STARLING REVIEW

Is dehydroepiandrosterone a hormone?

F Labrie, V Luu-The, A Bélanger, S-X Lin, J Simard, G Pelletier and C Labrie

Laboratory of Molecular Endocrinology and Oncology, Laval University Hospital Research Center (CRCHUL) and Laval University, Québec City, Québec G1V 4G2, Canada

(Requests for offprints should be addressed to F Labrie; Email: fernand.labrie@crchul.ulaval.ca)

Abstract

Dehydroepiandrosterone (DHEA) is not a hormone but it is a very important prohormone secreted in large amounts by the adrenals in humans and other primates, but not in lower species. It is secreted in larger quantities than cortisol and is present in the blood at concentrations only second to cholesterol. All the enzymes required to transform DHEA into androgens and/or estrogens are expressed in a cell-specific manner in a large series of peripheral target tissues, thus permitting all androgen-sensitive and estrogen-sensitive tissues to make locally and control the intracellular levels of sex steroids according to local needs. This new field of endocrinology has been called intracrinology. In women, after menopause, all estrogens and almost all androgens are made locally in peripheral tissues from DHEA which indirectly exerts effects, among others, on bone formation, adiposity, muscle, insulin and glucose metabolism, skin, libido and well-being. In men, where the secretion of androgens by the testicles continues for life, the contribution of DHEA to androgens has been best

evaluated in the prostate where about 50% of androgens are made locally from DHEA. Such knowledge has led to the development of combined androgen blockade (CAB), a treatment which adds a pure anti-androgen to medical (GnRH agonist) or surgical castration in order to block the access of the androgens made locally to the androgen receptor. In fact, CAB has been the first treatment demonstrated to prolong life in advanced prostate cancer while recent data indicate that it can permit long-term control and probably cure in at least 90% of cases of localized prostate cancer. The new field of intracrinology or local formation of sex steroids from DHEA in target tissues has permitted major advances in the treatment of the two most frequent cancers, namely breast and prostate cancer, while its potential use as a physiological HRT could well provide a physiological balance of androgens and estrogens, thus offering exciting possibilities for women's health at menopause.

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Introduction

Humans, along with the other primates, are unique among animal species in having adrenals that secrete large amounts of the inactive precursor steroids dehydroepiandrosterone (DHEA) and especially DHEA-sulfate (DHEA-S), which are converted into potent androgens and/or estrogens in peripheral tissues (Labrie 1991, Labrie *et al.* 1995a, 1996b, 1997d, 2000b, 2001, Luu-The 2001) (Fig. 1). In fact, plasma DHEA-S levels in adult men and women are 100–500 times higher than those of testosterone and 1000–10 000 times higher than those of estradiol, thus providing a large reservoir of substrate for conversion into androgens and/or estrogens in the peripheral intracrine tissues which naturally possess the enzymatic machinery necessary to transform DHEA into active sex steroids.

Adrenal secretion of DHEA and DHEA-S increases during adrenarche in children at the age of 6–8 years. Maximal values of circulating DHEA-S are reached between the ages of 20 and 30 years. Thereafter, serum DHEA and DHEA-S levels decrease markedly (Fig. 2) (Migeon *et al.* 1957, Vermeulen *et al.* 1982, Orentreich *et al.* 1984, Bélanger *et al.* 1994, Labrie *et al.* 1997e). In fact, at 70 years of age, serum DHEA-S levels are decreased to approximately 20% of their peak values, while they can decrease by 95% by the age of 85–90 years (Migeon *et al.* 1957).

The marked reduction in the formation of DHEA-S by the adrenals during aging (Migeon *et al.* 1957, Vermeulen & Verdonck 1976, Vermeulen *et al.* 1982, Orentreich *et al.* 1984, Bélanger *et al.* 1994, Labrie *et al.* 1997c) results in a dramatic fall in the formation of androgens and

Figure 1 (A) Schematic representation of the role of ovarian and adrenal sources of sex steroids in premenopausal women. After menopause, the secretion of estradiol by the ovaries ceases and then almost 100% of sex steroids are made locally in peripheral target intracrine tissues. (B) Schematic representation of the role of testicular and adrenal sources of androgens in 60-year-old men. ACTH, adrenocorticotropin; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; E₂, 17 β -estradiol; LH, luteinizing hormone; LHRH, LH-releasing hormone; CRH, corticotropin-releasing hormone.

estrogens in peripheral target tissues, a situation that has been proposed to be associated with age-related diseases such as insulin resistance (Coleman *et al.* 1982, Schriock *et al.* 1988) and obesity (Nestler *et al.* 1988, MacEwen & Kurzman 1991, Tchernof *et al.* 1995). On the other hand, much attention has been given to the benefits of DHEA administered to postmenopausal women, especially on the bone, skin, vagina and well-being after oral (Morales *et al.* 1994, Baulieu *et al.* 2000) and percutaneous (Diamond *et al.* 1996, Labrie *et al.* 1997b) administration.

It is thus remarkable that man, in addition to possessing very sophisticated endocrine and paracrine systems, has largely invested in sex steroid formation in peripheral tissues (Labrie *et al.* 1985, 1988, 1997a, Labrie 1991). In

fact, while the ovaries and testes are the exclusive sources of androgens and estrogens in lower mammals, the situation is very different in man and higher primates, where active sex steroids are in large part or wholly synthesized locally in peripheral tissues, thus providing target tissues with the appropriate controls which adjust the formation and metabolism of sex steroids to local requirements.

Transformation of the adrenal precursor steroids DHEA-S and DHEA into androgens and/or estrogens in peripheral target tissues depends upon the level of expression of the various steroidogenic and metabolizing enzymes in each cell of these tissues. This sector of endocrinology that focuses on the intracellular hormone formation and action has been called intracrinology

Figure 2 Effect of age (20–80 years old) on serum concentration of (A) DHEA, (B) DHEA-S, (C) DHEA-fatty acid esters (DHEA-FA) and (D) androst-5-ene-3 β ,17 β -diol (5-diol) in women (Labrie *et al.* 1997c; reproduced with permission from *Journal of Clinical Endocrinology and Metabolism*).

(Labrie *et al.* 1988, Labrie 1991) (Fig. 3). This situation of a high secretion rate of adrenal precursor sex steroids in men and women is thus completely different from all animal models used in the laboratory, namely rats, mice, guinea pigs and all others (except monkeys), where the secretion of sex steroids takes place exclusively in the gonads (Labrie *et al.* 1985, 1988, 1997a, Bélanger *et al.* 1989). One explanation for the delayed progress in the field of formation of sex steroids in peripheral target tissues or intracrinology is the fact that the adrenals of the animal models usually used do not secrete significant amounts of

adrenal precursor sex steroids, thus focusing all attention on the testes and ovaries as the exclusive sources of androgens and estrogens. The term intracrinology was thus coined (Labrie *et al.* 1988) to describe the synthesis of active steroids in peripheral target tissues where the action is exerted in the same cells where synthesis takes place without release of the active steroids in the extracellular space and general circulation (Labrie 1991).

Although orchietomy, estrogens or gonadotropin-releasing hormone (GnRH) agonists or antagonists (through blockade of secretion of bioactive LH) cause a 90–95% reduction in the concentration of circulating testosterone (Labrie *et al.* 1980, 1985, Waxman *et al.* 1983, Moghissi *et al.* 1984) (Fig. 4A), a much smaller effect is seen on the only parameter that directly reflects intra-tissular androgenic action, i.e. the intra-prostatic concentration of the potent androgen DHT. In fact, intra-prostatic DHT levels are reduced by only 50–70% following medical or surgical castration (Labrie *et al.* 1985, Bélanger *et al.* 1986) (Fig. 4A). Moreover, as illustrated in Fig. 4B, the plasma concentrations of the two main metabolites of androgens, namely ADT-G and 3 α -diol-G, remain at 28% and 37% of control, respectively, after castration in adult men (Bélanger *et al.* 1986), thus reflecting the high levels of adrenal precursors converted into DHT in the prostate. In agreement with the above-mentioned clinical findings, we have observed that plasma concentrations of DHEA and 4-dione comparable with those found in adult men exert potent stimulatory effects on androgen-dependent growth and gene expression in the rat ventral prostate (Labrie *et al.* 1988, 1989).

In women, the role of the adrenal precursors DHEA-S, DHEA and 4-dione in the peripheral formation of estrogens is even more important than the situation in men for androgens. In fact, in men, androgen secretion by the testes continues at a high level through life while, in women, estrogen secretion by the ovaries completely ceases at menopause, thus leaving the adrenals as the only source of sex steroids. In fact, the best estimate is that the intracrine formation of estrogens in peripheral tissues in women accounts for 75% of all estrogens before menopause, and close to 100% after menopause (Adams 1985, Labrie *et al.* 2003a). In addition to E₂, another important but still largely unrecognized estrogen is androst-5-ene-3 β ,17 β -diol (5-diol). This steroid of adrenal origin has in fact been shown to exert direct estrogenic effects in both normal and malignant estrogen-sensitive tissues at concentrations found in the circulation of normal adult women (Adams 1985, Poulin & Labrie 1986, Simard *et al.* 1988).

Discovery of the castration effect of GnRH agonists (Labrie *et al.* 1980) has rendered possible the 100% effective, yet reversible, abrogation of testicular and ovarian function, a uniquely well-tolerated approach that has now been available for 25 years for the therapy of androgen- and estrogen-sensitive diseases, especially prostate, breast and uterine cancer. These cancers account for

Figure 3 Human steroidogenic and steroid-inactivating enzymes in peripheral intracrine tissues. 4-DIONE, androstenedione; A-DIONE, 5- α -androstane-3,17-dione; ADT, androsterone; epi-ADT, epiandrosterone; E₁, estrone; E₁-S, estrone sulfate; 5-DIOL-FA, androst-5-ene-3 α ,17 β -diol fatty acid; 5-DIOL-S, androst-5-ene-3 α ,17 β -diol sulphate; HSD, hydroxysteroid dehydrogenase; TESTO, testosterone; RoDH-1, Ro dehydrogenase 1; ER, estrogen receptor; AR, androgen receptor; UGT2B28, uridine glucuronosyl transferase 2B28; Sult2B1, sulfotransferase 2B1; UGT1A1, uridine glucuronosyl transferase 1A1.

