≥ 19	0.96	0.45–2.03	29/20
				Trend: not significant		

CI = confidence interval; OR = odds ratio; RR = relative risk.

Table 4. Summary of three case-control studies of GGC/GGN repeat length polymorphisms in the androgen receptor circulating levels in relation to risk for prostate cancer

Study (reference No.)	No. of case subjects	No. of control subjects	GGC/N repeat comparison	OR/RR	95% CI	N (case subjects/control subjects)
Irvine et al. (163)	57	37	16	1.00	Referent	30/21
			Not 16	1.18	Not presented	27/16
Stanford et al. (184)	257	250	>16	1.00	Referent	56/75
			≤16	1.60*	1.07–2.41	201/175
Platz et al. (187)	582	794	Not 23	1.00	Referent	244/369
			23	1.20	0.97–1.49	338/425

*Odds ratio (OR) or relative risk (RR) is significantly different from referent value at the $P < .05$ level. CI = confidence interval.

Table 5. Summary of three case-control studies of the interaction of CAG and GGC/GGN repeat length polymorphisms in the androgen receptor circulating levels in relation to risk for prostate cancer

Study (reference No.)	No. of case subjects	No. of control subjects	CAG/GGC-N repeat comparison	OR/RR	95% CI	N (case subjects/control subjects)
Irvine et al. (163)	57	37	≥22/16	1.00	Referent	34/28
			<22/not 16	2.10 ($P = .08$)	Not presented	23/9
Stanford et al. (184)	257	250	≥22/>16	1.00	Referent	22/32
			≥22/≤16	1.15	0.56–2.35	32/41
			<22/>16	1.54	0.83–2.86	97/93
			<22/≥16	2.05*	1.09–3.84	98/77
			Trend: $P = .008$			
Platz et al. (187)	582	794	>23/not 23	1.00	Referent	66/119
			>23/23	1.17	0.77–1.77	90/133
			21–23/not 23	1.39	0.93–2.06	75/116
			21–23/23	1.22	0.82–1.83	152/185
			<21/not 23	1.49*	1.02–2.15	103/134
			<21/23	1.62*	1.07–2.44	96/107

*Odds ratio (OR) or relative risk (RR) is significantly different from referent value at the $P < .05$ level. CI = confidence interval.

cancer risk and elevated levels of testosterone and androstenedione or decreased levels of SHBG and estradiol were found each in only a single study [(175) for testosterone, SHBG, and estradiol; (171) for androstenedione], and they were not observed in eight (testosterone), four (SHBG and estradiol), or three (androstenedione) other studies. It is possible that relevant associations may have been missed in most studies, because the data for each individual hormone were not adjusted for concentrations of other hormones studied, even though there are many intercorrelations between circulating levels of these hormones. Only in the study by Gann et al. (175) were these types of adjustments applied, after which risk was significantly associated with increased circulating testosterone levels and testosterone/DHT ratio, as well as decreased concentrations of SHBG and estradiol, and, in men older than 61 years, DHT. A meta-analysis study by Eaton et al. (188) used most but not all studies included in this overview, as well as a study that was discounted here (169) and some unpublished data. They found no significant differences for the ratios of mean hormone levels between case patients and control subjects, with the exception of slightly elevated levels of $3\alpha,17\beta$ -androstenediol glucuronide. This analysis is essentially in agreement with the analysis of this overview, with the only consistent finding slightly elevated ratios between case patients and control subjects of $3\alpha,17\beta$ -androstenediol glucuronide in five of five studies (Table 2). However, Eaton et al. (188) did not take into account the risk estimates produced by these studies, which seriously limits its conclusions.

The results of three nested case-control studies on the rela-

tion between prostate cancer risk and two different polymorphisms in the human type II 5α -reductase enzyme gene (SRD5A2) do not support the notion of an association between risk and increased 5α -reductase activity (154,155,180). However, an infrequent polymorphism associated with increased 5α -reductase activity was more common in case patients than in control subjects in one case-control study (156), which indicates that associations between polymorphisms in the SRD5A2 gene and prostate cancer risk cannot be discounted. Data on a relation between prostate cancer risk and a polymorphism in the CYP17 gene, which encodes for the 17α -hydroxylase and $17,20$ -lyase activity involved in androgen biosynthesis, are contradictory (154,181). Furthermore, the functional significance of this polymorphism in males is not yet known. In four of five similar nested case-control studies of polymorphisms in trinucleotide repeats in the promoter region of the androgen receptor gene, an association was found between risk and short CAG repeat alleles—short CAG repeat lengths are associated with greater androgen receptor transactivation activity. However, this association was weak and significant in only one study. An association between risk and polymorphisms in androgen receptor GGC or GGN repeat lengths is not clear because results of three studies were inconsistent, and the functional significance of these polymorphisms is not known. There is possibly an interaction between CAG and GGC/GGN repeat length in relation to prostate cancer risk, but results of the three studies examining this possible interaction were inconsistent. Short CAG repeats were also correlated with advanced disease and/or early onset of prostate cancer (182–186,189,190).

Epidemiologic Evidence for Involvement of Steroid Hormones: Summary and Conclusions

Taken together, the results of the above summarized studies do not provide unequivocal or strong evidence for any particular association between prostate cancer risk and circulating levels of hormones or polymorphisms in genes that encode for proteins involved in steroid hormone action or metabolism. Only a few associations with prostate cancer risk have been observed consistently (in at least three studies), and they are weak at best: 1) slightly, but mostly not significantly, higher circulating testosterone and estrogen levels and lower DHEA (sulfate) levels in high-risk African-American men as compared with lower-risk European-American men, and 2) a CAG repeat length polymorphism in the androgen receptor gene with short repeat lengths associated with increased risk and increased receptor transactivation activity. The evidence for involvement of activity of the enzyme 5 α -reductase, which is critical in androgen action in the prostate, is inconsistent and contradictory.

Difficulties in Interpretation

Several important points should be considered in interpreting these observations: First, there are numerous potential problems with most studies that measure circulating hormone levels, such as the usually large intra- and interassay variability in the immunoassays used (122,191–194). Typically, only single blood samples are available, and within-subject variations over time and possible differences in circadian rhythms cannot be taken into account. Another problem is that there are many interrelationships between various hormones (144,174), which only an occasional study has taken into account during data analysis (175). Second, young Japanese men and Chinese men from Hong Kong are probably at least partially westernized in their lifestyle (194), and they can, therefore, not simply be compared with older Asian men. Young men that are hormonally studied today may have a prostate cancer risk that is different from the currently recorded risk in older men of the same population, as suggested by the rising prostate cancer rates in Japan (194). Third, the factors that cause the differences in prostate cancer risk between black, white, and Asian men in the United States may be different from those that determine differences in prostate cancer risk between Asian or African populations and populations in the United States or West European countries, as indicated earlier.

