duces fluctuations offers the hope that improved methods of delivering levodopa or development of long-acting dopaminergic drugs will reduce the "on–off" phenomenon.

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SERUM LEVELS OF SEX HORMONES IN VULVAR LICHEN SCLEROSUS, AND THE EFFECT OF TOPICAL TESTOSTERONE

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Abstract Lichen sclerosus is a chronic cutaneous disorder of unknown cause that most commonly occurs on the vulva in postmenopausal women. Earlier investigations suggested that the affected tissue is reversibly atrophic and that it has normal metabolic potential. The present study was designed to determine whether levels of sex steroid hormones in serum are altered in this disease and how they are affected by topical testosterone, a recognized form of therapy.

As compared with normal values for age, serum levels of dihydrotestosterone, free testosterone, and androstenedione were significantly decreased in patients with untreated vulvar lichen sclerosus. Dihydrotestosterone and testosterone levels rose and exceeded normal values after therapy. These results suggest that abnormal enzymatic activity (5α-reductase) may be responsible for this disease and indicate a need for similar studies of the disorder in other locations. (N Engl J Med 1984; 310:488-91.)

LICHEN sclerosus is a chronic cutaneous disorder that may affect any skin area in any race, at any age, and in either sex.[1-3] Most commonly, however, it occurs on the vulva in postmenopausal white women. Untreated, the condition is progressive and results in pruritus, dissolution of the labia minora, ecchymoses, and contracture of the introitus with subsequent dyspareunia.[4] Squamous carcinoma is a rare development,[5] and there are minor associations with achlorhydria, vitiligo, autoimmune thyroiditis, and pernicious anemia.[6]

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Knowing that testosterone has a stimulating effect on skin and skin derivatives, Cinberg used topical testosterone to treat vulvar lichen sclerosus in 14 postmenopausal women and reported satisfactory results.[7] Subsequently, other investigators[8-14] used 2 per cent testosterone propionate in petrolatum to treat a total of 142 biopsy-proved cases of vulvar lichen sclerosus and reported that improvement occurred in 135. Pasieczny has demonstrated that this treatment is also effective in men.[15] Although topical testosterone is not effective in all cases, it is now recognized as a standard method of therapy for this disease.[16]

Prompted by this clinical experience, we conducted a study to determine whether or not serum androgen and estrogen levels are altered in patients with un-

treated lichen sclerosus, as compared with normal values for age. A follow-up study was performed to determine whether the levels of these hormones change after initiation of topical testosterone therapy.

Methods

In 30 women with untreated lichen sclerosus who presented at the Vulvar Referral Unit of the University of Florida Department of Obstetrics and Gynecology, histologic confirmation of the diagnosis was obtained. The patients ranged in age from 19 to 87 years, with a mean of 58.6. All but five women were over age 50, and all were white. Informed consent was obtained, and serum samples were collected during the initial visit between the hours of 1 and 4 p.m. All patients were then given 2 per cent testosterone propionate in white petrolatum for application to the vulva twice a day. Additional serum samples were obtained from 10 of the 30 patients at varying intervals after the initiation of therapy. The 10 were representative of the overall study group.

For patients in whom subsequent samples were obtained, the symptomatic and objective clinical responses and side effects were documented.

Serum levels of the androgens testosterone, dihydrotestosterone, and androstenedione, and of the estrogens estrone and estradiol were quantitatively determined by specific radioimmunoassays, as previously described.[17,18] In addition, the levels of sex hormone–binding globulin and unbound testosterone were determined. Results were corrected for procedural losses.

Androgens

A 1.0-ml serum sample was extracted twice with diethyl ether after the addition of tracer amounts of tritiated testosterone, dihydrotestosterone, and androstenedione. The three androgens were isolated from the dried ether extract by chromatography on Sephadex LH-20 columns. Antiserum to testosterone-3-carboxymethyloxime conjugated to bovine serum albumin was prepared in rabbits and used for both testosterone and dihydrotestosterone estimations, along with the appropriate tritiated and standard steroids. Antiserum to androstenedione was obtained from Radioassay Systems Laboratories. The interassay coefficients of variation were 7.9 per cent (testosterone), 10.1 per cent (dihydrotestosterone), and 10.7 per cent (androstenedione).

Levels of non–protein-bound (free) testosterone were calculated on the basis of estimated levels of sex hormone–binding globulin, with ^{3}H-dihydrotestosterone used to estimate binding capacity, according to the method of Wiest et al.[19]

Estrogens

Estrone and estradiol were similarly extracted from 1.0 ml of serum with diethyl ether and were purified on Sephadex LH-20 minicolumns. Antiserum to 6-keto-estradiol was supplied by Dr. G. D. Niswender, Colorado State University. Estrone antiserum was prepared from rabbits. Validation of both, as performed in our laboratory, has been reported elsewhere.[18] The sensitivities of the two assays were 5 pg per tube, and the interassay coefficients of variation were 9.6 per cent (estrone) and 12.2 per cent (estradiol).