37.0% of all new cancers estimated to be diagnosed in 2005 in the USA (Jemal *et al.* 2005). However, the impact of the precursors of adrenal origin that continue to be secreted and transformed into androgens and/or estrogens in peripheral tissues, including the prostate, after medical or surgical castration, needs to be controlled in order to achieve the most efficient endocrine therapy needed to treat these cancers (Labrie 2002). Definitive proof of the importance of the androgens made in the human prostate (intracrinology) is illustrated by the fact that the first demonstration of a prolongation of life in prostate cancer in randomized studies was obtained when the effect of an LHRH agonist (medical castration) associated with a pure anti-androgen (in order to simultaneously block the androgens of adrenal origin) was found to be superior to the effect of an LHRH agonist alone (Labrie *et al.* 1982, Crawford *et al.* 1989, Bennett *et al.* 1999, Prostate Cancer Trialists' Collaborative Group 2000). Most importantly, the same treatment applied at the localized stage of the disease has led to a probable cure of the disease in more than 90% of patients (Labrie *et al.* 2002).

Since ovarian estrogen secretion ceases at menopause, the major role of peripheral estrogen formation in post-

menopausal women is clearly demonstrated, as mentioned above, by the observation of the major benefits of aromatase inhibitors in advanced breast cancer in postmenopausal women (Nabholtz *et al.* 2000, Goss *et al.* 2003, Mouridsen *et al.* 2003) as well as by the findings of a 76% decrease in breast cancer incidence in postmenopausal osteoporotic women who received the selective estrogen receptor modulator (SERM) raloxifene for 3 years (Cummings *et al.* 1999).

It should also be noted that the importance of the intracrine formation of androgens and estrogens extends to non-malignant diseases such as acne, seborrhea, hirsutism and androgenic alopecia as well as to osteoporosis and vaginal atrophy (Cusan *et al.* 1994, Labrie *et al.* 1997b). Another example of the relevance of intracrinology in non-malignant diseases is endometriosis (Bulun *et al.* 2000). In this regard, it has recently been demonstrated that aromatase is expressed aberrantly in endometriosis, while this activity is not detectable in the normal endometrium. Furthermore, another abnormality in this disease is the deficient expression of type 2 17 β -HSD, thus impairing the inactivation of E₂ into E₁. Consequently, the increased formation of E₂ by aromatase coupled with

Figure 4 (A) Effect of castration on the serum levels of testosterone (T), on one hand, and on the concentration of the active androgen DHT remaining in prostatic cancer tissue after castration, on the other hand. Note the relatively small effect (approximately 60%) of castration on intra-prostatic DHT concentration as compared with the 90% fall in serum testosterone. LHRH-A, LHRH agonist. (B) Plasma concentrations of androstane-3 α ,17 β -diol glucuronide (3 α -Diol-G) and androsterone glucuronide (ADT-G) in 20 intact (yellow bars) and 18 castrated (gold bars) men with prostate cancer. Patients were of similar age.

the decreased inactivation of E₂ by type 2 17 β -HSD leads to increased stimulation of the endometrium and endometriosis.

It is increasingly apparent that mammary cells possess complex regulatory mechanisms that allow for the strict control of the intracellular levels of both stimulatory and inhibitory sex steroids. For instance, our data show that DHT favors the degradation of E₂ into E₁, thus suggesting that the potent anti-proliferative activity of DHT in E₂-stimulated ZR-75-1 human breast cancer cells is, at least partially, exerted on 17 β -HSD activity (Adams 1985, Poulin *et al.* 1988, 1989, Couture *et al.* 1993). Conversely, we have found that estrogens cause a marked increase in the production of the glucuronidated androgen metabolites 3 α -diol-G, 3 β -diol-G and ADT-G in MCF-7 cells, thus decreasing the inhibitory androgenic activity (Roy *et al.* 1992). In fact, since glucuronidation is the predominant route of androgen inactivation, androgen-inactivating enzymes constitute an important site of regulation of breast cancer growth.

The skin is also an important target of intracrine sex steroid action. In fact, it is well recognized that the skin synthesizes androgens from inactive steroid precursors and that acne, seborrhea, hirsutism and androgenic alopecia are associated with excess androgens (Mauvais-Jarvis *et al.*

1969, Milne 1969, Wilson & Walker 1969, Bingham & Shaw 1973, Liang *et al.* 1983, Labrie 1991, Dumont *et al.* 1992b, Cusan *et al.* 1994). In fact, increased local biosynthesis of the potent androgen DHT from the weaker androgen testosterone by 5 α -reductase has been suggested to be one of the mechanisms involved (Kuttann *et al.* 1979). Although a series of studies have addressed the role of sex steroids in the control of hair growth and sebaceous gland physiology, the importance of skin as a site of regulated steroid biosynthesis and metabolism has received little attention. The presence of 3 β -HSD in rat skin has been reported (Flamigni *et al.* 1970, Muir *et al.* 1970) and local rat skin steroidogenesis has also been suggested to modulate sebaceous gland activity (Ebling *et al.* 1971). These early pioneering studies can now be carried further using the molecular biology tools that have become available (Zhao *et al.* 1990, 1991). Since human skin is composed of various cell populations showing sensitivity to androgens, especially the epidermis, hair follicles, sebaceous glands, sweat glands and dermis, antibodies developed against fragments of type 1 5 α -reductase have been used to localize the enzyme by immunohistochemistry (Luu-The *et al.* 1994). We have also found that 5 α -reductase is expressed in sweat and sebaceous glands, as well as in the epidermal cell layers, thus providing the

molecular basis for the important role of androgens in human skin and its appendages.

Intracrinology and its steroidogenic and steroid-inactivating enzymes

Steroidogenic enzymes

As mentioned above, transformation of the adrenal precursor steroids DHEA and DHEA-S into androgens and/or estrogens in peripheral target tissues depends upon the level of expression of the various steroidogenic and metabolizing enzymes in each cell of these tissues. Knowledge in this area has recently made major progress with the elucidation of the structure of most of the tissue-specific genes that encode the steroidogenic enzymes responsible for the transformation of DHEA and DHEA-S into androgens and/or estrogens in peripheral intracrine tissues (Labrie *et al.* 1988, 1992b, 1995b, 1997a, Peltoketo *et al.* 1988, Luu-The *et al.* 1989a, 1995b, Andersson & Russel 1990, Lachance *et al.* 1990, 1991, Labrie 1991, 2000b, Rhéaume *et al.* 1991, Pelletier *et al.* 1992, Milewich *et al.* 1993, Martel *et al.* 1994, Adamski *et al.* 1995) (Fig. 3).

Human 3 β -HSD isoenzymes and their genes Despite its essential role in the biosynthesis of all classes of hormonal steroids, the structure of the 3 β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 -isomerase gene family, hereafter called 3 β -HSD, was only elucidated in 1989 (Luu-The *et al.* 1989a, Lachance *et al.* 1990, 1991, Rhéaume *et al.* 1991). The membrane-bound enzyme 3 β -HSD catalyzes an essential step in the transformation of all 5-pregnen-3 β -ol and 5-androsten-3 β -ol steroids into the corresponding Δ^4 -3-keto-steroids, namely progesterone, as well as the precursors of all androgens, estrogens, glucocorticoids and mineralocorticoids.

In contrast with the results obtained using microsomes and purified enzymes which show that 3 β -HSD catalyzes the interconversion of 3 β -hydroxy- and 3-keto-5 α -androstane steroids (Luu-The *et al.* 1991), when intact transfected cells in culture are used without the addition of cofactors, an experimental procedure which better mimics the physiological conditions, 3 β -HSD catalyzes almost exclusively the oxidation of 3 β -hydroxy- into 3-keto-5 α -androstane steroids (Huang & Luu-The 2001b) while the reverse reductive reaction is catalyzed by another enzyme, namely, 3($\alpha \rightarrow \beta$)-HSE (Huang & Luu-The 2000, 2001b) and type 7 17 β -HSD (Liu *et al.* 2005).

Not only is 3 β -HSD found in the classical steroidogenic tissues (placenta, adrenal cortex, ovary and testis), but also in several peripheral tissues, including the skin, adipose tissue, breast, lung, endometrium, prostate, liver, kidney, epididymis and brain (Labrie *et al.* 1992a, Pelletier *et al.* 1992, Milewich *et al.* 1993, Martel *et al.* 1994), thus catalyzing the first step in the intracrine transformation of DHEA into 4-dione, the precursor of both androgens and

estrogens. The existence of multiple members of the 3 β -HSD gene family offers the unique possibility of tissue- and/or cell-specific expression of this enzymatic activity.

Following purification of 3 β -HSD from human placenta and development of antibodies against the enzyme in rabbits (Luu-The *et al.* 1990b), we have isolated and characterized a first 3 β -HSD cDNA type (Luu-The *et al.* 1989a) and its corresponding gene (Lachance *et al.* 1990). The second 3 β -HSD cDNA type, which corresponds to the almost exclusive mRNA species expressed in the adrenals and gonads, was chronologically designated human type 2 3 β -HSD (Rhéaume *et al.* 1991). The structure of the corresponding human type 2 3 β -HSD gene has also been elucidated (Lachance *et al.* 1991). The human 3 β -HSD genes corresponding to human cDNAs types 1 and 2 contain four exons and three introns within a total length of 7.7–7.8 kbp. These genes were assigned by *in situ* hybridization to the p13.1 region of chromosome 1 and are closely linked to D1S514 located at 1–2 cM of the centromeric marker D1Z5 (Morissette *et al.* 1995).