Another crucial issue is that circulating hormone levels and polymorphisms in critical genes provide very little information about concentrations at the molecular targets of these hormones in the prostate gland or about steroid hormone metabolic processes within the prostate. For example, less than 10% of circulating DHT is produced by the prostate, and a substantial proportion of serum 3 α ,17 β -androstenediol glucuronide is derived from nonprostate sources; these two steroids are, therefore, not very good indicators of prostatic 5 α -reductase activity (166–168). Also, aromatase activity has been identified in the human prostate and the LNCaP prostate cancer cell line (195–199), although there are reports of contradictory findings (200,201) that may be related to methodologic differences. In addition, estrogen levels in the human prostate exceed those found in the circulation (202). Thus, local formation of estrogens in the prostate may occur and may contribute to dysregulation of growth. Finally, although there is some information about the functional

significance of some polymorphisms in genes encoding for proteins involved in steroid hormone action or metabolism, their influence on the prostatic activity of steroid hormone metabolizing enzymes or the activity of steroid hormone receptors in the prostate is not known. In view of the highly complex and often tissue-specific mechanisms of regulation of gene expression, it is likely that these polymorphisms have only limited and probably cell type-specific influences on these regulatory processes.

Hypotheses

From the studies summarized above, four possible hypotheses emerge about steroid hormone factors associated with prostate cancer risk: 1) slightly elevated (bioavailable) testosterone serum levels, as indicated by studies comparing healthy low- and high-risk men (134,135,140,144,148); 2) increased peripheral and possibly prostatic activity of 5 α -reductase (135,143,153,175); 3) slightly increased serum levels of estrogens, as indicated by studies comparing healthy low- and high-risk men (63–65,134,140,144); and/or 4) increased androgen receptor transactivation activity related to short CAG repeats in the promoter region of the androgen receptor gene (163,182–184). However, there are contradictory data for each of these hypotheses, as indicated earlier and documented in Tables 1–5, and the observed associations were at best weak.

Circulating Androgens and Estrogens

Two of these four hypotheses implicate higher bioavailable circulating androgen levels in high-risk men compared with low-risk populations (191), which may be related to increased androgen production or exposure (136). For example, although body mass index or obesity does not appear to be a risk factor, there are some indications that muscle mass is positively associated with risk, perhaps reflecting exposure to endogenous androgens or anabolic steroids. However, studies of polymorphisms in the CYP17 gene (which encodes for the cytochrome P450C17 α -hydroxylase and 17,20-lyase activity involved in androgen biosynthesis) do not support the notion of a relation between risk and androgen production rates. As will be detailed later, the results of several animal model studies strongly support this contention, but more research is needed to confirm and further define this association in humans and to establish its underlying biologic mechanisms (increased androgen production or 5 α -reductase activity and decreased DHT catabolism) (191). Furthermore, elevated androgen levels do not universally occur in all high-risk groups. Meikle and colleagues (203,204) studied brothers (ages 47–75 years) and sons (ages 22–43 years) of prostate cancer patients who have a threefold to fourfold excess risk compared with unrelated control subjects of the same age ranges. They reported that serum levels of testosterone and DHT were significantly lower, rather than higher as one might expect, in these blood relatives of prostate cancer patients. Because circulating testosterone levels may thus be lower in men with a family history of prostate cancer than in other men, hormonal involvement in familial aggregation of prostate cancer risk seems paradoxical and the involvement of androgens in hereditary prostate cancer may be different from that in sporadic prostate cancer. Zumoff et al. (205) observed that circulating levels of testosterone, but not DHT, were markedly lower in prostate cancer patients younger than 65 years than in those patients 65 years and older. However, control subjects had tes-

tosterone levels that were similar to those of prostate cancer patients of 65 years and older. In several of the studies, summarized in Tables 1 and 2, findings were markedly different when comparing younger (18 to 25–40 years) and older healthy men (>40 years) or comparing younger (<62–70 years) and older men (>62–70 years) with prostate cancer and their age-matched controls. Circulating testosterone levels are also known to paradoxically decrease with aging, whereas prostate cancer risk increases (144–146,206). At the same time, SHBG levels increase with age and estrogen levels remain constant or increase (144,146,206). Thus, bioavailable estrogens and particularly testosterone decrease with increasing age and increasing risk for prostate cancer. This situation may explain the lower prostatic concentrations of DHT with aging reported by Krieg et al. (202) but is in contrast to increasing prostatic estrogen levels they observed with aging. These observations suggest that the role of androgens and estrogens in prostate carcinogenesis may differ in younger men (early onset prostate cancer) and in older men (late-onset cancer) and may be different in men that are at high risk because of familial predisposition and those at high risk associated with their ethnic background or living environment. It is also possible that risk-increasing effects of elevated circulating levels of androgens and estrogens may be effectuated early in life (134,135,141,143) or even before birth (142,207), rather than in the one or two decades preceding the diagnosis of prostate cancer.

Another risk factor may be increased androgen receptor activity related to genetic polymorphisms in the androgen receptor gene. Although body mass index or obesity does not appear to be a risk factor, there are some indications that muscle mass is positively correlated with risk, perhaps reflecting exposure to endogenous androgens or anabolic steroids. Heavy alcohol use accompanied with liver disease may increase risk and be related with decreased clearance of estrogens and elevated circulating estrogen levels. Estrogen levels were also elevated in healthy black men living in the United States compared with European-American men, and this is perhaps associated with the very high risk for prostate cancer of black men living in the United States. However, no association between risk and circulating estrogen levels was found in nested case–control studies (in predominantly European-American cohorts). Thus, the epidemiologic evidence for involvement of androgenic and estrogenic steroid hormones in human prostate carcinogenesis remains inconclusive (191).

Interactions of Environmental, Hormonal, and Racial/Ethnic Factors

The strongest single risk factor for prostate cancer appears to be a western lifestyle, particularly western dietary habits, including a high-fat intake. It is conceivable that dietary risk factors, such as fat, exert their enhancing effects mediated by a hormonal mechanism that involves androgens (11,15,208). For example, heavy alcohol use accompanied with liver disease may increase risk and be related with decreased clearance of estrogens and elevated circulating estrogen levels. However, it is unlikely that lifestyle is the sole factor that explains the differences in prostate cancer risk between Asian and American populations (10,11,136). Genetic factors are probably also important determinants of racial/ethnic disparity of sporadic prostate cancer, such as 5 α -reductase activity or increased androgen receptor

activity related to polymorphisms in the 5 α -reductase or androgen receptor genes.

The single most important combination of risk factors is to be of sub-Saharan African descent and to reside in the United States—African-Americans, as a group, have twice the risk of European-Americans. The reasons for the black–white disparity in prostate cancer rates in the United States are not understood. Environmental exposures (in the broadest sense of the term) are probably responsible for a large fraction of this disparity (10,11,15,209). A relation with similar racial disparities in exposure to potential carcinogens and high-risk dietary habits has been proposed (11,22,59). However, genetic factors, such as polymorphisms in the 5 α -reductase or androgen receptor genes may be related with increased 5 α -reductase activity or androgen receptor activity. However, environmentally influenced hormonal mechanisms may be involved as well, possibly acting *in utero* (207). For example, young, African-American men and pregnant, African-American women have been reported to have higher circulating levels of androgens and estrogens than European-Americans (142). Estrogen levels were also elevated in healthy black men living in the United States compared with European-American men, and this finding is perhaps associated with the very high risk for prostate cancer of black men living in the United States. However, no association between risk and circulating estrogen levels was found in nested case–control studies (in predominantly European-American cohorts).

