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The Relevance of the Pharmacologic Properties of a Progestational Agent to Its Clinical Effects as a Combination Oral Contraceptive

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Levonorgestrel (LNg) is known for its marked progestational/contraceptive activity. As shown in animal experiments, however, high doses of LNg are required to elicit an androgenic response; in contrast, considerably lower doses of LNg are required for antiovulatory (contraceptive) action. Thus, a large dose separation exists between androgenic and contraceptive activity. When LNg is combined with an estrogen, as in the contraceptive formulations, the androgenic response is attenuated or negated.

The results of recent clinical trials have demonstrated that the androgenic activity of LNg is not expressed at contraceptive doses, particularly when LNg is combined with ethinyl estradiol (EE), as in the low-dose monophasic/triphasic formulations (monophasic [Nordette®]: 150 mcg LNg/30 mcg EE; triphasic [Triphasil®/Trinordiol®]: six days, 50 mcg LNg/30 mcg EE; five days, 75 mcg LNg/40 mcg EE; ten days, 125 mcg LNg/30 mcg EE). Clinical evidence from several trials confirms that sex hormone-binding globulin levels are increased, plasma androgen levels are decreased, and acne is markedly improved with the use of Triphasil® and Nordette®, suggesting a non-androgenic profile.

INTRODUCTION

The 19-nortestosterone derivatives have more than one biological activity: e.g., progestational, anti-estrogenic, androgenic, anabolic. The merits of a 19-nortestosterone steroid as a contraceptive component are judged on its ovulation-inhibiting properties and progestational activity, and the margin of separation from other biological activities. Initially, the steroid is profiled in animal tests using a large dose range. When these steroids are combined with estrogen and used at contraceptive doses in humans, however, other steroid activities may not be manifested.

As an example, levonorgestrel (LNg) has been shown in animal experiments to have marked progestational and anti-ovulatory activity at low doses; however, in contrast, very high doses are required to evoke an androgenic response. In animals, when low doses of LNg are used in combination with estrogen at contraceptive doses, the androgenic activity of LNg is not manifested; in humans, a growing body of clinical evidence demonstrates a non-androgenic profile, substantiating the preclinical obser-

Abbreviations: A: androstenedione DHEA-S: dehydroepiandrosterone sulfate EE: ethinyl estradiol LNg: levonoregestrel OC: oral contraceptive RIA: radioimmunoassay SC: subcutaneous SHBG: sex hormone binding globulin T: testosterone TP: testosterone propionate

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vations. In light of these data, the androgenic property of LNg has little clinical relevance when used at contraceptive doses in combination with ethinyl estradiol (EE). The results reported from preclinical and clinical studies support this statement.

This paper is divided into two parts; Part I reports the preclinical demonstration in animals of the dose separation of anti-ovulatory and androgenic activities; Part II reports the preliminary clinical data from three studies on the effects of Triphasil® on acne, on sex hormone binding globulin (SHBG), and on plasma androgen levels.

PART I PRECLINICAL EVIDENCE: CONTRACEPTIVE (ANTI-OVULATORY) AND ANDROGENIC ACTIVITY OF LEVONORGESTREL IN ANIMALS

Inhibition of Spontaneous Ovulation in the Rat

Introduction Administration of progesterone and synthetic progestogens such as levonorgestrel, on diestrus of the four-day rat estrous cycle, can prevent the expected occurrence of ovulation [1]. This activity appears to occur via a mechanism involving the hypothalamic-pituitary axis, resulting in suppression of the ovulatory gonadotropin surge rather than through a direct action on the ovary.

Methods Adult female Sprague Dawley CD® rats (Charles River), weighing 200–250 g, maintained under controlled lighting conditions (lights on 0500 hours–1900 hours) and exhibiting at least two consecutive four-day estrous cycles, were administered various doses of levonorgestrel as a single subcutaneous (sc) injection at 1330 hours on diestrus. At the subsequent estrus, two days following treatment, the rats were sacrified by CO_2 asphyxiation, and the oviducts were excised and examined for ova under a dissecting microscope. The presence of a single ovum represents the criterion for ovulation.