We have observed that mutations in the type 2 3 β -HSD gene are responsible for classic 3 β -HSD deficiency, a form of congenital adrenal hyperplasia that impairs steroidogenesis in both the adrenals and gonads (Rhéaume *et al.* 1992, Simard *et al.* 1993, 1995). However, the absence of mutations in the type 1 gene provided the long-awaited molecular explanation for the persistence of peripheral steroidogenesis in these type 2 3 β -HSD-deficient patients, thus demonstrating the importance of peripheral sex steroid formation or intracrinology.

Human 17 β -HSDs The 17 β -HSDs are responsible for the formation and inactivation of all active androgens and estrogens (Fig. 3). As discussed above for 3 β -HSD, until relatively recently 17 β -HSDs as well as almost all other dehydrogenases were considered to be reversible enzymes that catalyze the interconversion of substrates and products, mainly because the enzymatic activity was usually characterized using tissue homogenates, subfractions or purified proteins with added oxidized (NAD⁺, NADP⁺) or reduced (NADH, NADPH) cofactors. These exogenous cofactors drive the reaction in the oxidative or reductive direction depending upon their oxidized or reduced state respectively. However, using a more physiologically relevant method of enzymatic activity analysis, namely intact transfected cells in culture without the addition of exogenous cofactors, the transfected enzyme catalyzes the reaction in a unidirectional manner (Luu-The *et al.* 1995a, 2001, Dufort *et al.* 1999, Huang & Luu-The 2000, 2001b). These findings agree with the isolation of multiple types of 17 β -HSDs where six catalyze the reductive reaction (types 1, 3, 5, 7, 12 and 13) and four catalyze the oxidative reaction (types 2, 4, 6 and 8).

The readers are referred to original manuscripts and reviews for information on type 1 (Peltoketo *et al.* 1988, 1992, Luu-The *et al.* 1989b, 1990a, Dumont *et al.* 1992a,

Lin *et al.* 1992, Zhu *et al.* 1993, Breton *et al.* 1994, Ghosh *et al.* 1995), type 2 (Luu-The *et al.* 1989b, Wu *et al.* 1993, Andersson *et al.* 1995), type 3 (Geissler *et al.* 1994), type 4 (Leenders *et al.* 1994, Adamski *et al.* 1995, de Launoit & Adamski 1999), type 6 (Biswas & Russell 1997), type 7 (Duan *et al.* 1996, Nokelainen *et al.* 1998, Krazeisen *et al.* 1999) and type 8 (Aziz *et al.* 1993, Kikuti *et al.* 1997, Luu-The 2001) 17 β -HSDs. The roles of types 9, 10 and 11 17 β -HSDs in the human remain to be determined while human types 12 and 13 17 β -HSDs are at a final stage of characterization (Liu *et al.* 2005). We will limit our review to type 5 17 β -HSD, an enzyme which plays an important role in the peripheral formation of androgens in both men and women.

Type 5 17 β -HSD Although type 3 17 β -HSD synthesizes testosterone from 4-dione in the Leydig cells of the testes, thus providing approximately 50% of the total amount of androgens in men, the same enzymatic reaction is catalyzed in the peripheral target tissues in both men and women as well as in the ovary by a different enzyme, namely type 5 17 β -HSD (Dufort *et al.* 1999). This enzyme is highly homologous with types 1 and 3 3 α -HSDs as well as 20 α -HSD (Dufort *et al.* 1999) and thus belongs to the aldo-keto reductase family.

In the postmenopausal ovary, hypertrophied stromal cells are localized mainly at the periphery and hilus (Russell & Bannatyne 1989). These stromal cells contain both 3 β -HSD and type 5 17 β -HSD, thus permitting the transformation of DHEA into 4-dione and then into testosterone. The amount of stromal hyperplasia in postmenopausal ovaries is correlated with the ovarian vein levels of 4-dione and testosterone (Sluijmer *et al.* 1998). These hyperplastic stromal cells are thus responsible for the synthesis of 4-dione and testosterone in the postmenopausal ovary.

Type 5 17 β -HSD is not only expressed in the ovary but it is also present in a large series of peripheral tissues including the mammary gland. The epithelium lining the acini and ducts of the mammary gland is composed of two layers, an inner epithelial layer and an outer discontinuous layer of myoepithelial cells. By immunocytochemistry, 3 β -HSD is seen in the epithelial cells of acini and ducts as well as in stromal fibroblasts (Fig. 5A). Immunostaining is also observed in the walls of blood vessels, including the endothelial cells. In the positive cells, the labeling is mainly cytoplasmic. No significant labeling could be detected in the myoepithelial cells. As shown in Fig. 5B, immunostaining for type 5 17 β -HSD gives results almost superimposable onto those obtained for 3 β -HSD, the cytoplasmic labeling being observed in both epithelial and stromal cells as well as in blood vessel walls (Pelletier *et al.* 1999). Studies performed at the electron microscopic level revealed that, in sections stained for 3 β -HSD or type 5 17 β -HSD, labeling was not associated with any specific membrane-bound organelles in the different reactive cell

types (Pelletier *et al.* 2001). The type 5 17 β -HSD structure has an eight-stranded α/β -barrel in its center, a typical folding motif in a large family of enzymes, with each inner β -strand connected to an outer α -helix. In addition, two β -strands (B1 and B2) form a β -hairpin turn preceding β 1 of the barrel, blocking the N terminus of the β -barrel; one α -helix (H1) interrupts between α 7 and β 8 and another one (H2) follows α 8 at the C terminus. Four large loops, namely loop-A (residues 24–33), loop-B (residues 117–143), loop-C (residues 217–238) and loop-D (residues 301–323), help to form the substrate and cofactor-binding sites at the C-terminal end of the α/β -barrel (Fig. 6). In addition, the refined models from the two ternary complexes have a root mean squared deviation of 0.61 Å for 311 Ca atoms from the enzyme protein and a maximum deviation of 3.1 Å at Ca of Gly315 (Qiu *et al.* 2004).

Human 5 α -reductase isoenzymes The enzyme 5 α -reductase catalyzes the 5 α -reduction of 4-dione, testosterone and other 4-ene-3-keto-steroids to the corresponding 5 α -dihydro-3-keto-steroids. The best known role of this enzyme is the transformation of testosterone into DHT, the most potent androgen, which is responsible for the differentiation of the male external genitalia and prostate as well as virilization at puberty. The major impact of 5 α -reductase in men, however, is its role in prostate cancer and benign prostatic hyperplasia. Two types of human steroid 5 α -reductases, chronologically identified as type 1 and type 2, were isolated from human prostatic cDNA libraries (Andersson & Russel 1990, Andersson *et al.* 1991). The structure of the human type 1 5 α -reductase gene was first elucidated by Jenkins *et al.* (1991). This gene is not responsible for 5 α -reductase deficiency, and is relatively insensitive to the inhibitor finasteride (Andersson *et al.* 1991). Type 2 5 α -reductase, on the other hand, is the isozyme responsible for male pseudohermaphroditism from 5 α -reductase deficiency and is sensitive to finasteride (Andersson *et al.* 1991, Wilson *et al.* 1993).

Considering the crucial role of type 2 5 α -reductase, we have elucidated the structure of its corresponding gene (Labrie *et al.* 1992b). The type 2 5 α -reductase gene contains five exons and four introns and shows splicing sites identical to those of the type 1 gene. Its coding region shares 57% homology with that of the type 1 5 α -reductase gene. Type 1 5 α -reductase is the predominant form expressed in human skin (Luu-The *et al.* 1994).

Steroid-inactivating enzymes

There is also good evidence that the DHT formed in peripheral tissues is essentially metabolized locally before its appearance in the circulation (Horton & Lobo 1986, Horton 1992). Phase I DHT catabolites include androstanedione, ADT, epiandrosterone, 3 α -diol and androstane-3 β ,17 β -diol, which are formed by the action

Figure 5 Human mammary gland immunostained for (A) 3 β -HSD and (B) type 5 17 β -HSD. Staining can be observed in the secretory epithelial cells of acini (A). Stromal cells (arrows) and capillaries (arrow heads) are also labeled. Magnification $\times 350$.

of a series of 3 α / β -HSDs and 17 β -HSD isoforms (Fig. 3) (Labrie *et al.* 2000a, Andersson 2001, Dufort *et al.* 2001, Luu-The 2001). However, most if not all of the androgen-target tissues express HSD isoforms that are capable of back converting the phase I metabolites into DHT, thus suggesting that a fine regulation of these enzymes is extremely important for controlling the concentration of DHT in androgen-target tissues.

The serum levels of the conjugates are increased after oral or topical administration of DHEA or 4-dione in the

presence of no change or minimal change in the blood levels of non-conjugated androgen metabolites (Labrie *et al.* 1997a). These observations further support the concept that 5 α -reduced androgen glucuronides found in the circulation are produced *in situ* in peripheral tissues after conversion of the adrenals and/or gonadal steroid precursors into DHT first and, subsequently, into phase I DHT metabolites without release of these intermediate steroid precursors and metabolites into the circulation (Horton & Lobo 1986, Labrie 1991, Horton 1992, Labrie *et al.* 2003a). Consequently, the glucuronidation of phase I metabolites by UDP-glucuronosyltransferase (UGT) enzymes in androgen-sensitive tissues should be considered as the end of the androgenic signal. In the circulation, two major phase II DHT metabolites, namely ADT-G and 3 α -diol-G, have been identified, but low amounts of DHT-G and 3 β -diol-G were also detected (Labrie *et al.* 1997a).