Conclusions

The epidemiologic evidence for involvement of androgenic and estrogenic steroid hormones in human prostate carcinogenesis remains inconclusive (191). The most promising hormonal risk factor candidates are elevated circulating testosterone and estrogen levels and polymorphisms in the androgen receptor gene associated with increased receptor transactivation activity. In addition, hormonal effects of dietary factors, such as fat, may play a critical role in prostate carcinogenesis in humans, as well as, perhaps, still unexplored/unknown polymorphisms in genes encoding for proteins involved in steroid hormone metabolism and hormone action.

PROSTATE CANCER AND STEROID HORMONES IN LABORATORY ANIMALS

Spontaneously occurring prostate tumors are rare in most species (5–7,210), with exception of the dog and, particularly, humans. It is not understood why prostate cancer is so common in men, whereas it is very rare in almost all other species. There are compelling reasons to implicate hormones, particularly androgenic and estrogenic steroids, in human prostate carcinogenesis, as indicated earlier. The same steroid hormones are also very powerful factors in the induction of prostate cancer in rodent species in which spontaneous prostate neoplasms are rare (15,56,211,212). Pertinent studies concerning the role of androgens and estrogens in experimental prostate carcinogenesis are summarized in the following sections.

It is important to first point out that the various lobes of the rat prostate differ in their propensity to develop prostate carcinomas, either spontaneously or induced by carcinogens or hormones (15,210,211). The rodent prostate, unlike the human or canine prostate, consists of distinct paired lobes: the ventral, dorsal, lateral, and anterior lobes; the dorsal and lateral lobes are

frequently referred to as the dorsolateral prostate, and the anterior lobe is more commonly termed the coagulating gland. In the human and canine prostate, these lobes have merged into one gland, in which different zones have been defined (213). A homologue of the rodent ventral lobe is not present in the human gland (214).

Hormonal Induction

Testosterone

Long-term administration of testosterone induces a low to moderate (5%–56%) incidence of prostate cancer in several rat strains (210,215–220) but not in all strains (221). The induced tumors were adenocarcinomas in all studies but one, in which some squamous cell carcinomas were also observed (218). These carcinomas appeared to develop from the dorsolateral prostate and/or coagulating gland but not from the ventral prostate lobe (210,215–219). The prostate carcinoma incidence in most of these studies was low (5%–20%) (215,218–220). Only the studies reported by Pollard et al. (216,222–225) with the use of the Lobund–Wistar strain sometimes had higher carcinoma incidences, but the incidences varied considerably (0%–60%). In the only other studies with the Lobund–Wistar strain, a low incidence of prostate cancer was found but a high incidence of seminal vesicle tumors was found (219,226). The actual dose of testosterone considerably fluctuated over time in many of these studies from five to 10 times control values down to control values (221,226), but, even when the level of circulating testosterone was kept steadily elevated by twofold to threefold, prostate carcinomas were induced (220). These data indicate that testosterone acts as a complete carcinogen for the rat prostate.

Estrogens and Testosterone

Noble (215) was the first to demonstrate that testosterone is carcinogenic for the rat prostate. He also established that sequential treatment with testosterone and estrogens was even more effective than testosterone per se in the Noble (or NBL) rat strain that he developed. Long-term treatment of NBL rats with a combination of testosterone and estradiol leads to a 100% incidence of adenocarcinomas, which develop from the periurethral ducts of the dorsolateral and anterior prostate (227–229). The development of these tumors is preceded by the appearance of epithelial dysplasia in these ducts and in the acini of the dorsolateral prostate in 100% of treated animals (228–230). Carcinomas developing from the acinar dysplasia in the periphery of the prostate gland have not been observed, but the absence of malignant progression of these lesions, which are morphologically similar to human prostatic intraepithelial neoplasia, has not been established with certainty (228,229). When diethylstilbestrol (DES) was combined with testosterone, the treatment resulted in widespread dysplasia in the ventral prostate, but less or no dysplasia in the dorsolateral prostate (230). Long-term treatment of NBL rats with DES and testosterone induced a low carcinoma incidence in the dorsolateral prostate and some early-stage carcinomas (carcinoma *in situ*) in the ventral lobe (229). When the combined testosterone and estradiol treatment was given to Sprague–Dawley rats, dysplasia developed in the same high frequency as in NBL rats, but the incidence of carcinomas was considerably lower (228,229). Thus, a very high incidence of prostate cancer results from the addition of estrogen to tes-

tosterone treatment, which by itself produces prostate cancer in 35%–40% of treated NBL rats.

Perinatal Estrogen Exposure

Carcinogenic effects of perinatal exposure to DES on the accessory sex glands in male experimental animals have been described in mice, rats, and hamsters (15,231–233). McLachlan and colleagues (231,234) found that 25% of the male offspring of CD-1 mice that had been treated with DES on days 9–16 of gestation had nodular enlargements of the coagulating gland, ampullary glands, and colliculus seminalis at an age of 9–10 months. In one animal, a lesion was found in the area of the coagulating gland and colliculus seminalis that resembled early neoplasia (234). Of eight prenatally DES-exposed male mice that survived for 20–26 months, one had an adenocarcinoma of the coagulating gland, three had hyperplasia of the coagulating gland, two had hyperplasia of the ventral prostate, one had a carcinoma of the seminal vesicle, and two had squamous metaplasia of the seminal vesicle (231,232). No such lesions occurred in control animals. Prenatal DES exposure of mice also induces testicular tumors (particularly of the rete testis) and non-neoplastic lesions in the testes and epididymis (235).

Treatment of Han:NMRI mice with DES or estradiol on the first 3 days of life resulted in a 90%–100% incidence of epithelial dysplasia of the periurethral glands and of the periurethral proximal parts of the dorsolateral prostate, coagulating glands, and seminal vesicles after 12–18 months (236,237). Subsequent treatment with DHT and estradiol from 9–12 months of age increased the severity of the dysplasia when the prostates were examined at 12 months, suggesting permanent estrogen hypersensitivity of these tissues. Arai et al. (232) treated Wistar rats with DES for the first 30 days of life. One group was neonatally castrated and the second group remained intact. Two of 11 castrated, DES-exposed rats developed squamous cell carcinomas in the area of the dorsolateral prostate, coagulating gland, and ejaculatory ducts, and all these animals had papillary hyperplasia and squamous metaplasia of the coagulating gland and ejaculatory duct. Squamous metaplasia was also found in some of eight noncastrated DES-exposed rats, but no hyperplasia or neoplasia developed. Vorherr et al. (238) obtained similar results in rats exposed prenatally and/or neonatally to DES.