Results and Discussion Levonorgestrel blocked ovulation in a dose-dependent fashion when administered on diestrus to the four-day cycling rat; the estimated 100 percent anti-ovulatory dose is 50–100 μg/rat (Table 1).

Androgenic/Anabolic (Myotrophic) Effects

Introduction Certain synthetic progestogens, because of their common structural origin as derivatives of 19-nortestosterone and their similarities to this steroid and testosterone, may possess a varying degree of androgenic and/or anabolic activity.

TABLE 1
Inhibition of Ovulation by Levonorgestrel in Rats When Administered on Diestrus

Treatment	Dose (μg/rat, sc)	No. Ovulating/Total	(% Inhibition)	$\bar{x}$ No. Ova Shed by Ovulating Rat
Oil	—	14/15	(7)	14.5
Levonorgestrel	10	5/5	(0)	13.2
	20	3/5	(40)	13.3
	50	2/10	(80)	11.0
	100	0/5	(100)	0.0

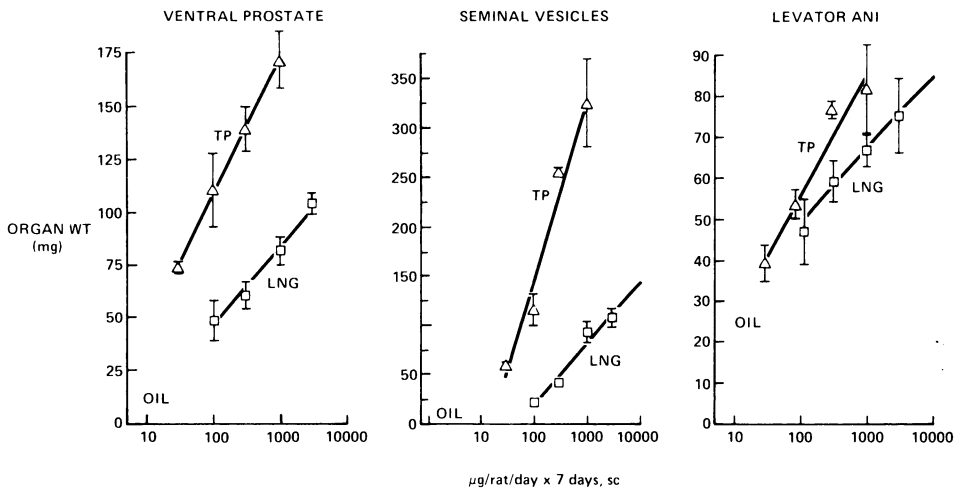

FIG. 1. Androgenic/anabolic evaluation of levonorgestrel in the immature castrated male rat.

Classical assays are used to evaluate such properties [2] and these tests were used to redefine LNg's properties.

Methods Immature male Sprague Dawley CD® rats (Charles River) were bilaterally castrated on day 27 of age. Treatment (subcutaneous) with various doses of LNg or the androgenic reference compound, testosterone propionate (TP), was begun 24 or 48 hours after surgery and continued for seven days. Groups of intact, untreated rats and castrate, vehicle-treated rats served as controls. The day following the final treatment, all rats were sacrificed by CO_2 asphyxiation. The ventral prostrate and seminal vesicles (minus contained fluid and the coagulating gland), both androgenic targets, and levator ani (anabolic target) were removed and the weights were recorded.

Results and Discussion Subcutaneous administration of LNg produced a dose-dependent increase in the weights of the ventral prostate and seminal vesicle that generally paralleled, but that was considerably less than, the response to testosterone propionate (Fig. 1). Notably, the highest doses of LNg tested, 1,000–3,000 μg, did not increase weights of these end organs to the extent observed with much lower daily doses of TP (50–100 μg). The response of the levator ani indicates that LNg resembles testosterone more closely in terms of anabolic effects than in terms of androgenic activity. The dose separation of anti-ovulatory (contraceptive) activity from the androgenic activity in rats is exemplified in the composite Fig. 2 (data derived from Table 1 and Fig. 1). LNg inhibits ovulation at single low doses (estimated ED^{100} = 50–100 μg/rat, sc) and stimulated weight increases of ventral prostate (androgenic target tissue) and seminal vesicles (anabolic target tissue) at much higher total doses (700–21,000 μg/rat, sc).

