Growth Suppression of Hamster Flank Organs by Topical Application of γ-Linolenic and Other Fatty Acid Inhibitors of 5α-Reductase

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Certain unsaturated aliphatic fatty acids, such as γ-linolenic acid, inhibit 5α-reductase activity *in vitro* and *in vivo*. Hamster flank organ growth, as measured by the increase in the area of pigmented macule, is dependent on androgen. When one of the paired flank organs of a castrated hamster was treated topically with testosterone, the treated organ, but not the contralateral flank organ, became larger and darker. Topical application of γ-linolenic acid to the testosterone-treated flank organ suppressed this testosterone effect. Other fatty acids that were not inhibitors of 5α-reductases were not active. Topical treatment of hamster flank organs with 5α-dihydrotestosterone also stimulated the growth of the organ. This 5α-dihydrotestosterone-dependent activity, however, was not significantly affected by γ-linolenic acid, suggesting that flank organ growth was dependent on 5α-dihydrotestosterone and that γ-linolenic acid acted by inhibiting 5α-reductase. With intact male hamsters, the endogenous androgen-dependent growth of flank organs is also suppressed by topical treatment with γ-linolenic acid. The effect of γ-linolenic acid is localized at the site of its application; topical application of γ-linolenic acid did not affect the androgen-dependent growth of other organs such as testis, epididymis, seminal vesicle, and prostate. γ-Linolenic acid, with low toxicity and absence of systemic effect, therefore may be potentially useful for treatment of androgen-dependent skin disorders. *Key words: androgen/unsaturated fatty acids/hair/melanocytes. J Invest Dermatol 109:152–157, 1997*

In the prostate and skin, testosterone (T) is converted to a more active metabolite 5α-dihydrotestosterone (DHT) by 5α-reductases (Anderson and Liao, 1968; Bruchovsky and Wilson, 1968; Gomez and Hsia, 1968). Inhibition of 5α-reductase represents an unique approach for developing therapeutic methods for androgen-dependent diseases, such as benign prostatic hyperplasia, prostatic cancer, acne, seborrhea, common baldness, hirsutism, and hidradenitis suppurativa. Various compounds have been shown to inhibit 5α-reductase activity (Liang and Heiss, 1981; Liang and Liao, 1992; Hirsch *et al*, 1993; Russell and Wilson, 1994; Liao and Hiipakka, 1995). Finasteride {Proscar; 17β-[N-(1,1-dimethylethyl)carbamoyl]-4-aza-5α-androst-1-en-3-one}, a 5α-reductase inhibitor, lowers the level of DHT in serum and the prostate, reduces prostate volume, and increases urinary flow in some patients (Stoner and Finasteride Study Group, 1992). We have reported that certain aliphatic unsaturated fatty acids, such as γ-linolenic acid (γ-LA; Liang and Liao, 1992), and catechin-3-gallates (Liao and Hiipakka, 1995), can inhibit 5α-reductase activity of liver and prostate of rats and humans *in vitro*.

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Abbreviations: γ-LA, γ-linolenic acid; SA, stearic acid; T, testosterone; 4-MA, 17β-(N,N-diethyl)carbamoyl-4-methyl-4-aza-5α-androstan-3-one.

5α-Reductase is found in many organs (Hiipakka *et al*, 1993; Russell and Wilson, 1994) including sebaceous gland of hamsters (Takayasu and Adachi, 1972) and human hair follicles (Randall, 1994). Two 5α-reductase isozymes have been identified in rats and humans (Russell and Wilson, 1994). The type 1 isozyme predominates in rat tissues such as liver, kidney, brain, and lung, whereas the type 2 enzyme is more abundant in rat testis and epididymis. Both isozymes are found in skins of the neonate, but the type 1 isozyme is the major form expressed in the skin after puberty. The type 1 isozyme is also expressed in balding scalp. The possibility that the type 2 isozyme plays an unique role in skin and hair growth cannot be excluded. Finasteride, a 4-azasteroid, is a competitive inhibitor of 5α-reductases and has an affinity 30-fold higher for isozyme 2 than for isozyme 1 (Russell and Wilson, 1994). In contrast, the green tea catechins, epicatechin-3-gallate, and epigallocatechin-3-gallate are more effective inhibitors of the type 1 enzyme, and γ-LA inhibits both isozymes equally well (Liao and Hiipakka, 1995).