Figure 6 Representation of type 5 17 β -HSD/testosterone/NADP structure. The testosterone molecule is displayed in yellow and the NADP molecule in red. Two β -strands (B1 and B2, colored in deep blue) form a β -hairpin turn at the N terminus of the β -barrel. Two additional α -helices (H1 and H2) are colored in yellow and brown. Four large loops, namely loop-A (L-A, blue), loop-B (L-B, green), loop-C (L-C, light green) and loop-D (L-D, red), form the substrate and cofactor-binding sites at the C-terminal end of the α / β -barrel. The figure was generated with program PyMOL (Qiu *et al.* 2004).

UGT2B enzymes in the human prostate

Conjugation of compounds, including steroids, by glucuronidation is a pathway that has been found in all vertebrates studied to date. More than 45 different UGT cDNA clones have been isolated from seven mammalian species, including 18 human UGT clones (Mackenzie *et al.* 1997, Levesque *et al.* 2001).

In the human prostate, the alveoli are composed of two cell types. The basal cells are small cells lining the periphery of the alveoli, whereas the luminal cells are large columnar cells in contact with the alveolar lumen. The two cell types play distinct roles in androgen formation and action (Fig. 7). The expression of type 1 3 β -HSD, type 5 17 β -HSD and types 1 and 2 5 α -reductase is detected in the basal cells, whereas, in the luminal cells, where the androgen receptor is exclusively observed, mostly 5 α -reductase activity is found (Pelletier *et al.* 1998, 2001). After castration, DHT concentrations in the prostate are

reduced by 50–60%, thus indicating that testosterone precursors, such as DHEA, are responsible for an important proportion of DHT in the prostate (Dufort *et al.* 1999). It is reasonable to suggest that DHT is formed locally in luminal cells from testosterone, which is provided by the circulation and/or metabolism of circulating adrenal steroid precursors (DHEA and 4-dione) in basal cells. Enzymes of the phase I DHT catabolism are also present in basal cells, but they are not detected in luminal cells, which occupy the largest proportion of the human prostate (Huang & Luu-The 2000, 2001a, Dufort *et al.* 2001). This absence of phase I catabolic enzymes in luminal cells favors large concentrations of DHT. Indeed, DHT concentrations in the prostate exceed by almost tenfold those of testosterone and phase I DHT metabolites (Bélanger *et al.* 1989, 1990). The two-cell mechanism provides the basis for the specific control of testosterone and DHT levels in the prostatic tissue.

In agreement with the presence of conjugating activity in this tissue, large concentrations of 3 α -diol-G and ADT-G were also reported (Pelletier *et al.* 2001). Finally, the expression of UGT2B15 and UGT2B17 was subsequently established in the prostate (Turgeon *et al.* 2001). The UGT2B17 protein is detected in basal cells, whereas UGT2B15 is only observed in luminal cells (Barbier *et al.* 2000). It is probable that 3 α -diol and ADT formed in basal cells are easily converted to glucuronides by UGT2B17, whereas the action of UGT2B15 would be limited to DHT in the luminal cells. Taking into account the low levels of UGT2B15 protein found in the prostate, this situation favors high concentrations of DHT in this tissue, in agreement with previous biochemical observations on the intra-prostatic levels of DHT (Fig. 4). In addition, because the affinity of DHT for the androgen receptor is approximately 1000-fold higher than that for UGT2B15, it is believed that UGT2B15 might conjugate only a fraction of the accumulated DHT formed in the luminal cells.

Role of DHEA in women

There is no medical problem related to women's health with a higher negative impact on morbidity (and frequently mortality) than menopause, a condition closely associated with declining sex steroid availability. The most widely recognized fact concerning menopause is that there is a progressive decrease and finally a rapid arrest of estrogen secretion by the ovaries. The cessation of ovarian estrogen secretion is illustrated by the marked decline in circulating E₂ levels. This easily measurable change in circulating E₂ levels coupled with the demonstrated beneficial effects of exogenous estrogens on menopausal symptoms (Grady *et al.* 1992, Greendale & Judd 1993, Lomax & Schonbaum 1993, Archer *et al.* 1999) and bone resorption (Weiss *et al.* 1980, Christiansen *et al.* 1982, Genant *et al.* 1990, Harris *et al.* 1991, Grady *et al.* 1992,

Field *et al.* 1993, Lindsay 1993, Archer *et al.* 1999, Women's Health Initiative 2002) has focused most of the efforts of HRT on various forms of estrogens as well as on combinations of estrogen and progestin in order to avoid the risk of endometrial cancer induced by estrogens administered alone.

The almost exclusive focus on the role of ovarian estrogens in women's reproductive physiology has removed attention from the dramatic 70% fall in circulating DHEA which already occurs between the ages of 20 to 30 and 40 to 50 years (Migeon *et al.* 1957, Vermeulen & Verdonck 1976, Vermeulen *et al.* 1982, Orentreich *et al.* 1984, Bélanger *et al.* 1994, Labrie *et al.* 1997d) (Fig. 2). In fact, since DHEA is transformed to both androgens and estrogens in peripheral tissues, such a fall in serum DHEA and DHEA-S explains why women at menopause are not only lacking estrogens but are also likely to have been deprived of androgens for a few years, as illustrated by the 50–60% decrease in serum ADT-G (Labrie *et al.* 1997c) (Fig. 2).

In a recent study nine androgens and their precursors and metabolites were measured by gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry in serum samples from 377 healthy postmenopausal women aged 55–65 years and 47 normally cycling 30- to 35-year-old premenopausal women. A decrease of 60% was then observed in the sum of ADT-G and 3 α -diol-G while serum DHEA was decreased by 54% in postmenopausal compared with premenopausal women (F Labrie and A Bélanger, unpublished data). Such findings based upon mass spectrometry data provide strong support and confirm our previous observations (Labrie *et al.* 1997c). Serum testosterone, on the other hand, did not decrease significantly from 0.18 $\pm$ 0.07 in premenopausal to 0.14 $\pm$ 0.07 ng/ml in postmenopausal women.

Since the serum levels of ADT-G and 3 α -diol-G in women are 70% of those found in men of the same age while serum testosterone in women compared with men is only about 3% (0.15 ng/ml in women versus 4.5 ng/ml in men), it is clear that serum testosterone is not a valid marker of androgenicity in women. This situation is somewhat analogous to the situation in castrated men where castration causes a 90–95% reduction in the concentration of serum testosterone while the intra-prostatic concentration of DHT as well as of serum ADT-G and 3 α -diol-G are only reduced by 50–70% (Fig. 4) (Labrie *et al.* 1985, Bélanger *et al.* 1986).

Completion of the identification and characterization of all the human UDP-glucuronosyl transferases has made possible the use of the glucuronide derivatives of androgens as markers of androgenic activity. In fact, UGT2B7, UGT2B15 and UGT2B17 are the three enzymes responsible for the glucuronidation of all androgens and their metabolites in the human (Bélanger *et al.* 2003). The relatively simple inactivation mechanisms of androgens

Figure 7 Distribution of the steroidogenic and steroid-metabolizing enzymes in the human prostate.

(Fig. 3) permits measurement of the sum of the metabolites of all androgens in the circulation, thus offering a precise assessment of the total androgenic activity in both women and men.

While the only means of determining androgenic activity in specific tissues is the direct measurement of the intra-tissular concentration of the active androgens, such measurements are not possible in the human except under exceptional circumstances such as in samples of cancer tissue obtained at surgery (Poortman *et al.* 1983, Labrie *et al.* 1985, Bélanger *et al.* 1989). However, while not permitting the assessment of androgenic activity in specific tissues, measurement by validated mass spectrometry techniques of the glucuronide derivatives of ADT and 3 α -diol permits an accurate assessment of total androgenic activity in the whole organism. In fact, since inactivation of the active androgens into ADT and 3 α -diol and their subsequent glucuronidation into ADT-G and 3 α -diol-G is the obligatory route of elimination of androgens (Coffman *et al.* 1990, Beaulieu *et al.* 1996, 1997, Carrier *et al.* 2000, Turgeon *et al.* 2000) (Fig. 8), this approach appears to be the best means of evaluating total androgenic activity in individual subjects and patients. The clinician can then reliably correlate these values of androgenic activity with the other clinical findings.

As mentioned above, the level of transformation of the adrenal precursor steroid DHEA into androgens and/or estrogens in peripheral target tissues depends upon the level of expression of the various steroidogenic enzymes in each cell of each of these tissues (Labrie 1991, Labrie *et al.* 2003a). This situation of a high secretion rate of adrenal precursor sex steroids by the adrenals in men and women is thus completely different from all animal models used in the laboratory, namely rats, mice, guinea pigs and all others (except monkeys), where the secretion of sex steroids takes place exclusively in the gonads and the adrenals do not secrete significant amounts of DHEA (Bélanger *et al.* 1989).

The classical concept of androgen and estrogen secretion in women assumed that all sex steroids had to be transported by the general circulation following secretion by the ovaries before reaching the target tissues. According to this classical concept, it was erroneously believed that the active steroids could be measured directly in the circulation, thus providing a potentially valid measure of the general exposure of the whole body to sex steroids. In fact, this concept is valid only for animal species lower than primates but it does not apply to the human, especially in postmenopausal women where all estrogens and almost all androgens are made locally from DHEA in the peripheral tissues which possess the enzymes required to synthesize

Figure 8 Schematic representation of the very important contribution of the precursor DHEA of adrenal origin to total androgenic activity in postmenopausal women with a parallel minor contribution of testosterone (TESTO) of ovarian and adrenal origins. By intracrine mechanisms, DHEA is transformed into testosterone and DHT in peripheral tissues and then into the inactive metabolites ADT and 3 α -diol before transformation into the water soluble glucuronide derivatives ADT-G, 3 α -diol-3 G and 3 α -diol-17 G by the UGTs 2B7, 2B15 and 2B17. These water-soluble metabolites are then released into the general circulation where they can be measured. A very small proportion of the testosterone and DHT made intracellularly by the steroidogenic enzymes of the intracrine pathway diffuse into the circulation. The height of the colored boxes is proportional to the concentration of each steroid.

active sex steroids. Such a local biosynthesis and action of androgens in target tissues eliminates the exposure of other tissues to androgens and thus minimizes the risks of undesirable masculinizing or other androgen-related side-effects. The same applies to estrogens, although we feel that a reliable parameter of total estrogen secretion (comparable with the glucuronides identified for androgens) has yet to be determined.