Conclusion from the Preclinical Studies

The anti-ovulatory activity of LNg occurs with doses considerably below those required to produce androgenic and anabolic responses from appropriate target tissues.

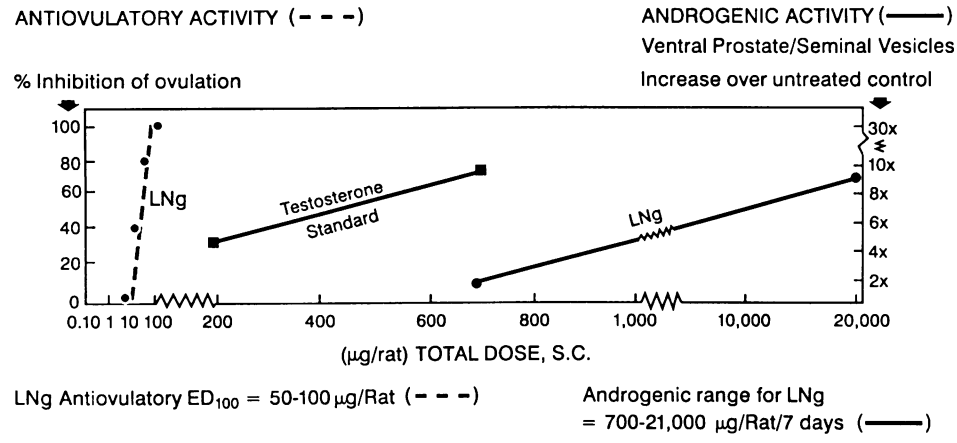

FIG. 2. The dose separation between anti-ovulatory activity and androgenic activity.

PART II CLINICAL EVIDENCE: THE EFFECTS OF LOW DOSE LNg ORAL CONTRACEPTIVES (OCs) ON ANDROGENS AND ACNE

Introduction

A prevailing concept has been that a patient who has acne should not use a contraceptive product containing a progestational agent with androgenic properties [3,4]. This idea, however, is a generalization based on an extension of animal data to humans and is not valid at the LNg doses used in combination with estrogen in the low-dose oral contraceptives.

Because acne is a known clinical side effect of elevated androgens, it was assumed that LNg-containing contraceptive products would exacerbate a pre-existing acne condition because LNg has androgenic properties, albeit at high doses in animals.

In contrast, clinical evidence has been accumulating that indicates the reverse is true: LNg at contraceptive doses in combination with EE consequently alleviates acne in most women, which suggests a non-androgenic profile.

During clinical trials with a low-dose LNg triphasic contraceptive (Triphasil®, Wyeth-Ayerst, Philadelphia, Pennsylvania), acne disappeared or was alleviated within three to six months in most women who had this skin problem at study entry.

The results of the study by Loudon and Biddell [5] in 271 women with acne showed that the condition disappeared or was improved within three to six months in 76 percent of the women who took the triphasic contraceptive. Other trials with the LNg triphasic OC have further supported these original observations [6–13]. Studies in women with acne who were taking the low-dose monophasic LNg product (Nordette®, Wyeth-Ayerst, Philadelphia, Pennsylvania) showed 24 percent to 35 percent improvement in acne within six months [14,15].

Cunliffe et al.[1] conducted a pilot study using the triphasic LNg product in women with acne. The results showed that sebum production was decreased by 20 percent and the mean grade of acne improved in all 20 patients studied during the three months of treatment. Because the reduced sebum production was not dramatic enough to account totally for the marked improvement in acne, another explanation was sought.

[1]Cunliffe WJ: A Study of the Effects of Trinordiol on the Sebum Excretion Rate in Woman with Acne. Data on File, Wyeth-Ayerst, 1985

Although some authors have not observed abnormal increases in plasma androgen levels in women with acne vulgaris [16,17], the majority of studies have provided evidence for elevated plasma androgen levels [18–22].