In the stumptail macaque, a monkey model of androgenic alopecia, finasteride given orally prevents frontal baldness (Diani *et al*, 1992). Topical treatment of the frontal scalp of stumptail macaque with 17β-(N,N-diethyl)carbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA), another 4-azasteroidal inhibitor of 5α-reductase, also prevents hair loss (Rittmaster *et al*, 1987). Topical 4-MA administration, however, increases in the T/DHT ratio in serum, indicating systemic effects of topical 4-MA. An inhibitor of 5α-reductase that is active topically and inactive systemically would

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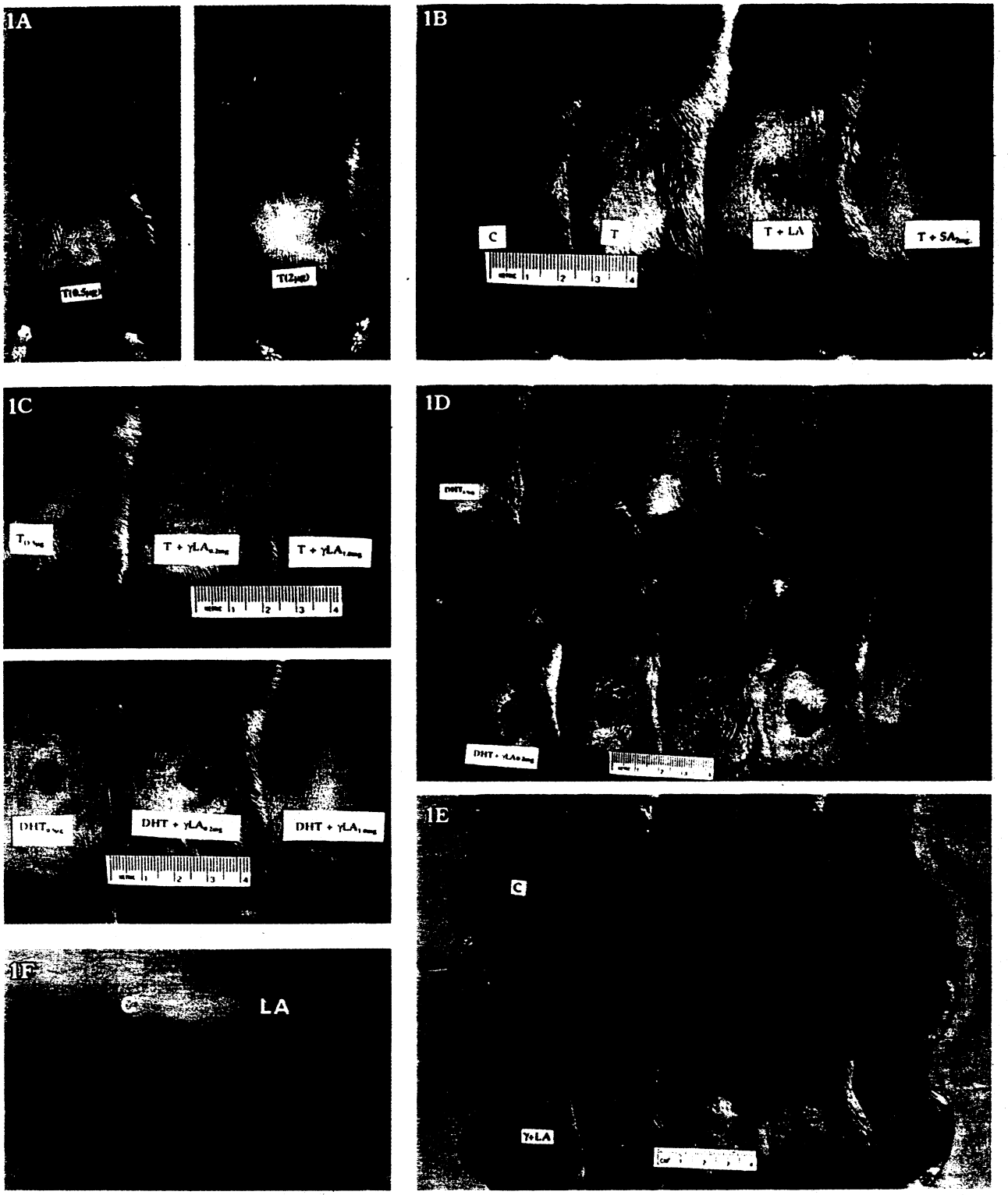