The results of these studies demonstrate that prenatal and neonatal estrogen exposure of rodents can be carcinogenic for the prostate. Data also suggest that these treatments may imprint permanent alterations in the hormonal sensitivity of the prostate that may play a role in the carcinogenic effect of perinatal estrogen exposure.

Induction by Chemical Carcinogens and Hormones

Exposure to Chemical Carcinogens Combined With Hormonal Stimulation of Cell Proliferation

Very few reports are available of induction of prostate tumors by chemical carcinogens administered systemically or via the oral or inhalation routes. Only two organic chemical carcinogens induce prostate adenocarcinomas on systemic administration, without any additional concomitant or subsequent treatment, *N*-nitrosobis(oxopropyl)amine (BOP) and 3,2'-dimethyl-4-aminobiphenyl (DMAB) (239,240). Direct application of chemical carcinogens to prostate tissue in experimental animals produces sarcomas or squamous cell carcinomas (7,241).

Short-term hormonal stimulation of cell proliferation in the prostate at the time of carcinogen administration has been demonstrated to increase the sensitivity of the target cells for tumor induction. Dorsolateral prostate adenocarcinomas have been produced at 5%–25% incidence when prostatic cell proliferation was stimulated in combination with treatment with indirect-acting carcinogens (such as DMAB and 7,12-dimethylbenz[*a*]anthracene) and direct-acting chemical carcinogens (such as *N*-methyl-*N*-nitrosourea [MNU]); these carcinogens do not induce these tumors when administered alone (220,242–245). However, in some studies, only a very small or no enhancing effect was found of stimulation of prostatic cell proliferation on prostate carcinoma induction by carcinogens (218, 221,246,247). Nevertheless, stimulation of cell proliferation appears to be co-carcinogenic for prostate cancer induction by many chemical carcinogens.

Testosterone as Tumor Promoter of Prostate Carcinogenesis

Long-term administration of testosterone to rats markedly enhances prostatic carcinogenesis following initial treatment with chemical carcinogens that target the prostate because of tissue-specific metabolism (DMAB and BOP) and/or concurrent hormonal stimulation of prostatic cell proliferation (70,71,211, 217–221,223,225,248). This enhancement may not occur if certain requirements are not adequately met (210,211,218). For example, after a single injection of BOP or MNU given to F344 rats without concurrent stimulation of prostatic cell proliferation, long-term testosterone treatment did not enhance prostatic carcinogenesis (221). High incidences (66%–83%) of adenocarcinomas of the dorsolateral and/or anterior prostate, but not the ventral prostate, were induced by chronic treatment with testosterone, following a single administration of MNU or BOP given during stimulation of prostatic cell proliferation in Wistar rats, or during and after 10 repeated biweekly injections of DMAB in F344 rats (70,71,210,211,218,220,221,248). This effect is somewhat strain dependent, because when the same treatments were given to Lobund–Wistar rats, rather variable prostate carcinoma incidences of between 50% and 97% were reported by Pollard and colleagues (217,223,225) and only a 21%–24% incidence was found by Hoover et al. (219) and Tamano et al. (226).

The enhancing effect of testosterone on prostate carcinogenesis is remarkably confined to the dorsolateral and anterior prostate, and no tumors occur in the ventral prostate. In fact, long-term testosterone treatment produces a shift of the site of DMAB- and BOP-induced carcinoma occurrence from exclusively the ventral lobe to predominantly the dorsolateral and anterior lobes (218,221,248). The dose–response relationship between testosterone dose and prostate carcinoma yield is very steep. A slight (less than 1.5-fold) elevation of circulating testosterone levels is sufficient for a near-maximal enhancement of the tumor response, and a twofold to threefold elevation is sufficient for a maximal response. These concentrations are within the normal range of circulating testosterone levels in the rat (220). Thus, testosterone is a powerful tumor promoter for the rat prostate.

Effects of Testosterone on Prostate Cancer Induction by Cadmium and Ionizing Radiation

Cadmium can be carcinogenic for the rat ventral prostate as demonstrated by Waalkes et al. (249,250). The selective sensi-

tivity of the ventral prostate lobe for the carcinogenic action of cadmium is most likely due to its lack of cadmium-binding proteins (251). A single injection of cadmium chloride produced *in situ* (noninvasive) carcinomas in the ventral lobe provided that cadmium-induced testicular toxicity was avoided, either by keeping the cadmium dose below 5 mg/kg, by intramuscular rather than subcutaneous administration of the cadmium, or by antagonizing the testicular toxicity of cadmium by simultaneous administration of sufficient amounts of zinc. These observations indicate that cadmium induces proliferative lesions in the rat ventral prostate only when testicular function, conceivably testosterone production, is intact. In addition, these data suggest that androgens also act as tumor promoters in this system, but this hypothesis has not been tested. Other mechanisms may also be involved, because, for example, testosterone considerably increases cadmium disposition and retention in the rat ventral prostate (252).

Local x-ray exposure of the pelvis has been shown to induce prostate carcinomas in ICR/JCL mice (253) and in Sprague–Dawley rats (254). Prostate carcinomas (33% incidence) developed only in rats that were castrated and received androgen replacement prior to irradiation, but intact and only castrated rats did not develop prostate cancer following irradiation. These observations suggest that testosterone treatment was required for tumor development, perhaps as tumor promoter (254).

Prostate Cancer and Steroid Hormones in Laboratory Animals: Summary and Conclusions

Stimulation of prostatic epithelial cell proliferation by androgens during exposure to chemical carcinogens increases the susceptibility of the rat prostate to cancer induction in a co-carcinogenic fashion. Testosterone appears to be a weak complete carcinogen, but it is a very strong tumor promoter for the rat prostate at near-physiologic plasma concentrations (220). The very powerful tumor-promoting activity of androgens perhaps explains their weak complete carcinogenic activity on the rat prostate. A slight elevation of circulating testosterone can lead to a marked increase in prostate cancer in rat models. This observation is highly relevant in view of the aforementioned possible weak association between human prostate cancer risk and slightly elevated circulating androgen levels found in some epidemiologic studies (191). Thus, the experimental data provide strong support for the concept that minimal increases in circulating androgens may have substantial enhancing effects on prostate cancer risk. The addition of estradiol to chronic treatment with testosterone strongly enhances the carcinogenic activity of the androgen for the rat dorsolateral prostate. The sensitivity for the carcinogenic effects of this hormone combination appears to be confined to the periurethral, proximal ducts of the dorsolateral and anterior prostate. The estradiol plus testosterone treatment also induces acinar lesions that are similar to human prostatic intraepithelial neoplasia. These observations strongly suggest a critical role for estrogens in prostate carcinogenesis. Perinatal estrogen exposure is also carcinogenic for the male rodent accessory sex glands. The periurethral, proximal ducts of the dorsolateral and anterior prostate and seminal vesicle and the intraprostatic urethral epithelium appear to be the most sensitive rodent male genital tract tissue to the carcinogenic effects of perinatal estrogen exposure. Of interest in this regard is the report by Driscoll and Taylor (255) of hypertrophy and squamous metaplasia of the prostatic utricle and prostatic ducts in

55%–71% of 31 infants that had been exposed to DES *in utero* and had died perinatally from unrelated causes. Such squamous metaplastic changes have also been reported to occur in human fetal prostatic tissue transplanted into nude mice that were subsequently treated with DES (256). These human observations suggest that the DES findings in rodents may have human relevance.