Today's knowledge of androgen physiology provides an explanation for the inconclusive studies on the role of androgens in women under various clinical conditions (Leiblum *et al.* 1983, Garland *et al.* 1992, Lipworth *et al.* 1996, Laughlin & Barrett-Connor 2000, Couzinet *et al.* 2001, Davis & Tran 2001, Labrie *et al.* 2003a, Miller *et al.* 2004, Tchernof & Labrie 2004).

We feel that the increased understanding of androgen and estrogen formation and action in peripheral target tissues called intracrinology (Labrie 1991, Luu-The *et al.* 1995b, Labrie *et al.* 1997a,b,c,d), as well as our recent

observations indicating the predominant role of androgens over that of estrogens in the prevention of bone loss after ovariectomy in the rat (Martel *et al.* 1998) and the observation of a similar situation in postmenopausal women (Labrie *et al.* 1997b), have paved the way for a timely and potentially highly significant progress in the field of sex steroid replacement therapy and aging. Such a possibility is well supported by our observations and those of others of a series of beneficial effects of DHEA in postmenopausal women (Morales *et al.* 1994, Diamond *et al.* 1996, Labrie *et al.* 1997b, Arlt *et al.* 1999, Baulieu *et al.* 2000).

Role of DHEA in bone physiology

A predominant role of androgens in bone physiology is well documented (Chesnut *et al.* 1983, Need *et al.* 1987, Savvas *et al.* 1988, Davis *et al.* 1995, Raisz *et al.* 1996, Labrie *et al.* 1997b, Martel *et al.* 1998, Baulieu *et al.* 2000,

Miller *et al.* 2002). In fact, both testosterone and DHT increased the transcription of α (I) procollagen mRNA in osteoblast-like osteosarcoma cells (Benz *et al.* 1991). Treatment with DHT has also been shown to stimulate endochondral bone development in the orchiectomized rat (Kapur & Reddi 1989). Bone mineral density measured in the lumbar spine, femoral trochanter and total body was increased more by estrogen plus testosterone implants than by E_2 alone over a 24-month treatment period in postmenopausal women (Davis *et al.* 1995).

Moreover, in established osteoporosis, anabolic steroids have been reported to help prevent bone loss (Henneman & Wallach 1957). Similarly, subcutaneous E_2 and testosterone implants have been found to be more efficient than oral estrogen in preventing osteoporosis in postmenopausal women (Savvas *et al.* 1988). Although the difference observed in that study has been attributed to the different routes of administration of the estrogen, the cause of the difference could well be the action of testosterone. As an index of increased bone formation, an increase in serum osteocalcin, a marker of bone formation, has been found in postmenopausal women receiving methyltestosterone plus estrogen, compared with estrogen alone (Raisz *et al.* 1996). Moreover, androgen therapy, as observed with nandrolone decanoate, has been found to increase vertebral bone mineral density in postmenopausal women (Need *et al.* 1989). Although androgens are gaining increasing support due to their unique actions in postmenopausal women, virilizing effects are observed with the use of testosterone (Burger *et al.* 1984, Studd *et al.* 1987).

In order to avoid the limitations of standard estrogen therapy (ERT) or hormone replacement therapy (HRT), we have studied the effect of DHEA administration to 60- to 70-year-old women for 12 months on bone mineral density, parameters of bone formation and turnover, serum lipids, glucose and insulin, adipose tissue mass, muscular mass, energy and well-being as well as on vaginal and endometrial histology (Diamond *et al.* 1996, Labrie *et al.* 1997b). DHEA was administered percutaneously to avoid first passage of the steroid precursor through the liver.

We have thus evaluated the effect of chronic replacement therapy with a 10% DHEA cream applied once daily for 12 months in 60- to 70-year-old women ($n=15$). Anthropometric measurements showed no change in body weight but a 9.8% decrease in subcutaneous skin fold thickness at 12 months ($P<0.05$) (Diamond *et al.* 1996). Bone mass density was increased by 2.3% at the hip, 3.75% at the hip Ward's triangle and 2.2% at the lumbar spine level (all $P<0.05$) (Labrie *et al.* 1997b). These changes in bone mineral density were accompanied by significant decreases at 12 months of 38% and 22% in urinary hydroxyproline and in plasma bone alkaline phosphatase respectively (all $P<0.05$). An increase of 135% over control ($P<0.05$) in plasma osteocalcin was concomitantly observed, thus suggesting a stimulatory effect of DHEA on bone formation.

DHEA, abdominal obesity and the metabolic syndrome

Abdominal obesity is associated with an increased risk of insulin resistance, type 2 diabetes and atherosclerosis, an association called the metabolic syndrome (Shimokata *et al.* 1989, Cefalu *et al.* 1995, Ferrannini *et al.* 1997, Kopelman 2000). Among other factors, hormonal changes, especially the declining secretion of DHEA and DHEA-S by the adrenals is thought to be a factor involved (Tchernof *et al.* 1996). In rat and mouse models, DHEA administration reduces visceral fat accumulation in diet-induced obesity (Yen *et al.* 1977, Cleary & Zisk 1986, Mohan *et al.* 1990, Hansen *et al.* 1997). A beneficial effect of DHEA has also been observed on the decrease in insulin resistance that occurs with age (Han *et al.* 1998).

In a study performed in postmenopausal women who received a DHEA cream for 12 months, we found that insulin resistance was decreased while subcutaneous fat at the level of the thigh was also decreased (Diamond *et al.* 1996). Moreover, the daily administration of 50 mg DHEA for 6 months in 65- to 78-year-old men and women decreased abdominal visceral fat by 10.2% in women and 7.4% in men (Villareal & Holloszy 2004). In the same study, abdominal subcutaneous fat was decreased by 6% in both women and men. Moreover, the responsiveness of serum insulin to the glucose tolerance test was decreased by 13% with no change in the glucose response, thus leading to a 34% improvement in the insulin sensitivity index following DHEA administration. No change in serum prostate-specific antigen (PSA) was observed in men receiving DHEA. An improvement in DHEA action has also been found in middle-aged men suffering from hypercholesterolemia (Kawano *et al.* 2003).

In a previous study performed by the same group, DHEA administration for 6 months decreased total body fat mass by 1.4 kg while fat-free mass was increased by 0.9 kg (Villareal *et al.* 2000). No change of body composition was found in studies where DHEA was administered for only 3 months (Flynn *et al.* 1999, Jedrzejuk *et al.* 2003) or 4 months (Arlt *et al.* 2001).

Effect of androgens on libido, hot flushes and quality of life

Community-based studies suggest self-reported sexual dysfunctions in women that ranges from 8 to 50% (Laumann *et al.* 1999). It is believed that low serum free testosterone is the diagnostic marker of 'female androgen insufficiency' (Bachmann *et al.* 2002) as indicated in some studies (Sherwin & Gelfand 1987, Davis *et al.* 1995, Shifren *et al.* 2000, Goldstat *et al.* 2003) and by expert opinions (Cameron & Braunstein 2004). In fact, the incidence of low libido and sexual dysfunction increases with age in women from the third decade (Laumann *et al.* 1999) as well as after ovariectomy (Nathorst-Boos & von Schoultz 1992). While psychosocial and health factors are involved in low arousal and low sexual desire

(Dennerstein *et al.* 1997), it is believed that low androgens play an independent role (Bachmann *et al.* 2002, Miller *et al.* 2004).

In fact, androgens are known to play a role in women's arousability and pleasure as well as intensity and ease of orgasm. Androgens are also involved in the neurovascular smooth muscle response of swelling and increased lubrication (Basson 2004). It should be remembered that DHEA is transformed into both androgens and estrogens in the vagina (Sourla *et al.* 1998; Berger *et al.* 2005). Estrogens, on the other hand, affect the vulval and vaginal congestive responses. Since estrogens also affect mood, they have an influence on sexual interest (Basson 2004).

In a community-based cross-sectional study of 1021 18- to 75-year-old women, no clinically significant correlation was observed between a low score of any domain of the profile of female sexual function and low serum levels of free testosterone or 4-dione. However, an association was found between low DHEA-S and low sexual responsiveness in women aged ≥ 45 years. There was also a significant correlation between low serum DHEA-S and low arousal, pleasure and orgasm. For women aged 18–44 years, a low domain score for sexual desire, sexual arousal and sexual responsiveness was associated with a serum DHEA-S below the 10th centile (Davis 2005).

Loss of libido and/or sexual satisfaction are common in early postmenopause. The addition of androgens to HRT is known to have beneficial effects on these problems (Greenblatt *et al.* 1950, Grody *et al.* 1953, Leiblum *et al.* 1983, Sherwin & Gelfand 1987, Sherwin 1988). Shifren *et al.* (2000) have found that transdermal testosterone administered by patch improved sexual frequency, pleasure and mood in surgically menopausal women. The effect was seen at a daily 300 μ g dose of testosterone, a dose that led to serum testosterone levels in the upper limit of normal. Testosterone treatment has also been studied in non-androgen-deficient women complaining of decreased libido (Goldstat *et al.* 2003). Such treatment with testosterone improved libido and sexual function as well as quality of life compared with placebo. Similarly, in menopausal women with normal levels of androgens, the addition of methyltestosterone to estrogen increased sexual desire and frequency as compared with estrogen alone (Lobo *et al.* 2003). Similar results have been observed with testosterone implants (Davis *et al.* 1995). Among women with dysfunction of sexual interest and desire, androgen therapy has been suggested for those having free serum testosterone levels within the lower quantile of the reference range (Bachmann *et al.* 2002). In fact, there is increased use of testosterone to treat hypoactive sexual desire disorder (HSDD) (Sherwin & Gelfand 1987, Davis *et al.* 1995, Shifren *et al.* 2000, Goldstat *et al.* 2003). A series of randomized clinical trials demonstrate that testosterone is effective in women with HSDD.