Some combination OCs can reduce androgen levels (e.g., total testosterone, androstenedione, dehydroepiandrosterone sulfate [DHEA-S]) [6–9,14]. It was speculated that, in women with acne, the combination contraceptives reduce androgens partly by raising sex hormone binding globulin (SHBG) and consequently reducing circulating free androgens, resulting in the improvement of acne. Consequently, multi-center and individual trials were designed to examine the effects of the triphasic preparation on acne grade and on peripheral androgen levels in non-hirsute women as well as on SHBG levels. Preliminary data are reported here from three separate studies.

Methods and Materials

Study No. 1 Dr. Cunliffe[2] conducted a comparative and investigator-blinded parallel study in which 60 patients were randomly assigned to the LNg triphasic (Triphasil®/Trinordiol®),[3] or to Diane®[4] (a therapy for acne as well as a contraceptive). The two drugs were taken for 21 days, followed by a seven-day tablet-free interval, and this regimen was maintained for six months. Baseline measurements were taken at the initial assessment visit. Efficacy, safety, and patient assessments were made after two, four, and six months. Efficacy assessments included counting the number of non-inflamed lesions, papules and pustules, macules, and a total acne count.

Only preliminary data on the Triphasil® (also known as Trinordiol®) patients completed at this time are reported here. The full trial results will be the subject of a separate publication.

Study No. 2 Drs. Aschner and Sabogal[5] conducted a comparative parallel trial in 20 patients that was part of a large multi-center, multinational study. Women were between 15 and 35 years of age and met the usual inclusions/exclusions for oral contraceptives. In addition, women had to express the desire for contraception and have mild to moderate acne without hirsutism. Women were randomized to either Triphasil® or Diane®. The drugs were taken on a 21-day-on, seven-day-off regimen for six months. Baseline measurements of acne, hormones, and SHBG were obtained at the initial visit and repeated at follow-up visits at one, two, three and six cycles and one month post-treatment. Clinical assessments included a physical/gynecological examination, pretreatment; routine general laboratory chemistries; hormone tests (androgens and luteal phase progesterone), sex hormone binding globulin; and acne assessment at each visit. Androgen tests included total and free testosterone, dehydroepiandrosterone sulfate (DHEA-S), and androstenedione. Assays were performed using standard radioimmunoassay (RIA) methods.

Study No. 3 Dr. Lemay and colleagues [23] conducted an open, non-compara-

[2]Cunliffe WJ: The Effect of Trinordiol®, Diane® or tetracycline on Acne in Healthy Young Women. Data on File, Wyeth-Ayerst, 1989

[3]Triphasil®/Trinordiol®; 0.050 mg LNg and 0.030 mg EE × 6 days, 0.075 mg LNg and 0.040 mg EE × 5 days, 0.125 mg LNg and 0.030 mg EE × 10 days. Philadelphia, PA, Wyeth-Ayerst

[4]Diane® 2 mg cyproterone acetate and 0.050 mg ethinyl estradiol × 21 days/months. Berlin, West Germany, Schering AG

[5]Aschner P, Sabogal M: The effects of Trinordiol® and Diane® in young women with acne. Data on File, Wyeth-Ayerst, 1989

tive trial in 40 patients to determine the effect of Triphasil®[6] on acne as measured by both subjective and objective measurements as well as the efficacy of Triphasil® as a contraceptive.

The usual inclusion/exclusion criteria for OC use applied. In addition to physical and gynecologic examinations, baseline measurements were taken for clinical chemistries and hormone levels for androgens and progesterone, and SHBG.

Acne was graded by count and severity and categorized as comedonal or papulopustular. The total count times the grade number constituted the acne severity scores. In most instances, photographs were taken before and after six months of triphasic OC administration. The same, most severely affected side of the face was photographed each time.

Acne was counted on the face, anterior chest, and back. Because a contraceptive was being used, only women with a pretreatment ovulatory cycle as judged by increase in luteal phase progesterone were enrolled. Plasma levels of androstenedione, DHEA-S, total testosterone, free testosterone, and SHBG were determined by RIA before treatment, at one, two, three, and six months of treatment. Diary cards were kept by all patients to verify compliance, and to record any bleeding irregularities as well as any side effects. Follow-up visits took place after cycles 1, 2, 3, and 6, at which time acne, androgen levels, and SHBG levels were determined at each visit.