MECHANISMS OF HORMONAL PROSTATE CARCINOGENESIS

As stipulated before, there are compelling reasons to assume that androgens play a critical role in prostate carcinogenesis, and there is experimental evidence to suggest that estrogens are involved as well (56). Because of the hormonal nature of these steroids, receptor mediation has been proposed as the major mechanism by which androgens and estrogens act in the causation of prostate cancer (257). For androgens, mechanisms other than those mediated by androgen receptors seem unlikely, except for the generation of estrogens via aromatization. For estrogens, however, nonreceptor-mediated genotoxic effects are conceivable, in addition to receptor-mediated processes (56). These various potential mechanisms are discussed in the following sections.

Stromal–Epithelial Interactions

First, it is important to emphasize that considerable evidence indicates interactions between epithelial and stromal cells in the normal prostate. Such interactions are undoubtedly critical and may be essential in prostate carcinogenesis as well, because prostatic mesenchyme is known to be a mediator of androgen action in the developing and adult rodent prostate and possibly the human prostate (258,259). No studies, however, have directly addressed the role of stromal–epithelial interaction in human or rodent prostate carcinogenesis. Krieg et al. (202) studied steroid hormone concentrations in stromal and epithelial compartments of normal human prostates from subjects varying from 20 to 80 years of age. DHT concentrations in the epithelium decreased considerably with aging, but they remained stable in stromal cells, whereas testosterone concentrations appeared unaffected by age in either compartment. These data suggest that the activity of 5 α -reductase in the epithelium decreases with aging but remains intact in the stroma. However, concentrations of estradiol and estrone in the stroma, but not the epithelium, increased markedly with aging. These observations suggest that the prostatic stroma is an important site for both androgen and estrogen action and metabolism, such as aromatase activity, which seems to increase with aging because estrogens accumulate with aging and androgen levels remain stable. This is unlike the concentrations of estrogens and androgens in the circulation or in epithelial cells, where both decrease. Thus, it is conceivable that with aging and increasing risk for prostate cancer the prostatic stroma continues to be an important androgen signal mediator to the epithelium and is an increasingly important local producer of estrogens.

Role of Androgens in Prostate Carcinogenesis

The results of the earlier summarized rodent experiments clearly indicate carcinogenic and strong tumor-promoting properties of androgens, and the results of a limited number of epidemiologic studies provide some support for the notion that androgens may have such effects in humans. However, the

mechanisms of the carcinogenic and tumor-promoting effects of androgens on the rodent prostate are not known with certainty. The very steep relationship between testosterone dose and prostate carcinoma response in rat models is suggestive of involvement of an androgen receptor-mediated mechanism (220). Other mechanisms may nevertheless be involved as well. For example, Ripple et al. (260) observed increases in indicators of oxidative stress in androgen-sensitive LNCaP human prostate cancer cells exposed to DHT, although it is possible that these effects were androgen receptor mediated.

Stimulation of Cell Proliferation and Carcinogenic and Tumor-Promoting Effects of Androgens

The postulated role of androgens in human prostate carcinogenesis has been ascribed to their androgen receptor-mediated stimulating effects on prostatic cell proliferation (136,257). No direct evidence, however, is available that elevation of circulating testosterone leads to increased cell proliferation in the human prostate. It has been well established that androgen administration to castrated rodents causes elevation of prostatic cell proliferation similar to that observed in cell cycle synchronization experiments with cells *in vitro*. However, the increase in prostatic cell proliferation caused by testosterone or DHT administration in castrated rodents is only transient, and after a few days cell turnover returns to its normal very low levels (261). Thus, continued androgen treatment of rodents does not result in permanently elevated cell proliferation rates in the male accessory sex glands, but rather appears to support differentiation. Furthermore, DHT may even suppress prostatic cell proliferation in intact rats (228). Thus, a mere continuous stimulation of cell proliferation is unlikely to be the major mechanism of the enhancing effects of testosterone on prostatic cancer induction in rodents and possibly humans.

Conceivably other, nonhormonal factors affect prostatic cell proliferation. For example, over the lifetime of a man, the prostate undergoes repeated inflammatory insults (prostatitis) with reactive cell proliferation and generation of reactive oxygen species as possible consequences (262,263), and sexual activity conceivably also affects prostatic cell turnover. Support for a cell proliferation hypothesis is provided by rodent experiments that indicate that increased prostatic cell proliferation at the time of exposure to carcinogens can enhance the sensitivity of the tissue to the carcinogenic effects of these agents (220,242–245). Stimulation of cell proliferation during carcinogen exposure increases the likelihood that promutagenic DNA damage, such as carcinogen–DNA adducts, will be fixed as permanent mutations. In humans, increased cell proliferation may thus enhance the carcinogenic effects of low-level exposure to environmental and endogenous carcinogens.

The rate of cell proliferation at the time of carcinogen exposure may be only one of several androgen-related factors that determine sensitivity of the prostate to cancer induction by carcinogens through androgen-receptor mediated mechanisms. For example, Sukumar et al. (264) have hypothesized that prostate cells that harbor critical genetic alterations, such as activating point mutations in oncogenes or inactivating alterations in tumor suppressor genes, may be selectively sensitive to induction of the cell proliferation, rather than cellular differentiation, by androgens. However, this hypothesis has not been critically tested. These cells could thus have a selective growth advantage over normal cells, which do not respond to chronic

testosterone treatment with sustained proliferation (221). It is also possible that androgens, in addition to other factors, influence the effectiveness of indirect-acting carcinogens that are metabolically activated and otherwise metabolized in the prostate itself.

Role of Androgen Metabolism and Androgen Receptor Sensitivity

Pertinent to any hypothesis implicating androgens in prostate carcinogenesis are considerations related to androgen receptor function and androgen metabolism, from steroid biosynthesis, to conversion of testosterone to DHT and to DHT catabolism. Ross et al. (136) have developed the idea that genetically determined differences in the activities of steroid biosynthetic enzymes, 5 α -reductase, and enzymes that metabolize DHT, as well as in androgen receptor activity are major determinants of risk both at the population and at individual levels [see also (18)]. Functional polymorphisms in the genes that encode for these enzymes and the androgen receptor have been hypothesized to underlie this notion (18,136).