Figure 1. Androgen stimulation and the effect of fatty acids on hamster flank organ. *(A)* The right flank organs of castrated male hamsters were treated with 0.5 μg *(Left)* or 2 μg *(right)* of T daily for 17 d to show that topical application of T to the right flank organ promoted the growth of the right flank organ but not the untreated left organ. *(B)* The right flank organs of castrated male hamsters were treated with ethanol (C), 0.5 μg of T (T), 0.5 μg of T and 1 mg of γ-LA (T + LA), or 0.5 μg of T and 2 mg of SA (T + SA) daily for 18 d to show that γ-LA but not SA inhibited the T-dependent growth of flank organs. *(C)* The right flank organs of castrated male hamsters were treated with 0.5 μg of T *(upper)* or DHT *(lower)* alone (animals at the left), with 0.2 mg (animals in the middle), or with 0.5 mg of γ-LA (animals at the right) daily for 19 d to show that γ-LA selectively inhibited the T-dependent, but not significantly the DHT-dependent, growth of flank organs. *(D)* The right flank organs of castrated male hamsters were treated with 5 μl of ethanol containing 0.5 μg of DHT (upper group) alone or 5 μl of ethanol containing 0.5 μg of DHT and 1.0 mg of γ-LA (lower group) daily for 19 d to show that γ-LA was not very effective in inhibiting the DHT-dependent growth of hamster flank organs. *(E)* The right flank organs of intact male hamsters were treated with ethanol alone (upper group) or 0.2 mg of γ-LA (lower group, γ + LA) daily for 23 d to show that γ-LA inhibited flank organ growth in intact hamsters. *(F)* The flank organ of an intact hamster was treated with ethanol alone (C to the left) and the flank organ of another intact hamster was treated with 1.0 mg of γ-LA (LA to the right) to show that hair of the γ-LA–treated flank organ was markedly lighter in color and shorter in length than hairs of the flank organ treated with ethanol alone.

Table I. γ-LA but not SA Inhibits T-stimulated Growth of the Pigmented Macules of Hamster Flank Organs[a]

Treatment of Right Flank Organ	Pigmented Macule (mm^2)[b]	
	Untreated (L)	Treated (R)
C (control, 5 μl ethanol)	5.1 ± 1.9	4.2 ± 0.5
C + γ-LA (1 mg)	4.2 ± 0.6	4.1 ± 0.3
C + S-A (1 mg)	4.4 ± 0.4	4.9 ± 0.9
C + S-A (2 mg)	4.6 ± 1.4	5.0 ± 0.8
T (0.5 μg per 5 μl ethanol)	3.6 ± 0.5	32.7 ± 9.2
T + γ-LA (1 mg)	4.1 ± 0.3	15.3 ± 3.9[c]
T + S-A (1 mg)	4.3 ± 0.6	27.7 ± 4.4[d]
T + S-A (2 mg)	4.2 ± 0.4	30.1 ± 7.1[d]

[a] Right flank organ (R) was treated daily with 5 μl of ethanol (control) or 5 μl of ethanol containing 0.5 μg of T with or without γ-LA or SA at the doses indicated for 18 d. The left flank organs (L) were not treated. Five castrated male hamsters were in each treatment group. At the end of treatment period, the area of the pigmented macule was determined.
[b] Values are the mean ± SD.
[c] γ-LA inhibition was 53% ($p < 0.01$).
[d] Effect of SA was not significant ($p > 0.05$).

Table II. Specific Effect of Various Fatty Acids on T-stimulated Growth of the Pigmented Macules of Hamster Flank Organs[a]

Fatty Acid	Pigmented Macule Area (mm^2)[b]			
	Left (control)	Right (+ T)	Inhibition[c] %	p Value[d]
None (control)	4.8 ± 1.1	49.6 ± 9.3	—	—
Undecylenic acid (C11:1,10)	4.7 ± 1.2	41.7 ± 11.9	16	>0.05
Palmitic acid (C16:0)	5.1 ± 2.9	37.2 ± 7.0	25	>0.05
Oleic acid (C18:1, cis-9)	4.7 ± 0.5	28.1 ± 9.3	43	<0.01
Elaidic acid (C18:1, trans-9)	4.9 ± 1.1	47.0 ± 6.6	5	>0.05
Linoleic acid (C18:2, cis-9-12)	5.4 ± 1.6	23.9 ± 5.6	52	<0.01
Linolelaidic acid (C18:2, trans-9,12)	4.1 ± 0.7	46.6 ± 9.8	6	>0.05
α-Linolenic acid (C18:3, cis-9,12,15)	4.1 ± 1.2	27.6 ± 9.2	44	<0.01
γ-L-A (C18:3, cis-6,9,12)	4.4 ± 1.6	17.0 ± 7.1	66	<0.01
Arachidonic acid (C20:4, cis-5,8,11,14)	4.7 ± 1.6	35.7 ± 8.8	28	>0.05
Erucic acid (C22:1, cis-13)	4.6 ± 1.5	35.4 ± 5.1	29	>0.05
Nervonic acid (C24:1, cis-15)	4.0 ± 1.5	39.9 ± 6.0	10	>0.05