Results

Study No. 1 Results from the Cunliffe study[2] show that most patients experienced a reduction in acne; of the 13 patients reported, ten showed improvement, one showed no change, and two became worse. Overall, a reduction of acne amounting to 56.7 percent was observed in these patients (Table 2).

Study No. 2 Preliminary results from the multi-center trial conducted in Colombia by Aschner and Sabogal[5] are reported here. Reports on the ten patients who took Triphasil® showed that a marked reduction in the acne count was accompanied by reductions in androgen levels (Table 2). Overall, a mean reduction of acne of 92 percent occurred in these patients. All but one of the ten patients treated showed marked reduction in acne. In addition, SHBG increased 16 percent over pretreatment, total testosterone (T) decreased 19 percent, free testosterone decreased 34 percent, and DHEA-S decreased 28 percent.

Study No. 3 The preliminary results from Lemay and associates [23] (Canada) on 34/40 patients are reported (Table 2) and the total results will be published when studies of all patients are completed. Table 3 shows that, after three months of treatment, androgens showed the following reductions when compared to pretreatment: total T, reduction of 0.12 ng/ml (21 percent); free T, 0.5 percent (25 percent); A, 0.9 ng/ml (33 percent); and DHEA-S, 946 μg/dl (32 percent); SHBG showed an increase of 51 nmol/l (108 percent); after six months these decreases were 0.09 ng/ml (16 percent), 0.6 percent (30 percent), 1.3 ng/ml (48 percent), 1,039 μg/dl (36 percent), respectively, and SHBG increased 54 nmol/l (114 percent). As one example, Fig. 3 depicts the percentage decrease in androgen levels, acne count, and severity of one patient, patient X, before and during treatment with Triphasil®. Figure 4 shows photographs of patient X before and after six months of treatment with Triphasil®. From the photographs, the improvement in facial acne is evident. The reduction in

[6]Triphasil formulation in Canada as used in this study contained dl-norgestrel.

TABLE 2
Acne Improvement and Change in Androgens and SHBG in Patients Treated with Levonorgestrel Triphasic for Six Months

Study No.	Investigator	Country	No. Pts.	Improvement (%)	% Change from Values Before Treatment				
					SHBG	Total T	Free T	A	DHEA-S
1	Cunliffe[2]	UK	13	56.7	—[a]	—	—	—	—
2	Aschner and Sabogal[5]	Colombia	10	92	↑16	↓19	↓34	—	↓28
3	Lemay [23][b]	Canada	34	54	↑114	↓16	↓30	↓48	↓36

[a]—, Not done
[b]Triphasic formulation used contained dl-norgestrel

TABLE 3
Androgens* and SHBG*

Cycle	T	FT	A	DHEA-S	SHBG
Normal	0.2–0.8 ng/ml ± SD(*n*)	0.6–1.7 % ± SD(*n*)	0.9–2.2 ng/ml ± SD(*n*)	800–3,000 μg/dl ± SD(*n*)	— nmol/l ± SD(*n*)
Pre	0.58 ± .03 (33)	2.0 ± .06 (33)	2.7 ± 0.2 (33)	2,919 ± 212 (33)	47 ± 4.9 (33)
Cycle 1	0.44 ± .03 (32)	1.5 ± .04 (32)	1.9 ± 0.2 (32)	2,320 ± 192 (32)	96 ± 6.2 (32)
Cycle 2	0.46 ± .03 (33)	1.5 ± .04 (33)	2.0 ± 0.2 (33)	2,233 ± 194 (33)	93 ± 6.5 (31)
Cycle 3	0.46 ± .03 (32)	1.5 ± .05 (32)	1.8 ± 0.2 (32)	1,973 ± 186 (31)	98 ± 7.3 (33)
Cycle 6	0.49 ± .03 (22)**	1.4 ± .05 (22)	1.4 ± 0.1 (22)	1,880 ± 179 (26)	101 ± 9.9 (25)

*Statistically significant at 3 and 6 cycles, $p < 0.01$

**$p < 0.05$

( ) = number of patients

T, testosterone; FT, free testosterone; A, androstenedione; DHEA-S, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin

Reproduced from preliminary data with permission of Drs. Andre Lemay and Lucile Turcot-Lemay; full paper in preparation

acne severity and count, pari passu with reduction in androgen levels shown in Fig. 3, are objective evidence supporting the improvement seen in the photographs of patient X. In this patient there was a 29 percent reduction in total T, 28 percent reduction in free T, 12 percent reduction in androstenedione (A), 56 percent reduction in DHEA-S, and 148 percent increase in SHBG at three months; at six months, total T had increased 12 percent over pretreatment values, free T decreased 39 percent, DHEA-S decreased 72 percent, and A decreased 58 percent. The observed trend in acne improvement and androgen reduction was the same for the subsequent patients studied in this trial.