The evidence for these polymorphisms being important in human prostate carcinogenesis has been summarized in detail and evaluated together with the results of endocrinologic studies in earlier sections. The overall conclusions were that to date there is inconsistent and conflicting evidence that functional polymorphisms in the 5 α -reductase gene and differences in 5 α -reductase activity are important determinants of prostate cancer risk. However, there is stronger evidence to suggest that risk is associated with a functional polymorphism in the androgen receptor gene, short lengths of CAG repeats in the transactivation domain of the protein that are linked with increased transactivation activity *in vitro* (137–139,163,164,182–185). However, this association is weak at best. Several other polymorphisms identified in genes encoding for the androgen receptor and other androgen metabolizing enzymes studied have been unevenly distributed among populations that differ in prostate cancer risk (152–156,160,162) or to be associated with risk in case–control studies (153–155,163,170,182). These studies concerned polymorphisms with unknown functional significance in genes encoding the type II 3 β -hydroxysteroid dehydrogenase that catabolizes DHT, the cytochrome P450C17 α enzyme that has 17 α -hydroxylase and 17,20-lyase activity involved in androgen biosynthesis, and CCG or GGN repeats in the promoter region of the androgen receptor gene. The study of these types of polymorphisms is a rapidly evolving field of investigation and will no doubt lead to significant and relevant new findings in the near future (136).

The observation of slightly, but mostly not significantly, higher circulating testosterone levels in high-risk African-American men compared with lower-risk European men suggests that their rates of androgen biosynthesis may be higher. Although lower as well as higher testosterone concentrations have been found in lower-risk Asian or African men compared with European- or African-Americans, testosterone levels were lower in Asians living in Asia than in American populations regardless of their ethnicity in the only two studies that included Asian populations. In addition, directly measured testosterone production rates were lower in Chinese in China than in both Chinese-Americans and European-Americans (148). These observations are consistent with the hypothesis that environmental factors, such as diet, determine prostate cancer risk at the

population level by influencing androgen production such that they are lower in low-risk than in high-risk circumstances (208).

Assessing the role of androgen in prostate carcinogenesis is complicated by the fact that the prostatic stroma is an important site for androgen action and metabolism in the prostate in addition to the epithelium. For example, epithelial DHT concentrations decline dramatically with aging, but they remain stable in the stroma even though the source for intraprostatic DHT, circulating testosterone, also diminishes with aging. This observation suggests that stromal 5 α -reductase activity remains stable, whereas epithelial activity of this enzyme declines with aging. These observations illustrate the difficulties in interpreting the results of studies of circulating androgenic (or other) hormone levels of genomic polymorphisms in relevant genes, because they do not necessarily provide relevant information about what is going on at the level of the prostatic epithelial cell and its important immediate environment, the prostatic stroma.

Role of Estrogen in Prostate Carcinogenesis

The results of the earlier summarized epidemiologic studies provide limited evidence for an association between prostate cancer risk and circulating levels of estrogens, which appear to be higher in men of African descent younger than 50 years of age than in European-American men. This observation suggests that estrogens may be involved in prostate carcinogenesis, because men of African descent living in an American environment have the highest risk for prostate cancer of any population.

Most of the direct evidence in support for a role of estrogens in prostate carcinogenesis comes from studies with treatment of NBL rats with testosterone and estradiol (229,265). It appears that the estrogen-related mechanisms underlying this effect are a mixture of estrogen receptor-mediated and nonreceptor processes, which are discussed in the following paragraphs. In addition, there is evidence to suggest that the mechanisms involved in hormonal induction of rat prostate cancer, originating from periurethral prostatic ducts, are different from those involved in the induction by testosterone and estradiol of dysplastic lesions developing in the dorsolateral prostate acini.

As alluded to earlier, there is evidence for the presence of the CYP19 enzyme aromatase in the human prostate, which could provide a local source of estrogens from conversion of testosterone (195–199), but there are contradictory reports (200,201). The local production of estrogens in the human prostate is possibly a stromal process, and stromal aromatase activity may increase with aging (202). Data on the presence of aromatase in the rodent prostate are also somewhat contradictory, because aromatase activity has been reported in the rat ventral prostate and a transplantable rat prostate carcinoma (266), but it was not detectable in mouse prostate (267). These discrepancies, which may be due to interspecies or methodologic differences, point to the need for further research.

Estrogen Receptor-Mediated Mechanisms

Estrogen receptors are found in the prostate, and Lau et al. (268) demonstrated that both the estrogen receptor- α and - β are present in the rat prostate. Thus, direct receptor-mediated effects of estrogens on the prostate are plausible. However, rodent studies that used antiestrogen treatments (tamoxifen and ICI-182,780) have yielded contradictory results about the involve-

ment of estrogen receptor mechanisms in prostate carcinogenesis. The prostate tumor-promoting effects of testosterone may involve estrogen generated by aromatization. However, simultaneous administration of testosterone and tamoxifen failed to alter the prostate carcinogenesis-enhancing effect of the androgen in an experiment in rats injected with DMAB prior to the hormone treatment (269). However, ICI-182,780 blocked the induction of epithelial dysplasia in the prostatic periphery in NBL rats treated with testosterone and estradiol for 16 weeks (270); the effects of this antiestrogen on induction of periurethral prostate carcinomas are not known.

Leav et al. (228) and Ofner et al. (230) showed that dorso-lateral prostatic tissue with epithelial dysplasia from NBL rats treated with testosterone and estradiol for 16 weeks accumulates estradiol and 5α -androstane- $3\beta,17\beta$ -diol, a weak estrogenic agonist; this accumulation of estrogenic species does not occur in the ventral lobe, which also does not develop dysplasia. In rats treated with testosterone and DES for 16 weeks, dysplasia developed more distinctly in the ventral than in the dorsolateral prostate, as indicated earlier. This development coincided with a preferential accumulation of estradiol and 5α -androstane- $3\beta,17\beta$ -diol in the ventral prostate (230). These observations suggest that increased levels of estradiol and the weakly estrogenic androgen metabolite in prostatic target tissue may be causally related with the development of hormone-induced dysplasia and perhaps carcinomas in the NBL rat model (230).

In tissue with epithelial dysplasia from the dorsolateral prostatic periphery that was derived from rats treated with testosterone and estradiol for 16 weeks, elevated levels of nuclear, but not cytosolic, type II (intermediate-affinity) estrogen-binding sites, but not type I (high-affinity) binding sites, have been found (228,271). The type II estrogen receptor is a cell proliferation marker believed to be a key factor in normal and aberrant growth regulation in female estrogen target tissues. These data indicate that protracted stimulation of cell proliferation may be involved in the formation of hormone-induced rat prostate dysplasia (228,271). Indeed, mitotic indices in testosterone plus estrogen-treated NBL rat dorsolateral prostate were increased over control values; this increased mitotic activity was largely confined to the dysplastic lesions (228,271).

One well-established effect of estrogen treatment in rodents is stimulation of prolactin secretion. This finding raises the possibility that some or even all estrogen effects on the rodent prostate may be mediated through elevation of prolactin secretion, and there is some experimental support for this notion. Transplantation of a prolactin-producing pituitary tumor into rats treated with DMAB enhanced the formation of atypical hyperplasia, a preneoplastic lesion, but not carcinomas, in the ventral prostate, and treatment with bromocriptine, a prolactin secretion-suppressing agent, counteracted this effect (272). Bromocriptine also lowered the formation of ventral prostatic atypical hyperplasia and carcinomas in rats treated with only DMAB. Development of epithelial dysplasia in the dorsolateral prostatic periphery of NBL rats treated with testosterone and estradiol for 16 weeks was also blocked by bromocriptine, but effects on periurethral carcinoma development were not studied (273). Thus, there is evidence to suggest that prolactin may modulate the induction of preneoplastic lesions in the rat prostate, but the relevance of these findings for prostate cancer development are not clear.