In addition, the detailed benefits of androgens added to ERT or HRT have been described on general well-being,

energy, mood and general quality of life (Sherwin & Gelfand 1985, Sherwin 1988). Improvements in the major psychologic and psychomatic symptoms, namely irritability, nervousness, memory and insomnia have been observed following addition of androgens to ERT (Notelovitz *et al.* 1991). It should also be mentioned that androgenic compounds have been found to be beneficial for the treatment of the mastalgia frequently caused by HRT (Pye *et al.* 1985). In fact, ERT may result in severe breast pain which may lead to discontinuation of therapy.

The androgenic effect of DHEA should also be useful in reducing hot flushes. In fact, androgen therapy is successful in reducing hot flushes in hypogonadal men (De Fazio *et al.* 1984). Moreover, the addition of androgens has been found to be effective in relieving hot flushes in women who had unsatisfactory results with estrogen alone (Sherwin & Gelfand 1984). Hot flushes are one of the main reasons women initially seek HRT therapy, and estrogen is very effective at alleviating this symptom. Other studies have also shown a beneficial effect of DHEA on hot flushes (Baulieu 1999, Stomati *et al.* 2000).

A clear example of the nature of androgen deficiency of adrenal origin is provided by cases of adrenal insufficiency. Arlt *et al.* (1999) have studied the effect of 50 mg DHEA daily and placebo for 4 months in a population of women suffering from adrenal insufficiency. Treatment with DHEA raised serum testosterone in the low normal range. Such treatment increased the frequency of sexual thoughts, interest and satisfaction. Well-being, depression and anxiety were also improved. In a study where DHEA was administered at a high 300 mg daily dose, a greater subjective mental ($P < 0.016$) and physical ($P < 0.030$) stimulation was observed in response to an erotic video (Hackbert & Heiman 2002). In a study performed in women receiving 50 mg DHEA daily, improved libido was observed in women aged 70 years or more but not in those aged 60–70 years (Baulieu 1999).

Additional potential benefits of DHEA

The 70–95% reduction in the formation of DHEA and DHEA-S by the adrenals during aging results in a dramatic reduction in the formation of androgens and estrogens in peripheral target tissues, which could well be involved in the pathogenesis of age-related diseases such as insulin resistance (Coleman *et al.* 1982, Schriock *et al.* 1988) and obesity (Nestler *et al.* 1988, MacEwen & Kurzman 1991, Tchernof *et al.* 1995). Low circulating levels of DHEA-S and DHEA have also been found in patients with breast cancer (Zumoff *et al.* 1981) and DHEA has been found to exert anti-oncogenic activity in a series of animal models (Schwartz *et al.* 1986, Gordon *et al.* 1987, Li *et al.* 1993). DHEA has also been shown to have immunomodulatory effects *in vitro* (Suzuki *et al.* 1991) and *in vivo* in fungal and viral diseases (Rasmussen *et al.* 1992), including HIV (Henderson *et al.* 1992). On the other hand, a stimulatory

effect of DHEA on the immune system has been described in postmenopausal women (Casson *et al.* 1993).

DHEA has been shown to have important effects on the skin of aged individuals, the most salient of which is an increase in sebum production (Labrie *et al.* 1997b). The index of sebum secretion was 79% increased after 12 months of DHEA therapy with a return to pretreatment values 3 months after cessation of treatment. This has been shown in a number of studies performed in women, particularly those >70 years old who are physiologically hyposeborrheic and thus found an improvement in their skin with DHEA administration. The DHEA-induced increase in sebum production observed in our study is probably due to the fact that the sebaceous glands contain all the steroidogenic enzymes necessary to catalyze the transformation of DHEA into the androgen DHT, and that this androgen is the main stimulator of sebaceous gland activity (Labrie *et al.* 2000a, 2003a).

Apart from sebum production, other beneficial effects of DHEA on the skin have been noticed. To date, evaluation of the dermatological aspects of DHEA administration have only been performed in some detail in one study, the DHEA study in which male and female subjects between the ages of 60 and 79 years were orally administered 50 mg DHEA, once daily for 1 year. In that study, Baulieu *et al.* (2000) evaluated skin hydration, skin pigmentation and skin thickness. Skin surface hydration significantly increased for the whole DHEA-treated population examined after 12 months of treatment. Skin surface hydration is considered a real benefit for the skin, especially in aged individuals since in these subjects the dryness makes the skin rough. DHEA also significantly decreased facial skin pigmentation (yellowness) for the whole population. This decrease was more pronounced in women >70 years who are more concerned by age-related pigment changes. The two other components of skin colour remained stable during the duration of the study (i.e. lightness and redness).

Measurements of mid-thigh fat and muscle areas by computed tomography have shown a 3.8% decrease ($P<0.05$) in femoral fat and a 3.5% increase ($P<0.05$) in femoral muscular area at 12 months (Diamond *et al.* 1996). There was no significant change in abdominal fat measurements. These changes in body fat and muscular surface areas were associated with a 12% decrease ($P<0.05$) of fasting plasma glucose and a 17% decrease ($P<0.05$) in fasting plasma insulin levels. Treatment with DHEA had no undesirable effect on the lipid or lipoprotein profile. In fact, there was an overall trend for a 3–10% decrease in total cholesterol and its lipoprotein fractions. Plasma triglycerides were not affected.

DHEA administration stimulated vaginal epithelium maturation in eight out of ten women who had a maturation value of zero at the onset of therapy while a stimulation was also seen in the three women who had an intermediate vaginal maturation before therapy. Most

importantly, the estrogenic stimulatory effect observed in the vagina was not found in the endometrium which remained completely atrophic in all women after 12 months of DHEA treatment (Labrie *et al.* 1997b).

The present data suggest that the beneficial effects of DHEA therapy in postmenopausal women are exerted through the transformation of the steroid precursor into androgens and/or estrogens in specific intracrine target tissues, thus limiting the possibility of side-effects. As an example, the absence of stimulation of the endometrium by DHEA (Labrie *et al.* 1997c, Baulieu *et al.* 2000) should eliminate the need for progestin replacement therapy, thus avoiding the fear of progestin-induced breast cancer in postmenopausal women (Women's Health Initiative 2002). The observed stimulatory effect of DHEA on bone mineral density and the increase in serum osteocalcin, a marker of bone formation, are of particular interest for the prevention and treatment of osteoporosis and indicate a unique activity of DHEA on bone physiology, namely a stimulation of bone formation, while ERT and HRT can only reduce the rate of bone loss. In the light of the Women's Health Initiative study, the indication and benefits of HRT should be evaluated with care and adapted to the clinical situation of each woman.

The effects of DHEA are a combination of estrogen-like and androgenic effects

Androgen therapy, as observed with nandrolone decanoate, has been found to increase vertebral bone mineral density as well as cortical bone mineral content in postmenopausal women (Need *et al.* 1989). Androgenic side-effects, however, were recorded in 50% of patients. Such data are of interest since while almost all present therapies are limited to a reduction of bone loss, an increase in bone mass was found with the use of the anabolic steroid nandrolone. A similar stimulation of bone formation by androgens has been suggested in a hypogonadal male (Baran *et al.* 1978). A stimulation of bone formation in postmenopausal women treated with DHEA for 12 months is reported by Labrie *et al.* (1997b).

Most importantly, it has been observed that androgens exert a direct anti-proliferative activity on the growth of ZR-75-1 human breast cancer cells *in vitro* and that such an inhibitory effect of androgens is additive to that of an anti-estrogen (Poulin & Labrie 1986, Poulin *et al.* 1988). Similar inhibitory effects have been observed *in vivo* on ZR-75-1 xenographs in nude mice (Dauvois *et al.* 1991). Androgens have also been shown to inhibit the growth of 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma in the rat, this inhibition being reversed by the simultaneous administration of the pure anti-androgen flutamide (Dauvois *et al.* 1989). Taken together, these data indicate the involvement of the androgen receptor in the inhibitory action of DHEA on breast cancer.

Figure 9 Comparison of the effects of standard ERT (estrogen) and DHEA on parameters of menopause.

Since the endometrium remained atrophic after 12 months of treatment of postmenopausal women with DHEA (Labrie *et al.* 1997b), the proposed novel approach with DHEA (Fig. 9) should eliminate the need to use a progestin to protect against endometrial proliferation, thus avoiding the recently demonstrated stimulatory effect of progestins on breast cancer (Bergkvist *et al.* 1989, Clarke & Sutherland 1990, Musgrove *et al.* 1991, Horwitz 1992, Colditz *et al.* 1995, Magnusson *et al.* 1999, Persson 1999, Ross *et al.* 2000, Women's Health Initiative 2002).