Statistical Analyses

Average serum hormone levels in the 30 patients with lichen sclerosus were compared with values normal for age in our laboratory by the two-tailed paired t-test. In 10 patients comparisons between initial hormone levels and those found during testosterone therapy were also made by the two-tailed paired t-test. A P value less than 0.05 was considered to be significant.

Results

Serum hormone levels in the patients before treatment, as compared with normal values for their age groups, are presented in Table 1. Significant differences were observed in androgen levels but not in estrogen levels. Although the levels of total testosterone did not differ between the patients and the controls, the levels of serum dihydrotestosterone and androstenedione were significantly lower in the patients ($P<0.0001$) and the levels of free testosterone were significantly higher ($P<0.02$). In five postmenopausal patients receiving exogenous estrogen (one orally and four vaginally), the results did not differ significantly from those in the total group, nor did exclusion of these patients from the group alter the results. Because of the small sample size, insignificant results cannot be considered definitive. Further evaluation of more cases would be needed before a valid negative conclusion could be drawn.

Sex hormone–binding globulin levels were assayed in all patients and did not differ significantly from values normal for age (Table 1). No significant change was noted between values before and during treatment (Table 2).

Hormone levels in 10 patients receiving testosterone therapy for 3 to 18 months are shown in Table 2. During the period of testosterone application, both total testosterone and dihydrotestosterone levels increased significantly, resulting in values higher than those in untreated normal women. The increase in levels of free testosterone was not significant. There were no significant differences in androstenedione and estrone levels, but estradiol levels showed an unexplained decrease. The pre- and post-treatment dihy-

Table 1. Initial Serum Hormone Levels in Patients with Vulvar Lichen Sclerosus, as Compared with Expected Values According to Age.*

	Dihydrotestosterone	Testosterone		Androstenedione	Estrone	Estradiol	Sex Hormone–Binding Globulin
		Total	Free				
	ng/dl	*ng/dl*	*pg/ml*	*ng/ml*	*pg/ml*		*$\times 10^{-8}$ M*
Patients	7.2±0.4	25.5±0.9	6.4±0.6	0.57±0.06	70.7±1.6	34.3±1.0	9.622±1.905
Expected normal values	12.9±0.3	22.5±0.4	2.2±0.05	0.87±0.07	55.7±0.8	39.9±1.4	8.300±0.127
No. of patients	29	30	28	29	30	30	28
P value	<0.0001	NS	<0.02	<0.0001	NS	NS	NS
SI multiplication factor (nmol/liter)	0.0344	0.0346	0.0035	3.4916	0.0036	0.0036	—

*Values are presented as means ±S.E.M. Significance was determined with the two-tailed paired t-test. NS denotes not significant.

Table 2. Serum Hormone Levels before and during Testosterone Therapy in Patients with Vulvar Lichen Sclerosus.*

	No. of Patients	Dihydrotestosterone	Testosterone		Androstenedione	Estrone	Estradiol	Sex Hormone-Binding Globulin
			Total	Free				
		ng/dl	ng/dl	pg/ml	ng/ml	pg/ml		$\times 10^{-8}$ M
Initial level	10	7.0±0.9	22.5±3.0	4.1±0.8	0.57±0.07	76.0±11.7	49.4±14.8	7.745±1.119
Follow-up level	10	28.2±3.1	87.5±26.2	20.6±8.0	0.49±0.06	59.3±8.7	24.7±7.9	10.356±2.031
P value		<0.001	<0.05	NS	NS	NS	<0.05	NS
SI multiplication factor (nmol/liter)		0.0344	0.0346	0.0035	3.4916	0.0036	0.0036	--

*Values are presented as means ±S.E.M. Significance was determined with the two-tailed paired t-test. NS denotes not significant.

drotestosterone levels are shown in Figure 1.

Pubic hirsutism accompanied by slight facial acne developed in 1 of the 10 patients, but side effects were not observed in the other nine. Two patients (ages 25 and 56) did not have a clinical response despite a rise in their serum androgen levels.

Discussion

Only a limited number of basic investigations in lichen sclerosus have been completed. That the disease may recur in normal myocutaneous-pedicle grafts translocated to a previously affected vulvar site suggests that the process is independent of the local neurovascular supply.[20] Immunohistology has demonstrated the frequent deposition of fibrin in the upper dermis,[21] and a specific protease has been implicated in the disappearance of elastic fibers in this disease.[22] Normal levels of nucleic acid activity have been documented,[23,24] and radioactive phosphorus uptake is not diminished.[25] Glucose metabolism, alkaline phosphatase levels, and adenosine triphosphatase activity in slices of affected tissue exceed the values noted in skin from normal menopausal women.[26,27] The appearance of atrophy in this condition thus belies the demonstrated metabolic potential of the tissue.