In conclusion, several lines of evidence are available to sug-

gest that estrogen receptor-mediated mechanisms contribute to the induction of prostate cancer by hormonal treatments, but conclusive data in this regard are largely lacking.

Nonreceptor Mechanisms

Estrogens have been shown to be capable of producing DNA damage in target tissues susceptible to estrogen-induced carcinogenesis, independent of their interaction with the estrogen receptor, as discussed in detail elsewhere in this monograph. In the kidney of male hamsters treated with DES, Liehr and colleagues (275) have found a direct DES-DNA adduct and indirect estrogen-generated DNA adducts perhaps of endogenous origin and of undetermined structure detectable by ^{32}P -postlabeling (276). Both observations are thought to be related to the formation of catechol estrogens that undergo redox cycling during which reactive intermediates and reactive oxygen species are generated and lipid peroxidation can be initiated (274). Similar observations have been made in the prostate of NBL rats treated for 16 weeks with testosterone plus estradiol. This treatment enhanced the formation of a chromatographically unique endogenous adduct selectively in the periurethral region of the rat dorsolateral prostate, which is the site of the carcinogenic effect of this treatment [(277) Bosland et al., unpublished data; see also Chapter 4]. Ho and Roy (278) reported increased single-strand DNA breaks and accumulation of fluorescent lipid peroxidation products in the dorsolateral prostate of NBL rats after this treatment, but they did not separately analyze the periurethral and peripheral areas of the prostate. In addition, substantially elevated levels of 8-hydroxydeoxyguanosine and, to a lesser extent, lipid hydroperoxides have been found at the periurethral tissue but not in the peripheral area of these glands (Bosland et al., unpublished data; see also Chapter 4). Lower, but still elevated, levels of the endogenous DNA adduct detectable by ^{32}P -postlabeling, 8-hydroxydeoxyguanosine, and lipid hydroperoxides were also found in the periurethral prostate of rats treated with only testosterone, perhaps due to formation of estrogens by aromatization (Bosland et al., unpublished data). The enhancement of endogenous DNA adduct formation, oxidative DNA damage, and lipid peroxidation selectively at the site of tumor formation and preceding it strongly suggests that these effects are causally involved in the carcinogenic effect of the hormone treatment. It is likely, but as yet unproven, that the exogenously administered estrogens or formation of estrogen via aromatization of testosterone and a genotoxic mechanism are critical to the carcinogenic effect of this hormone combination for the prostate, rather than other mechanisms, including receptor mediation.

This hypothesis implies that catechol estrogen formation occurs at the relevant site within the prostate, as indicated earlier and discussed in detail elsewhere in this monograph. Lane et al. (212) demonstrated that microsomes isolated from testosterone plus estradiol-treated NBL rat dorsolateral prostate do not appear to be able to generate the catechol estrogens 2-hydroxy and 4-hydroxy-estradiol and -estrone. However, because periurethral prostate tissue was not incorporated in the analysis and the relevance of such microsomal assays for the *in vivo* situation is unclear, these data do not refute the possibility of catechol estrogen formation in the periurethral prostate. Furthermore, it is conceivable that the mechanisms of induction of dysplasia in the dorsolateral glandular prostate (estrogen receptor-mediated, possibly not involving estrogen-generated genotoxic processes) are

different from those involved in generation of the periurethral prostatic carcinomas (estrogen-generated genotoxicity, but possibly no estrogen receptor mediation). This idea leads to the hypothesis that 1) testosterone acts as a tumor promoter and estrogens act as genotoxic “tumor initiators” in the testosterone plus estradiol-treated NBL rat model of (periurethral) prostate carcinogenesis, and 2) the androgen also acts as enhancer of induction of dysplasia (periphery) in this model, which requires conjunct action of estrogen via estrogen receptors. However, these hypotheses remain to be critically tested.

The human relevance of these findings in the testosterone plus estradiol-treated NBL rat model remains unclear at present. However, oxidative DNA damage and lipid peroxidation reflective of reactive oxygen damage have been observed in the human prostate (279), and signs of increased oxidative stress have been found in patients with prostate cancer as compared with control subjects (280). Whether these observations are estrogen-exposure related or associated with other risk factors, such as a high fat diet (263), is not known, but they suggest that endogenous oxidative stress may be important in human prostate carcinogenesis and are consistent with involvement of estrogen-generated oxidative DNA damage.

Perinatal Estrogen Exposure: Imprinting

As summarized earlier, perinatal estrogen exposure of mice resulted in epithelial dysplasia of the periurethral proximal parts of the dorsolateral and anterior prostate and of the seminal vesicles (236,237) as well as carcinomas in these areas (234). In addition, mice that were neonatally estrogenized hyperresponded to secondary estrogen treatment (estradiol) with the development of considerable squamous metaplasia in these same tissues, but control subjects responded with little or no squamous change (236). These very same tissue areas possess estrogen receptors, which indicate their estrogen sensitivity (236). However, the activity of estradiol hydroxysteroid oxidoreductase, a marker of estrogen sensitivity, and incorporation of tritiated thymidine in epithelial compartments of these tissues were not changed in response to secondary treatments with estradiol in neonatally estrogenized mice (236,237). In response to secondary androgen treatment (DHT), tritiated thymidine incorporation was markedly increased selectively in stromal cells of the anterior and ventral prostate, indicating a lasting effect of neonatal estrogen exposure on the androgen responsiveness of the stromal component of the mouse prostate (236). These observations suggest that perinatal estrogen exposure of mice imprints lasting alterations in estrogen and androgen responsiveness of the male accessory sex glands.

The exact mechanism of these complex imprinting effects is not clear. Perinatal estrogen treatment may act indirectly on the male accessory sex glands by imprinting permanent alterations in the secretion of pituitary hormones and testicular androgen, or directly by, e.g., imprinting altered expression of androgen, estrogen, and prolactin receptors or changes in steroid metabolism in the accessory sex gland, which all may result in modified development of these glands (281–284). For example, in neonatally estrogenized mice, luteinizing hormone and follicle-stimulating hormone plasma levels were found to be elevated (282), whereas circulating testosterone levels were decreased (281,284) or unaltered (282). However, no abnormalities in circulating estrogen and androgen levels were found in boys that had been exposed to DES *in utero* (285). Prostatic DHT forma-

tion by 5 α -reductase was found to be impaired in adult mice neonatally treated with DES (267). Nuclear androgen receptor levels in these mice were decreased in the dorsal and ventral prostate but not affected in the lateral lobe, and the number of androgen receptor-positive stromal cells was increased in all three lobes (284). The significance of these findings for the carcinogenic effects of perinatal estrogen exposure to mice is not clear. Although the exact mechanisms of the carcinogenic effects of perinatal estrogen exposure for the prostate remain unclear, there appear to be lasting direct and indirect effects of this treatment on the mouse prostate. The human relevance of the findings in mice remains unclear at present, but *in utero* estrogen exposure is likely to occur in humans (142).