DISCUSSION

Tables 2 and 3 list the preliminary results from these ongoing studies[2,5] [23], whereas Table 4 lists published reports on the effects of triphasic [5–13] and low-dose monophasic [14,15] LNg contraceptives on acne, androgen levels, and SHBG. As indicated in compilation Table 4, both the LNg-containing triphasic and the low-dose LNg monophasic contraceptive have been shown to raise SHBG levels significantly in normal women and in women with acne, resulting in lower levels of free testosterone and other plasma androgens. The present studies shown in Tables 2 and 3 corroborate these findings and further show the simultaneous rise in SHBG, reduction in androgen

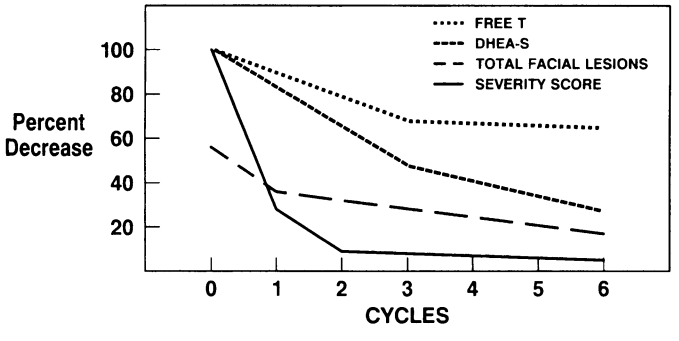

FIG. 3. The correlation between the improvement in total facial lesions and acne severity score over six months compared with the decrease in androgen levels over six months (**Free T, DHEA-S**) in patient X (adapted with permission from [23]).