[a] Right flank organ was treated daily with 5 μl of ethanol containing 0.5 μg of T with or without 1 mg of test fatty acid for 21 d. The left flank organ was treated with 5 μl of ethanol alone (control). Five castrated male hamsters were in each group. At the end of treatment period, the area of the pigmented macule was determined.
[b] Values are the mean ± SD.
[c] Percent decrease in the size of right (+T) flank organ treated with fatty acid compared to the right (+T) flank organ not treated (control).
[d] Statistical significances in means between control and fatty acid treated macules.

daily. The left flank organ received only ethanol vehicle. After 21 d, γ-LA (C18:3, cis-6,9,12) showed 66% inhibition and was most active among fatty acids tested. α-LA (C18:3, cis-9,12,15) was less active than γ-LA. Oleic acid (C18:1, cis-9) and linoleic acid (C18:2, cis-9,12) were active, whereas their trans-isomers, elaidic acid (C18:1, trans-9) and linolelaidic acid (C18:2, trans-9,12), were inactive. Palmitic acid (C16:0), arachidonic acid (C20:4, cis-5,8,11,14), and erucic acid (C22:1, cis-13) exhibited weak activity. Inhibition by undecylenic acid (C11:1,10) or nervonic acid (C24:1, cis-15) was not significant. In the absence of T, none of the fatty acids tested stimulated or inhibited the growth of the pigmented macules.

Effect of γ-LA on Hamster Flank Organ Growth Stimulated in Castrated Hamsters by T, or DHT, or in Intact Hamsters by Endogenous Androgen To investigate whether inhibition of 5α-reductase is the primary reason for γ-LA inhibition, we compared the ability of γ-LA to inhibit T- and DHT-stimulated growth of flank organs in castrated hamsters. If γ-LA acts by inhibition of 5α-reductase, only T- but not DHT-stimulated growth of pigmented macules would be inhibited.

To compare the effect of γ-LA on T- or DHT-stimulated growth of the pigmented macule, the right flank organs of castrated hamsters were treated with 0.5 μg of T or DHT with or without 0.2–1 mg of γ-LA each day. **Table III** and **Fig 1*C*** and ***D*** show that DHT was as effective as T in stimulating flank organ growth. γ-LA inhibition was clearly inhibitory when T was used but not when DHT was used. **Figure 1*E*** shows that flank organ growth of intact male hamsters could also be inhibited by treatment with daily application of 2 mg of γ-LA for 23 d.

Localized Effect of Topically Applied γ-LA on Flank Organ Growth The pigmented macules of intact 4-wk-old hamsters grow linearly from about 8 mm^2 to about 30 mm^2 within 2 wk, apparently in response to increased production of endogenous androgen **(Fig 3)**. The effect of topical application of 1 or 2 mg of γ-LA on the right flank organ slowed the growth of the organ considerably. The growth of left flank organ was not affected. In another experiment, shown in **Table IV**, 4-wk-old male hamsters were treated daily for 15 d with ethanol alone on the left flank organ and 0.5 mg or 1.0 mg of γ-LA on the right flank organs. γ-LA significantly inhibited only the growth of the pigmented macule of the right flank organ but not the left organ. **Figure 1*F*** shows that γ-LA also affects hair growth of the treated flank organ; the hairs in