The potential approach of HRT with DHEA is based upon the recent progress achieved in our understanding of sex steroid physiology in women and the recognition that women, at menopause, are not only deprived of estrogen due to the arrest of estrogen secretion by the ovaries, but have already been submitted for a few years to a decreasing exposure to androgens. In fact, normal women produce an amount of androgens equivalent to two-thirds of the androgens secreted in men (Labrie *et al.* 1997a). The pool of androgens in women decreases progressively from the age of 30 years in parallel with the decrease in the serum concentration of DHEA and DHEA-S (Labrie *et al.* 1997c). Consequently, it appears logical to use both androgenic and estrogenic replacement therapy at peri-

and postmenopause, thus maintaining a physiological balance between these two classes of sex steroids in each cell and tissue, a goal which can only be met by the local formation of androgens and estrogens in peripheral tissues from a steroid precursor such as DHEA (Fig. 9). In Fig. 9, comparison is made with the positive and negative effects of DHEA versus classical ERT.

It should also be mentioned that our data obtained in the rat clearly demonstrated that DHEA can provide beneficial effects which are lacking with the use of a SERM alone (Labrie *et al.* 2003b). In fact, while a SERM has effects limited to inhibition of bone resorption, the addition of DHEA stimulates bone formation (an effect not found with a SERM or an estrogen) and further reduces bone resorption above the effect achieved with a SERM alone. In addition to an increase in bone formation, DHEA has also been shown in postmenopausal women to stimulate vaginal maturation and decrease skin dryness.

Role of DHEA in men

Prostate cancer is the most frequently diagnosed cancer and the second cause of cancer death in men in North America (Jemal *et al.* 2005). In fact, one in eight men will

be diagnosed with prostate cancer during his lifetime. At the present rate, of the male population living in the USA, prostate cancer will kill more than 3 million men. Prostate cancer is thus a major medicosocial problem comparable with that of breast cancer in women. In fact, it was predicted that 30 350 men will die from prostate cancer in the USA in 2005.

The serious and frequently lethal cardio- and cerebrovascular complications of estrogens (VACURG 1967, Robinson & Thomas 1971, Peeling 1989), on one hand, and the psychological (Lunglmayr *et al.* 1988, Cassileth *et al.* 1989) as well as the physical limitations of orchiectomy, on the other hand, have generally delayed endocrine treatment until late stages of the disease when pain and debility had developed. Typically, at such a late stage, the large and disseminated tumors show poor and short-lived responses, thus limiting the success of endocrine therapy. In fact, similar to treatments for all other types of cancers, androgen blockade loses its effectiveness with increasing size of the tumors (Chen *et al.* 1996).

As indicated by a high proportion of positive responses achieved after only partial blockade of androgens by orchiectomy (Nesbit & Baum 1950, Staubitz *et al.* 1954, VACURG 1967, Mettlin *et al.* 1982, Murphy *et al.* 1983), prostate cancer is the most sensitive of all hormone-sensitive cancers to endocrine therapy. This uniquely high sensitivity of prostate cancer to androgens should be exploited optimally in order to best succeed in the fight against this disease.

In the course of our attempts to find an explanation for the lack of a stimulatory effect of chronic administration of GnRH agonists on gonadal functions, we made the unexpected observation that treatment of adult male rats for a few days led to variable degrees of inhibition of serum testosterone levels accompanied by a relatively small but usually significant inhibition of ventral prostate, seminal vesicle and testis weight (Auclair *et al.* 1977a,b). It should be mentioned that when we were treating rats with a GnRH agonist some 28 years ago we were expecting to observe larger seminal vesicles and a prostate of increased volume. Most unexpectedly, the opposite observation was made: the prostate, the seminal vesicles and the testicles became smaller instead of larger after a few days of treatment with a GnRH superagonist.

While experiments performed in the rat were simply suggestive of an inhibitory effect of GnRH agonists on testicular functions, we discovered in 1979 at our Clinic at the Laval University Medical Center that medical castration is achieved in men following chronic administration of GnRH agonists (Labrie *et al.* 1980).

Soon after our observation (Labrie *et al.* 1980) that administration of the GnRH agonist buserelin led to an almost complete inhibition of serum testosterone and DHT levels within 2 weeks following administration by the intranasal route, a less than optimal route of administration (Labrie *et al.* 1980), a detailed comparison of the

effect of various doses of the same GnRH agonist was performed after administration by the intranasal and subcutaneous routes (Faure *et al.* 1982). It is well recognized that medical castration with a GnRH agonist is equivalent to orchiectomy for prostate cancer therapy (Prostate Cancer Trialists' Collaborative Group 2000). In a comparison of 11 trials in which a GnRH agonist was used and in 17 trials in which orchiectomy was used, no difference was seen in the response or survival rate (Prostate Cancer Trialists' Collaborative Group 2000).

Two equally important sources of androgens are present in men

An important advance in our understanding of the biology and endocrinology of prostate cancer and its major impact on cancer treatment is the observation that humans and some other primates are unique among animal species in having adrenals that secrete large amounts of the inactive precursor steroids DHEA, its sulfate DHEA-S and some 4-dione, which are converted into potent androgens in a large series of peripheral tissues, including the prostate (Fig. 1B).

As indicated above, the local synthesis of active steroids in peripheral target tissues has been named intracrinology (Labrie *et al.* 1988, 2003a, Labrie 1991). The active androgens made locally in the prostate exert their action by interacting with the androgen receptor in the same cells where their synthesis takes place without being released in significant amounts in the extracellular environment or the general circulation. Contrary to the previous belief that the testes are responsible for 90–95% of total androgen production in men (as could be inferred from the 90–95% decrease in serum testosterone observed after castration), it is now well demonstrated that the prostatic tissue efficiently transforms the inactive steroid precursors DHEA-S, DHEA and 4-dione into the active androgens testosterone and DHT locally in peripheral tissues, including the prostate, without significant release of the active androgens in the circulation. In fact, the prostate makes its own androgens at a level comparable with the androgens of testicular origin (Fig. 1B).

Combined androgen blockade (CAB) in advanced disease

The first treatment shown to prolong life in prostate cancer was the combination of a GnRH agonist to block androgen secretion by the testes in association with an effective dose of a pure anti-androgen such as flutamide, nilutamide or bicalutamide (Labrie *et al.* 1982, 1985). These anti-androgens (sometimes called non-steroidal anti-androgens) block the action of the androgens produced locally in the prostate by interfering at the level of the androgen receptor.

An interesting observation is that the first demonstration of the benefits of CAB on survival (Labrie *et al.* 1982, 1985) has been achieved in the most difficult group of

Figure 10 Summary of meta-analyses comparing CAB (combination of medical or surgical castration) associated with a pure anti-androgen or non-steroidal anti-androgen (NSAA), namely flutamide or nilutamide versus medical or surgical castration alone. Adapted from Klotz *et al.* (2001). Caubet=Caubet *et al.* (1997); Debruyne=Debruyne *et al.* (2001); Bennett=Bennett *et al.* (1999).

patients to treat, namely those suffering from metastatic or advanced disease. These data have been obtained with flutamide and nilutamide. Although, in principle, the clinical results should be similar for bicalutamide, the two anti-androgens flutamide and nilutamide are those first demonstrated in prospective and randomized studies to prolong life, to increase the number of complete and partial responses, to delay progression and to provide better pain control (thus improving quality of life) in metastatic prostate cancer when added to surgical or medical castration compared with castration alone (Crawford *et al.* 1989, Denis *et al.* 1993, 1998, Janknegt *et al.* 1993, Caubet *et al.* 1997, Dijkman *et al.* 1997, Bennett *et al.* 1999, Prostate Cancer Trialists' Collaborative Group 2000, Debruyne *et al.* 2001, Klotz 2001, 2003, Schmitt *et al.* 2001, Aprikian *et al.* 2003). In the first large scale randomized study, patients who were treated with flutamide and the GnRH agonist lupron lived, on average, 7.3 months longer than those who received lupron plus placebo (Crawford *et al.* 1989).

Analysis of all the studies performed with flutamide and nilutamide associated with medical or surgical castration compared with castration plus placebo shows that overall survival (deaths from all causes) is increased by an average of 3–6 months following the addition of a pure anti-

androgen (Crawford *et al.* 1989, Denis *et al.* 1993, 1998, Janknegt *et al.* 1993, Caubet *et al.* 1997, Dijkman *et al.* 1997, Bennett *et al.* 1999, Prostate Cancer Trialists' Collaborative Group 2000, Schmitt *et al.* 2001) (Fig. 10). Since about 50% of patients in that age group (65 to 80 years old) die from causes other than prostate cancer, this 3–6 month difference in overall survival corresponds to an average of 6–12 months of life gained when cancer-specific survival is calculated. These additional months, or sometimes years, of life are obtained by simply adding a pure anti-androgen (flutamide, nilutamide or bicalutamide at a proper dose) to castration. Considering that such statistically significant benefits on survival are obtained, even at the very advanced stage of metastatic disease, these data demonstrate, as mentioned earlier, the particularly high level of sensitivity of prostate cancer to androgen deprivation.

As illustrated in Fig. 10, all the meta-analyses of all the data have shown significant ($2P<0.05$) or highly significant ($2P<0.01$) advantages of CAB versus castration alone in advanced prostate cancer (Caubet *et al.* 1997, Bennett *et al.* 1999, Prostate Cancer Trialists' Collaborative Group 2000, Debruyne *et al.* 2001, Klotz 2001, Schmitt *et al.* 2001). However, when the studies providing the most rigorous data are analyzed (Caubet *et al.* 1997), a 20%

advantage in overall survival is observed. Moreover, these differences are not those obtained strictly when comparing CAB versus castration but they rather compare immediate versus deferred CAB since most patients received an anti-androgen at the time of progression with castration alone.

It is of interest to mention the first results of a Japanese study (Akaza *et al.* 2004) showing improved PSA normalization (79.4% versus 38.6%) at 12 weeks and time to treatment failure (96.1 versus 67.7 weeks) in advanced prostate cancer patients who received the combination of a GnRH agonist and 80 mg/day bicalutamide versus the GnRH agonist and placebo. The risk of progression during follow-up was thus reduced by 54% in the CAB group compared with chemotherapy. This study, however, is not sufficiently mature to calculate the effect on survival but the early effects observed are in line with previous studies.