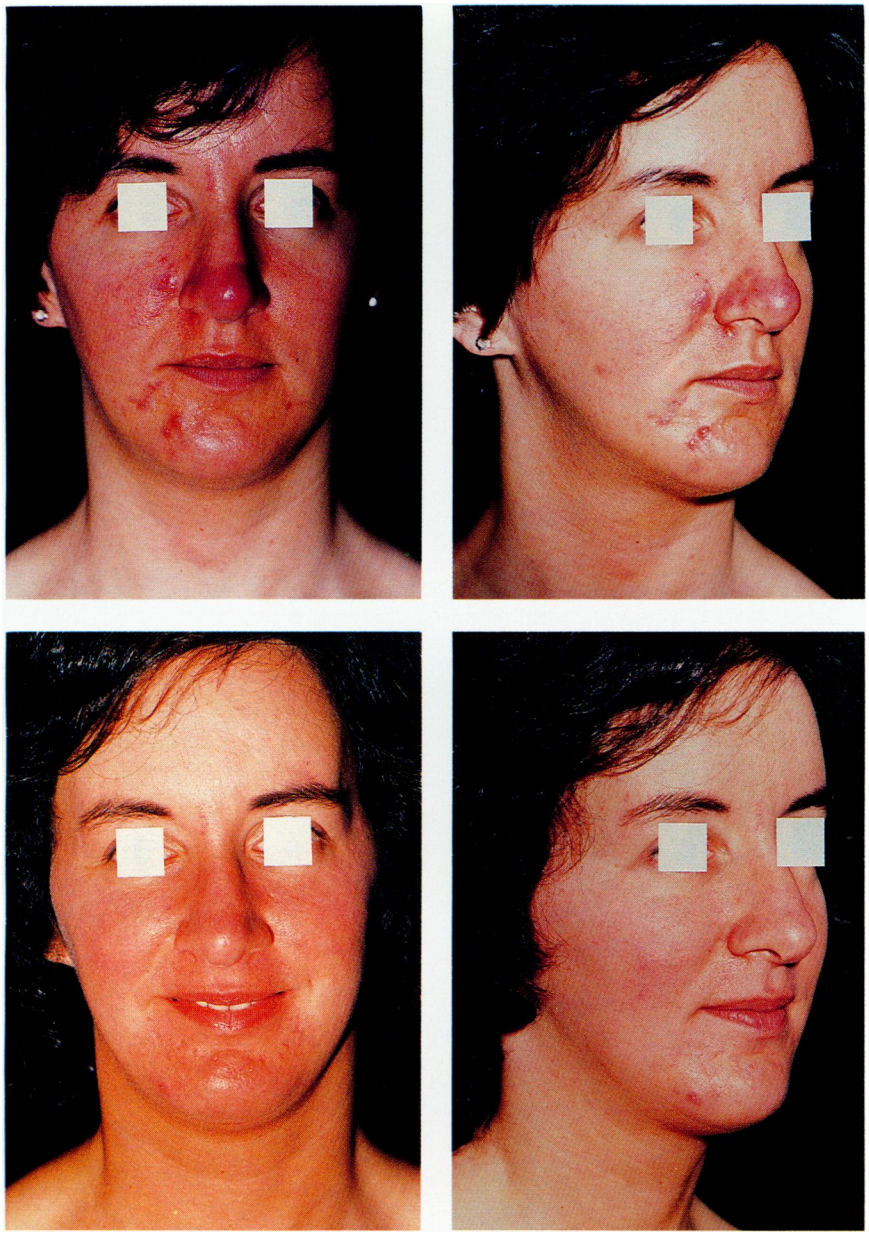

FIG. 4. Photographs of patient X shown before treatment (**A** and **B**) and after six months of triphasic treatment (**C** and **D**) (taken from [23] with permission).

levels, and reduction in acne. Improvement in acne was observed in all of the studies, but the magnitude of the change differs. Patient response time also varies, but, if improvement is to occur, it will be apparent by three to six months of treatment; these response differences are attributed to many variables. Recent evidence has implicated elevated plasma androgen levels in acne vulgaris. Since androgenic steroids can be derived from the ovaries, the adrenal, and fat, including the skin (Fig. 5) [24], we must

TABLE 4
Acne Improvement and/or Changes in SHBG and Androgens in Women Taking Levonorgestrel Triphasic

Investigator	Country	No. Pts.	Type of Patient	Acne Improvement (%)	% Change from Values Before Treatment				
					SHBG	Total T	Free T	A	DHEA-S
			Levonorgestrel Triphasic[a]						
Gaspard et al. [6]	Belgium	24	Normal	—[b]	↑98	↓37	↓43	—	—
Gaspard et al. [7]	Belgium	18	Normal	—	↑85	↓36	↓45	↓39	↓26
Vermeulen and Thiery [8][c]	Belgium	12	Normal	—	↑66	↓23	↓24	—	↓36
Jung-Hoffmann and Kuhl [9]	Germany	22	Normal	—	↑92	↓16	↓35	—	—
Loudon and Biddell [5]	UK	271	Acne	76	—	—	—	—	—
Allen et al. [10][d]	Canada	36	Acne	83	—	—	—	—	—
Woutersz et al. [11, 12]	U.S.	525	Acne	58	—	—	—	—	—
Upton [13]	Europe Multi-center	324	Acne	96	—	—	—	—	—
			Low-Dose Levonorgestrel Monophasic[a]						
Palatsi et al. [14]	Finland	15	Acne	24	↑27	↓36	↓34	—	↓14
Carlborg [15]	Sweden	37	Acne	35	—	—	—	—	—

[a]Six months' treatment
[b]—, not done
[c]Three months' treatment
[d]Levonorgestrel used as dl-racemate in this study