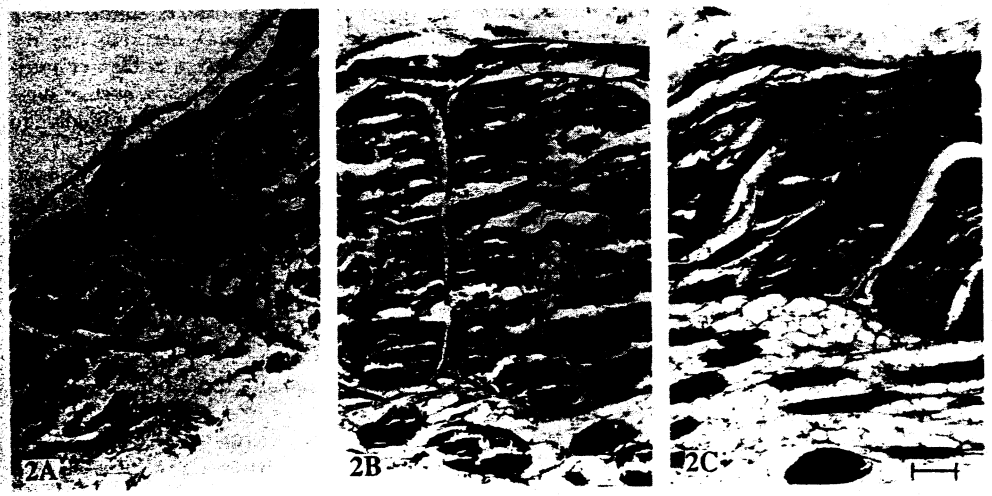

Figure 2. Histologic analysis of hamster flank organ treated topically with T and γ-LA are related to growth of the flank organs. Flank organs treated daily with control ethanol solution *(A)*, T (0.5 μg) *(B)*, or T with γ-LA (1 mg) *(C)* as shown in **Table I** were excised, fixed, sectioned, and then stained with hematoxylin and eosin. *Scale bar*, 300 μm.

Table III. γ-L-A Inhibits T- but not DHT-dependent Growth of Pigmented Macules of Hamster Flank Organs[a]

Experiment	Treatment	Pigment Macule (mm^2)[b]	% Inhibition[c]	Value[d]
IA	T (control)	32.9 ± 2.6		
	T + γ-LA (0.2 mg)	22.7 ± 4.3	31	>0.05
	T + γ-LA (0.4 mg)	24.9 ± 5.8	24	>0.05
	T + γ-LA (0.6 mg)	18.0 ± 3.4	45	<0.01
IB	DHT (control)	33.7 ± 8.6		
	DHT + γ-LA (0.2 mg)	32.1 ± 3.1	3	>0.05
	DHT + γ-LA (0.4 mg)	28.9 ± 3.2	14	>0.05
	DHT + γ-LA (0.6 mg)	27.0 ± 3.9	20	>0.05
IIA	T (control)	31.3 ± 7.1		
	T + γ-LA (1.0 mg)	15.5 ± 1.4	50	<0.01
IIB	DHT (control)	28.6 ± 7.9		
	DHT + γ-LA (1.0 mg)	21.7 ± 4.3	24	>0.05
IIIA	T (control)	29.2 ± 5.8		
	T + γ-LA (1.0 mg)	17.6 ± 3.3	40	<0.01
IIIB	DHT (control)	34.0 ± 8.1		
	DHT + γ-LA (1.0 mg)	25.1 ± 7.6	26	>0.05

[a] Right flank organ was treated daily with 5 μl of ethanol containing 0.5 μg of T or DHT with or without the indicated amount of γ-LA for 19 d. The left organ was not treated. Five castrated hamsters were in each group. At the end of treatment period, the area of the pigmented macule was determined.
[b] Values are the mean ± SD.
[c] Calculated by comparing with the control organ not treated with γ-LA.
[d] Statistical significances in means between control and γ-LA–treated macules.

Table IV. γ-LA Inhibits the Growth of Pigmented Macules of Intact Male Hamsters[a]

	Left Flank Organ		Right Flank Organ		Inhibition by γ-LA[c]	
Group	Treatment	Pigmented Macule[b] (mm^2)	Treatment	Pigmented Macule[b] (mm^2)	%	Value[d]
1	Vehicle	24.7 ± 2.1	Vehicle	23.5 ± 3.8	NA[e]	NA[e]
2	Vehicle	28.6 ± 4.0	γ-LA (0.5 mg)	19.0 ± 3.6	34	<0.01
3	Vehicle	29.2 ± 4.8	γ-LA (1.0 mg)	10.8 ± 2.7	54	<0.01

[a] Intact pre-pubertal male hamsters, 4 wk old, were used. Each group had 10 hamsters. The left flank organ received 5 μl of ethanol only (vehicle) and used as control. The right flank organ was treated with 5 μl of ethanol (group 1) or 5 μl of ethanol containing 0.5 mg (group 2) or 1.0 mg (group 3) of γ-LA. Treatment was carried out daily for 15 days. At the end of treatment period, the area of the pigmented macule was determined.
[b] Values are the mean ± SD.
[c] Calculated for the right organ by comparing with the left organ not treated with γ-LA.
[d] Statistical significances in means between right and left flank organ sizes.
[e] Not applicable.

the γ-LA–treated flank organ was lighter in color and shorter in length than those of the control.