OVERALL CONCLUSIONS

With the exception of “exposure” to a western lifestyle, including a high-fat diet, an African-American “environment,” and, perhaps, venereal disease and unknown factors related to farming and employment in armed services and nuclear industry, there are no known exogenous exposures that are associated with prostate cancer risk, and none of these circumstances constitute exposures to specific chemicals or factors. Familial aggregation of prostate cancer risk is consistently observed and confers a considerable increase in risk but explains less than 10% of all cases. Putative susceptibility loci have been identified, but there are no indications that these loci are related to hormonal factors. This lack of known specific risk factors is remarkable in view of the high frequency of this malignancy in western countries. It may indicate that there are many exogenous risk factors for prostate cancer that are too ubiquitous and overlapping to be detectable by epidemiologists. However, it is possible that there are strong endogenous determinants of prostate cancer risk that are “overpowering” most exogenous risk factors in epidemiologic analyses.

Androgenic hormones and androgen receptor mechanisms are prime candidates to be such important endogenous factors, but the epidemiologic evidence in favor of this view is weak. Elevation of bioavailable and bioactive androgens in the circulation and in the target tissue as an important risk factor is biologically very plausible. The results of several animal model studies strongly support this contention. Some experiments indicate that substantial enhancement of prostate carcinogenesis can be produced by very small elevations of circulating testosterone, which, if also valid for humans, may explain why the epidemiologic associations between circulating androgen levels and prostate cancer are weak at best. Evidence is also available indicating that increased transactivation activity of the androgen receptor may be associated with increased prostate cancer risk, both at the population and individual levels. However, more research is needed to confirm and further define these associations in humans and to further unravel the biologic mechanisms underlying the increased risk that may be associated with elevated circulating androgen levels and increased androgen receptor sensitivity.

African-American men have a twofold higher risk than European-American men do. The unknown environmental and possibly genetic factors that determine the high prostate cancer risk in African-American men may act through modifying their hormonal status. Indeed, circulating levels of androgens and, in men younger than 50 years, estrogens appear to be higher in men of African descent than in European-American men. Such endo-

crine mechanisms perhaps act as early as *in utero*, because circulating levels of androgens and estrogens have been shown to be slightly higher in young men and in pregnant African-American women than in European-American women.

Hormonal stimulation of prostatic epithelial cell proliferation enhances the susceptibility of the rat prostate to chemical carcinogens. Testosterone at near-physiologic plasma concentrations is a weak complete carcinogen and a strong tumor promoter for the rat prostate. The very strong tumor-promoting activity of androgens possibly explains their weak complete carcinogenic activity. The mechanism of the tumor-inducing and -promoting activities of androgens for the rat prostate is unknown. It is unlikely that chronic stimulation of prostatic cell proliferation rates by androgens is involved. However, it is possible that prostatic epithelial cells that carry critical genetic alterations have a selective growth advantage over normal cells and do not respond to androgens by differentiation, as normal cells would, but by proliferation.

Chronic exposure to testosterone plus estradiol is strongly carcinogenic for the dorsolateral prostate of some rat strains, whereas testosterone alone is only weakly carcinogenic. The mechanism of this carcinogenic effect in the rat prostate is incompletely understood, but it appears to involve estrogen-generated oxidative stress and genotoxicity and also requires androgen- and estrogen receptor-mediated processes, such as changes in sex steroid metabolism and receptor status. There is evidence for the presence of the enzyme aromatase in the human and rat prostate, providing a local source of estrogens, which in humans seem to increase in activity with aging. Perinatal estrogen exposure is carcinogenic for the rodent male accessory sex glands. Hyperplastic and squamous metaplastic changes have been reported in human genital tract tissue following prenatal DES exposure, indicating that prenatal exposure to DES may also target the human prostate. The mechanisms of these prenatal estrogen effects are not clear, but they may involve permanently imprinted changes in hormone production and tissue hormone sensitivity.

From these observations, the following multifactorial general hypothesis of prostate carcinogenesis emerges: Androgens act as strong tumor promoters via androgen receptor-mediated mechanisms to enhance the carcinogenic activity of strong endogenous genotoxic carcinogens, including reactive estrogen metabolites and estrogen- and prostatitis-generated reactive oxygen species, and possibly unknown weak environmental carcinogens. All these processes are modulated by a variety of environmental factors, such as diet, and by genetic determinants, such as hereditary susceptibility genes and polymorphic genes that encode receptors and enzymes involved in the metabolism and action of steroid hormones.

FUTURE RESEARCH NEEDS

This overview clearly indicates that, although steroid hormonal factors are strongly implicated in prostate carcinogenesis, we know very little about their involvement. Considerable research is needed to further our understanding of this relationship. Some promising areas for future research are summarized below. One aspect to be mentioned up front is that the African-American population offers unparalleled but vastly underexploited opportunities for such research, which may also lead to new insights in the possible prevention of prostate cancer in this underrepresented but disproportionately affected group.

- 1) To resolve the uncertainties about the importance of circulating hormone levels, additional, large, nested case-control studies are needed by using cohorts of men belonging to diverse racial/ethnic and other groups that differ substantially in risk of prostate cancer with serial measurements of circulating steroid and other hormones (both over time to assess consistency and trends and within 24-hour periods to assess circadian rhythm variations).
- 2) To address the functional significance of polymorphisms in genes encoding for enzymes involved in steroid hormone biosynthesis and metabolism, studies are needed of correlations between circulating hormone levels and these polymorphisms in relation to prostate cancer risk.
- 3) Even more important than the studies mentioned above, there is an urgent need to develop strategies to examine the relationships of circulating hormone levels and genetic polymorphisms in genes encoding for relevant enzymes with intraprostatic hormone levels, activities of steroid hormone metabolizing enzymes, and androgen receptor mechanisms.
- 4) To determine the importance of estrogen-generated gene damage, studies in humans and animal models are needed of DNA damage and mutations in the prostate associated with exposure to estrogens (and possibly other steroid hormones) in relation to risk of prostate cancer.
- 5) To assess the involvement of gene-environment interactions in prostate carcinogenesis, studies are needed in humans and animal models of the effects of diet and other environmental factors on circulating and prostatic hormone levels, intraprostatic activities of steroid hormone metabolizing enzymes, and prostatic androgen receptor function.
- 6) To expand understanding of the importance of genetic polymorphisms in prostate carcinogenesis, intensification is needed of searches for new relevant polymorphisms in genes encoding for enzymes involved in steroid hormone biosynthesis and metabolism, as well as factors involved in androgen receptor function, including determination of their function.
- 7) Finally, to facilitate many of the research needs listed above, there is the need for establishing banks of adequate DNA, serum, and prostatic tissue samples in large, well-documented, relevant cohorts of aging men.

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