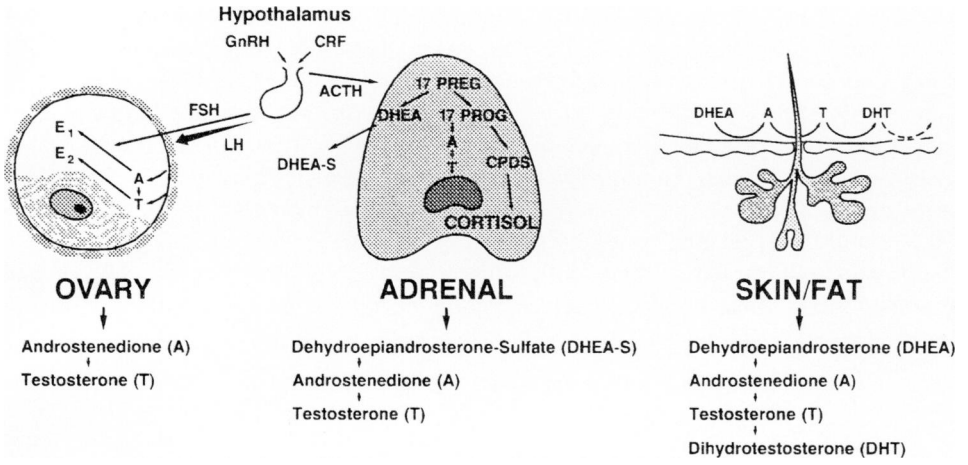

FIG. 5. The sources of androgen in the woman. (Adapted from [24] with permission.) **GnRH,** gonadotropin-releasing hormone; **CRF,** corticotropin-releasing factor; **FSH,** follicle-stimulating hormone; **LH,** luteinizing hormone; **ACTH,** adrenocorticotropic hormone; **E_1,** estrone; **E_2,** estradiol; **17 Preg,** pregnenolone; **17 Prog,** 17-hydroxyprogesterone; **CPDS,** 11-deoxycortisol.

assume that all sources are involved in androgen production in these women. It is known that estrogen or estrogen-dominant products raise sex hormone binding globulin (SHBG) levels; whereas androgen dominance impedes the estrogen-induced rise in SHBG in a dose-related manner. The higher the level of SHBG, the more testosterone is bound and the less free testosterone is available for target tissue receptor interaction. Consequently, since LNg OCs raise SHBG, we must conclude that the LNg OCs are estrogen-dominant and the LNg triphasic is much more estrogen-dominant than the low-dose LNg monophasic.

The women in these studies were not hirsute and thus the decrease in DHEA-S is puzzling. The adrenal appears to be involved because of the changes in DHEA-S thought to be predominantly of adrenal origin. The levels of pretreatment androgens in this population, although high, are borderline normal and not suggestive of a tumor. If one considers the sources of androgen in the female as shown in Fig. 5 [24], one can speculate that the skin sensitivity may also be involved. Triphasil® exerts a mild anti-androgen effect on peripheral target tissue, i.e., the sebaceous glands of the skin (sebum reduction).[1] The ovary certainly is affected by the LNg triphasic OC indirectly and perhaps directly by effecting ovarian steroidogenesis. The women in these trials had normal ovulatory menstrual cycles on admission to the trial, precluding disruption of the pituitary-ovarian axis. The theory of Lucky et al. [4], which postulates exaggerated adrenarche perhaps due to hypertrophy of the zona reticularis, is also possible in these women. Further studies are required to define more clearly the mechanism(s) of action of these OCs, such as effects on steroid enzyme levels. The clinical effect of LNg low-dose OCs by whatever mechanism, however, is to raise SHBG, reduce androgens, and improve acne.

CONCLUSIONS FROM THE CLINICAL STUDIES

Published clinical data from many sources (Table 4) show that the use of LNg triphasic OC leads to an increase in SHBG, to a reduction in plasma androgen levels,

and to an improvement in acne. These data lend support to the concept that LNg monophasic and triphasic preparations do not manifest androgenic properties at the doses and combinations (with estrogen) used in these low-dose OCs. Because an increase in SHBG is distinctly an estrogenic response, and because both low-dose monophasic and triphasic LNg products increase SHBG, these formulations are not considered androgenic. The improvement observed in acne, a disorder induced and supported by androgen, with the use of low-dose LNg monophasic/triphasic preparations, should not be interpreted as suggesting that these contraceptives are a new therapy for acne; rather, these data suggest that LNg low-dose monophasic and triphasic preparations are the preferred treatment for women who require contraception and who also have acne.

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