After daily treatment of intact hamsters with ethanol alone or with 1 mg or 2 mg of γ-LA in ethanol for 15 d, there was no significant difference in the weights of the testis (control group, 3.52 ± 0.35 g; 1-mg γ-LA group, 3.39 ± 0.47 g; 2-mg γ-LA group, 3.40 ± 0.32 g), epididymis (0.50 ± 0.06 g, 0.48 ± 0.07 g, and 0.48 ± 0.05 g, respectively), or seminal vesicles plus prostate (0.57 ± 0.13 g, 0.63 ± 0.21 g, and 0.60 ± 0.14 g).

DISCUSSION

We have previously shown that only certain fatty acids are potent inhibitors of 5α-reductase in a cell-free system (Liang and Liao, 1992). This structural specificity appears to be retained in the inhibition of the growth of pigmented macules of hamster flank organs by fatty acids **(Tables I, II)**. For example, among the 18-carbon fatty acids (C18), γ-LA, with three cis double bonds at positions 6, 9, and 12, is the most active fatty acid tested in both the enzyme assay system and flank organ test. α-LA with three cis double bond at positions 9, 12, and 15; linoleic acid with two cis double bonds at positions 9 and 12; or oleic acid with one cis double bond at position 9 are less active than γ-LA in both assays. In either assay system, C18 fatty acids without a double bond (SA) **(Table I, Fig 1*B*)** or with one or two trans double bonds (elaidic acid or linolelaidic acid) are not active, whereas their cis isomers are active **(Table II)**. These findings suggest that the number and position of double bonds and their stereo structure is important for the inhibitory activity. The length of fatty acid also seems to be important; arachidonic acid (C20), erucic acid (C22), and nervonic acid (C24) are not as active as C18 unsaturated fatty acids. The fact that the structural specificity for inhibition of 5α-reductase in cell-free systems and that for inhibition of flank organ growth are very similar suggests that γ-LA inhibition of flank organ growth is due to inhibition of 5α-reductase in the flank organ. This is consistent with the finding that γ-LA inhibited T-dependent, but not DHT-dependent, growth of flank organs **(Table III, Fig 1*C,D*)**. In line with this view, we also found that γ-LA can inhibit the endogenous androgen-dependent flank organ growth in the intact male hamsters **(Table IV, Fig 1*E*)**, which appeared to indicate that the flank organ growth is dependent on the local conversion of T to DHT, which is the case for prostate growth in rodents and human. In a separate study, we found that γ-LA (at 10–50 μM) can inhibit the conversion of T to DHT by minced hamster flank organ and by prostate cancer cells in culture (Liang and Liao, 1992). Our study, however, does not rule out the possibility that γ-LA can also inhibit DHT action at high concentrations. For example, certain unsaturated fatty acids, such as oleic acid and arachidonic acid, may inhibit androgen receptor binding to androgens (Kato, 1989).

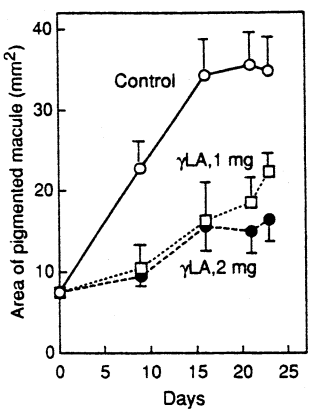

Figure 3. γ-LA inhibition of the growth of pigmented macules of intact male hamsters during maturation. The right flank organs of intact pre-pubertal male hamsters, 4-wk-old, were treated topically with ethanol alone (control) or with 1 or 2 mg of γ-LA (γLA) daily for 24 d. Each group contained 10 animals. The size of pigmented macule of the right flank organ was then measured. *Error bars*, SD (n = 10).

Our data suggest that γ-LA can inhibit androgen action on both dermal melanocytes and hair follicles of the flank organ. Our close inspection of pigmented macules revealed that the dark pigment is concentrated at the orifice of hair follicles, rather than distributed in the interfollicular areas of skin. Histologic examinations also showed that pigment is localized both in the hair shaft and in the upper dermis around the orifice of the hair follicle. γ-LA may inhibit T stimulation of the dermal melanocyte and the hair follicle, because the γ-LA–treated flank organ had shorter hairs and exhibited lighter color **(Fig 1*F*)**.