In the present study, women with vulvar lichen sclerosus were shown to have significantly decreased serum levels of dihydrotestosterone and one of its precursors, androstenedione. After the topical application of 2 per cent testosterone propionate, the serum levels of both dihydrotestosterone and testosterone were significantly increased. In contrast, Zelle found no abnormal plasma androgen levels in five patients after 4 to 17 months of topical testosterone therapy.[9]

In normal women, dihydrotestosterone is largely derived by peripheral synthesis from plasma precursors. An important androgen, its production rate is one third that of testosterone.[28] It is well established that 5α-reduction of testosterone to dihydrotestosterone occurs in the accessory sex organs in men and in the skin of both men and women. Wilson and Walker reported regional variation in the rate of dihydrotestosterone formation.[29] 5α-Reductase activity was found to be considerably higher in perineal tissues than in nonperineal areas. Subsequent in vitro studies have shown that 5α-reductase activity in pubic skin is androgen-dependent,[30] is higher in men than in women, increases at puberty,[31] is higher in hirsute women,[32,33] apparently is absent in patients with com-

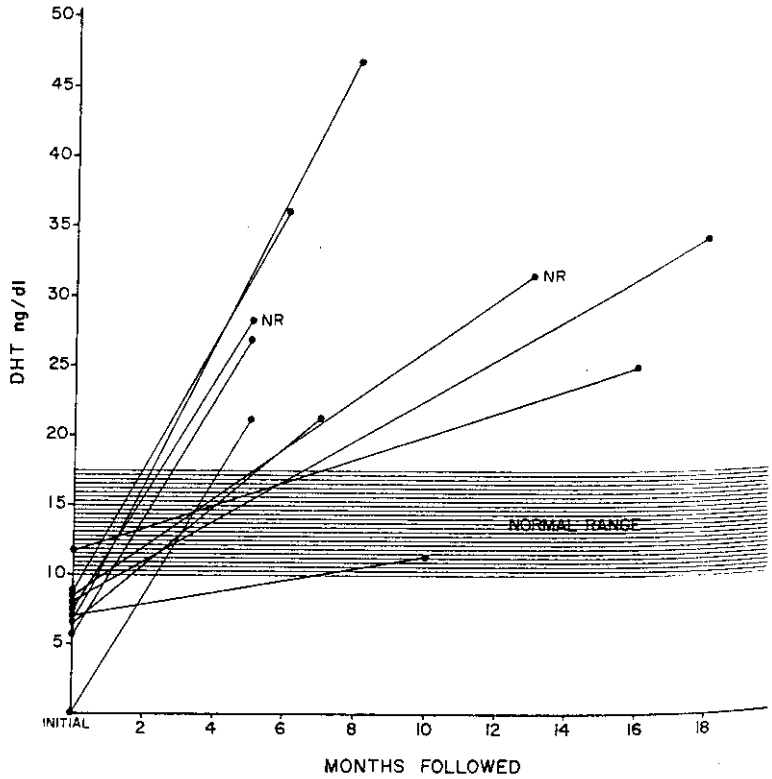

Figure 1. Serum Dihydrotestosterone (DHT) in 10 Patients before and after Testosterone Therapy.

NR denotes no response to therapy. Multiplication factor to convert DHT values to nanomoles per liter is 0.0344.

plete testicular feminization, and may be induced by androgens in men with hypogonadism.[30] Furthermore, androgen-specific intracellular receptor proteins have been demonstrated in vulvar skin fibroblasts.[34-36] Twice the number of high-affinity binding sites are present in fibroblasts from genital sites, as compared with those from nongenital sites.[37]

Since serum dihydrotestosterone levels were suppressed in our patients and were restored after testosterone therapy, accompanied by clinical improvement in most cases, it is possible that reduced 5α-reductase activity, at least at a local level, may have contributed to the onset of lichen sclerosus. Additional 5α-reductase activity may have been induced by a therapeutic increase in the levels of precursor testosterone, resulting in a significant rise in the level of dihydrotestosterone. This hypothesis is compatible with the clinical observation that some children with lichen sclerosus improve spontaneously at puberty.[3] The increased levels of androgens associated with adrenarche may act to stimulate 5α-reductase activity at the vulvar site.

However, in our patients there was no correlation between androgen levels during treatment and either a clinical response or development of side effects. Slight hirsutism developed in 1 of the 10 patients in the treatment group, but the rise in her androgen level was not excessive. Two other patients did not have a clinical response, although their androgen levels during treatment were among the highest in the group. The number of androgen receptor sites may vary greatly from patient to patient. These random clinical responses and side effects may indicate that the number and saturation of receptor sites are more important than absolute circulating hormone levels.

Although the patient population in this study was limited to women with the more common vulvar manifestations of the disease, women with lichen sclerosus in other locations and men with balanitis xerotica obliterans should be studied as well.

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