Concerning the costs of treatment, as recently published by Aprikian *et al.* (2003), the cost per month of prolonged survival in prostate cancer achieved with the simple addition of a non-steroidal anti-androgen to castration (GnRH agonist or orchiectomy) is 50% of that of vinorelbine for lung cancer, 10% of the cost of renotecan for colon cancer and 10% of the cost of trastuzumab for breast cancer. Moreover, the non-steroidal anti-androgens have minimal toxicity while vinorelbine and irinotecan are associated with severe grade 3 and 4 clinical toxicities and trastuzumab has cardiac side-effects when associated with anthracyclines. As Klotz (2003) said, 'We should embrace the modest survival benefit of CAB in advanced prostate cancer and offer it to the appropriate patients.'

In addition to the prolongation of survival, all the studies have shown that the decrease in bone pain is more rapid and more complete and that progression of the cancer is delayed, thus improving quality of life, when CAB is used compared with monotherapy. Moreover, CAB is the only treatment shown to prolong life in advanced disease. There is thus no other choice if one wants to prolong life. It should also be realized that there is no treatment of similarly advanced cancers that provides 3–6 months of prolongation of life or 6–12 months of additional cancer-specific survival with such a good quality of life. To the living population of males in the USA, where 3 million are expected to die from prostate cancer, 6 additional months of life correspond to the addition of 1.5 million years of life, while 12 additional months correspond to 3.0 million years of life.

High probability of cure of localized prostate cancer by treatment with CAB

Despite the important advance observed with monotherapy (GnRH agonists) in localized prostate cancer, namely at least a one-third reduction in deaths from prostate cancer (Peto & Dalesio 2003), can we achieve better results?

Based upon the observation that 50% of androgens are left in the prostate after castration alone (Figs 1B and 4), it is reasonable to suggest that superior results can be achieved with the combination of a GnRH agonist and a pure anti-androgen. There are already data indicating that patients with minimal metastatic disease derive greater benefits than those with extensive metastatic disease (Crawford *et al.* 1989, Denis *et al.* 1998, Soloway 1998).

Using CAB in localized and locally advanced disease, the evidence obtained even indicates that long-term control or cure of the disease can be obtained in at least 90% of patients (Labrie *et al.* 2002). In fact, while almost all studies performed so far in localized prostate cancer have used monotherapy (medical or surgical castration) (Bolla *et al.* 1997, Pilepich *et al.* 1997, Granfors *et al.* 1998, Messing *et al.* 1999, Hanks *et al.* 2000, D'Amico *et al.* 2004), there are strong scientific reasons to believe that even much better results can be expected with CAB (Labrie *et al.* 1985, Caubet *et al.* 1997, Bennett *et al.* 1999, Labrie 2000a,b, Prostate Cancer Trialists' Collaborative Group 2000).

Since we have already obtained evidence for the high efficacy of long-term and continuous CAB in localized prostate cancer (Labrie *et al.* 1999a), it was felt important to examine the long-term outcome of these patients as assessed by biochemical failure (PSA progression) following cessation of continuous CAB previously administered for periods up to 11.3 years. The effect of CAB on long-term control or possible cure of prostate cancer was thus evaluated by the absence of biochemical failure or the absence of a PSA rise for at least 5 years following cessation of continuous treatment. A total of 57 patients with initial localized or locally advanced disease thus received CAB for periods ranging from 1 to 11 years. CAB was then discontinued and the patients followed for a minimum of 5 years. Among the 20 patients with stage T2–T3 cancer initially who stopped treatment after continuous CAB for more than 6.5 years, only two PSA rises occurred for a non-failure rate of 90% (Fig. 11). For the 11 patients who had received CAB for 3.5–6.5 years, the non-failure rate was only 36%. It is of major interest that serum PSA increased within 1 year after cessation of CAB in all 11 patients with stage B2/T2 cancer initially treated with CAB for only 1 year, thus showing that active cancer remained present after short-term androgen blockade limited to 1 year despite undetectable PSA levels. Most importantly, in all patients who had biochemical failure after stopping CAB, serum PSA rapidly decreased again to undetectable levels soon after CAB was restarted and PSA remained at such low levels afterward. Of these 57 patients, only one patient had died of prostate cancer at the last follow-up (Labrie *et al.* 2002).

These are remarkable results observed in patients with localized prostate cancer. Treatment, however, must be continuous, without interruption and should last for many years. It is important to mention that the major survival

Figure 11 Effect of treatment duration of localized prostate cancer with continuous CAB on the probability of long-term control or cure of the disease as determined by no recurrence of a rise in PSA for at least 5 years after CAB cessation. The point at 4-7.5 years of treatment (33%) refers to three patients treated with CAB for 3-5-0 years and followed-up for at least 5 years, the point at 5-7.5 years refers to eight patients treated continuously with CAB for 5-0-6-5 years before cessation of treatment, the point at 8-2.5 years refers to eight patients treated continuously for 6-5-9-0 years and the point at 11 years refers to 12 patients treated for 10-11-7 years with continuous CAB before stopping treatment. All patients were followed-up for at least 5 years after continuous CAB or until a rise in PSA. Only one patient died of prostate cancer and 18 have died of other causes (Labrie *et al.* 2002).

benefits observed following androgen blockade, even in localized or locally advanced disease, are always associated with long-term (many years of non-interrupted) treatment (Bolla *et al.* 1997, Labrie *et al.* 1999b, 2002, Messing *et al.* 1999). In fact, an important observation is that when PSA increases following cessation of treatment, administration of CAB was successful in all cases in decreasing PSA to undetectable levels again, thus showing that, even after a long duration of treatment, resistance to CAB had not developed. In fact, resistance to CAB is the common finding in prostate cancer metastasized to the bone while it does not occur for the cancer localized in the prostate or in the prostatic area.

The present results obtained in prostate cancer patients diagnosed with localized disease and treated continuously for many years with CAB are not too different from the results that we have recently obtained with human breast tumor xenografts in nude mice where complete estrogen blockade achieved with a highly potent anti-estrogen led to the disappearance or cure of the tumors in 61% of cases within a few months (Roy *et al.* 2003). In fact, in both breast and prostate cancer, when the estrogens in breast cancer and the androgens in prostate cancer are blocked efficiently, cure of the disease can be achieved with hormonal therapy.

As mentioned above, however, the success of therapy requires long-term and continuous treatment before complete apoptosis or total cell death is achieved. Such results

clearly indicate that intermittent androgen blockade should remain experimental and should not be used outside clinical trials. Breast and prostate cancers have many characteristics in common and much can be learned from looking at the results obtained in each of them. In fact, when we consider the biology of these two cancers, there are many common features, especially the high level of sensitivity to hormones.

Most importantly, the present data indicate that possible cure of the disease can be obtained in most patients with localized prostate cancer treated continuously with CAB for more than 8 years, thus raising hopes for the successful treatment of patients who fail after surgery, radiotherapy or brachytherapy where no or minimally effective alternative therapeutic approach exists.

Major impact of blockade of androgens derived from DHEA in prostate cancer

The life-saving benefits of androgen blockade in prostate cancer have been largely underestimated. When compared with other cancer therapies, the results obtained are quite remarkable. In agreement with the data summarized above, a recent analysis of all clinical trial data attributes part of the improving outlook in the field of prostate cancer to early detection and prompt radical prostatectomy, but mostly gives the credit to follow-up hormone therapy. 'Hormonal treatment as a whole works ridiculously well' (Peto & Dalesio 2003), as reported by Arnst (2003). In fact, while death rates decreased by 1-1% per year from 1993 to 2001 for all cancers combined, prostate cancer showed a larger decrease at 3-6% (Mehring 2004). Although improvements in surgery and radiotherapy are likely to play a role, a study by Frank R Lichtenberg using National Cancer Institute data obtained from 2-1 million cancer patients has concluded that cancer-fighting drugs improved survival rates, especially for cancer of the prostate, where drug innovations have been the greatest (Mehring 2004).

It is important to note that androgen blockade is not only cytostatic, as was previously believed. In fact, androgen blockade is also cytotoxic or tumoricidal in localized disease. Moreover, it is important to remember that resistance to androgen blockade does not occur or is extremely rare in localized disease under treatment with CAB. Clearly, resistance to androgen blockade is a phenomenon typical of metastatic disease in the bones where the environment is very different and where the growth factors present in large amounts stimulate cancer growth, even in the absence of androgens. This knowledge about the absence of development of resistance to CAB in localized prostate cancer is extremely important. In fact, it is often erroneously believed that early androgen blockade should not be administered because resistance to treatment will develop and one might as well wait to use androgen blockade at a later stage of the disease. In fact, deferring

treatment implies that very often it will then be too late because, following migration of the cancer to the bones, resistance to treatment will occur automatically. It should be realized that when prostate cancer is first detected, even by screening, the cancer is not small since its diameter is of the order of 1 cm or more. This is the most appropriate time to treat with the very strong hope of a cure. The results summarized above indicate that androgen blockade, more specifically CAB, is probably the most efficient treatment of localized prostate cancer but the start of treatment should not be delayed.

It is important to remember that by avoiding the psychological limitations of surgical castration and the serious side-effects of high doses of estrogens, GnRH agonists are playing a leader role in the very efficient fight against prostate cancer. With the presently available techniques, screening can diagnose prostate cancer at a clinically localized stage in 99% of cases (Labrie *et al.* 1996a, 2002). Such an early diagnosis permits immediate treatment with a curative intent, CAB being a truly efficient alternative. Most importantly, CAB must be used immediately in patients who fail radical prostatectomy, radiotherapy or brachytherapy. Using this strategy, based upon today's available diagnostic and therapeutic approaches, death from prostate cancer can already be an exception (Labrie 2002).

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