Findings in male patients with congenital deficiency of 5α-reductase (Imperato-McGinley and Guatier, 1986; Russell and Wilson, 1994) and investigations of 5α-reductase inhibitors in

animals indicate that an inhibitor of 5α-reductase with systemic activities would be teratogenic to a male fetus. For this reason a topical preparation of 5α-reductase inhibitor that does not produce systemic activity is desirable for treating androgen-dependent skin diseases. Our study indicates that topical γ-LA treatment of flank organ can inhibit T action locally without causing a systemic effect. γ-LA is an essential fatty acid with a high safety profile. Toxicity studies have shown that γ-LA is tolerated well by rats (2.5 g γ-LA per kg body weight per d) and dogs (5 g γ-LA per kg body weight per d) when γ-LA was given in the form of evening primrose oil (contain about 9% γ-LA) orally for 1 y (Everett *et al*, 1988). In our study, topical application of γ-LA did not irritate the skin. Therefore, γ-LA, with low toxicity and absence of systemic effect, appears to be useful for treatment of androgen-dependent skin disorders.

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be ideal for treatment of androgen-dependent dermatologic disorders.

The paired hamster flank organs, one on each side of the costovertebral angle, are highly sensitive to androgen stimulation (Hamilton and Montagna, 1950). The androgen-sensitive components of the flank organ include dermal melanocytes, sebaceous glands, and hair follicles (Frost *et al*, 1973). This animal model has been widely used for testing androgenic (Voigt and Hsia, 1973), and anti-androgenic (Chakrabarty *et al*, 1980; Luderschmidt *et al*, 1984; Weissmann *et al*, 1985) compounds. The unique advantage of this animal model is that a test compound can be applied topically to only one of the flank organ leaving the other flank organ as a control. If a test compound has systemic effects, both flank organs will be affected. In this study, we show that topical application of γ-LA suppresses only the androgen-dependent growth of the treated hamster flank organ without showing systemic effects on the contralateral flank organ and that this effect is very likely due to local inhibition of 5α-reductase.

MATERIALS AND METHODS

Chemicals Fatty acids were obtained from Sigma (St. Louis, MO). T and DHT were purchased from Steraloids (Wilton, NH). The purity of compounds were analyzed by thin layer chromatography. After 5 wk of storage at 4°C, the solutions of γ-LA in ethanol had two additional polar products, indicating oxidation of γ-LA. To avoid oxidation, all compounds (T, DHT, and fatty acids) were dissolved in ethanol, placed in a vial wrapped with aluminum foil, and stored at 4°C. Air in the vials was displaced with nitrogen gas by placing one or two drops of liquid nitrogen into each vial before they were capped. Nitrogen was replaced each time the vials were opened. Thin layer chromatographic examination of γ-LA solutions prepared in this manner and refrigerated for 3 wk revealed no detectable alteration of γ-LA. All solutions were prepared fresh every week.

Animals and Treatment Pre-pubertal male Syrian golden hamsters, intact or castrated at 4 wk old, were obtained from Harlan Sprague Dawley (Madison, WI). Bilateral orchiectomy was performed under anesthesia. Hamsters were housed individually in plastic cages, had free access to Purina rodent chow and water, and were maintained on a 12-h light/12-h dark cycle. Hamsters were kept on a longer light period (16-h light/8-h dark cycle) to ensure maximum stimulation of sexual characteristics (Wuest and Lucky, 1989) for studies following intact 4-wk-old hamsters during sexual maturation. Hamsters were used 1–2 wk after castration. Hamsters were divided into 5–10 animals per group. Hair on the lower back of each animal was shaved with an electric hair clipper weekly to expose flank organs. A treatment solution (5 μl with ethanol as vehicle) was applied topically to the right flank organ once a day with a Pipetteman and a polypropylene disposable tip. The treatment solution contained (i) ethanol alone as control, (ii) an androgen (T or DHT), (iii) a fatty acid, or (iv) a combination of an androgen and a fatty acid. The concentration of γ-LA was between 1% (0.05 mg in 5 μl of ethanol per application) and 40% (2.0 mg in 5 μl of ethanol per application). Unless described otherwise, the left flank organ was not treated. The surface of the flank organ was wiped with an alcohol pad to remove residual compound before each treatment. At the end of each experiment (15–24 d), animals were sacrificed by CO_2 asphyxiation or an intraperitoneal injection of phenobarbital (65 mg per ml per animal). Flank organs from both the treated and untreated sides were examined 1 d after the last treatment by the methods described below. The body weight of each animal was recorded before and after treatment. γ-LA, at the daily dose of 0.05 mg to 2.0 mg, did not appear to irritate the skin and did not affect the body weight increase of the animals during the experimental period when they were compared with the control animals not treated with γ-LA.

Determination of the Area of the Pigmented Macule of Flank Organs and Statistical Analysis of Data In this report, the growth of the flank organ was determined by measuring the length of the long axis and the short axis of the pigmented spot (pigmented macule) with a caliper with digital display (Digimatic by Mitutoyo, Kawazaki, Japan). The surface area (mm^2) of the spot was calculated by the product of the long axis times the short axis (Gomez and Frost, 1975). For each experiment, the mean and standard deviation (SD) of the left (untreated) and right (treated) macule sizes (mm^2) were computed separately by treatment group. Within each experiment, an overall F-test was used to test the null hypothesis that the mean size of the treated macules was the same in all groups. Tukey's honest significant difference procedure was used to test individual pairwise differences between means (Hochberg and Tamhane, 1987). In addition, the difference in size between the left and right macules was computed for each animal. These difference data were then analyzed by the same methods used to analyze the treated macule sizes. The hypothesis of no difference between left and right was tested separately within each treatment group by using the standard t statistic. p Values were evaluated by using Tukey's test based on the studentized range statistic. p Values were also multiplied by the number of treatment groups to account for the multiple testing. p Values smaller than 0.05 was considered significant.

Histology The skin containing the flank organ was excised, fixed in 10% formalin, and sectioned along the long axis of the flank organ. The tissue sections were stained with hematoxylin and eosin for microscopic examinations.

RESULTS

Stimulation of Pigmented Macules in Castrates by T We applied different doses of T (0, 0.5, 2, or 5 μg per animal daily) to the right flank organ of castrated hamsters (five animals per group). After 24 d of treatment, the area of the pigmented macule was 2.4 ± 1.4 mm^2 (mean ± SD) for the control group, 45.6 ± 8.0 mm^2 for the 0.5-μg T group, 69.4 ± 13.7 mm^2 for the 2-μg T group, and 66.4 ± 4.2 mm^2 for the 5-μg T group. **Figure 1*A*** shows representative hamsters in the 0.5-μg and 2-μg T groups. Only the T-treated right flank organs, not the untreated left flank organs, were stimulated, darker, and larger in size. The submaximal dose of 0.5 μg T per flank organ per day was chosen for most of the studies described in this report.

Effects of γ-LA and Stearic Acid (SA) on T Stimulation of Pigmented Macules γ-LA is a potent inhibitor of 5α-reductase in the *in vitro* enzyme assay system (Liang and Liao, 1992; Liao and Hiipakka, 1995), whereas SA, its saturated isomer, is inactive. We therefore compared the effect of these two fatty acids on the T-stimulated growth of the pigmented macule. Castrated hamsters were divided into eight groups, five animals per groups. The right flank organs were treated daily with 0.5 μg of T with or without 1 mg of γ-LA or 1 or 2 mg of SA. Daily application of γ-LA for 18 d prevented the growth of the pigmented macules by about 50% **(Table I)**. There were no significant differences in the size of pigmented macules among the control group and those treated with either γ-LA or SA alone in the absence of T. **Figure 1*B*** shows representative animals in this experiment. γ-LA inhibition of T-dependent growth of pigmented macules was evident from the fact that the pigmented macules on castrated animals treated with both γ-LA and T were lighter in color and smaller than those on animals treated with T alone. In contrast, SA did not inhibit the T-dependent growth of the pigmented macule. The contralateral flank organs and the body weights were not affected. A dose-dependent response curve was established by determining the effect of application of 0.01–2 mg of γ-LA per flank organ daily on stimulation of pigmented macule growth by T (0.5 μg per flank organ per d). γ-LA was clearly inhibitory at a daily dosage of 0.05 mg or higher, and 50% inhibition was reached at about 1 mg. At a daily dosage of 2–4 mg of γ-LA, inhibition was 60–70%.

γ-LA Inhibition of T-dependent Growth of Sebaceous Glands The effect of T and γ-LA on the growth of sebaceous glands was examined histologically. The flank organ contained clusters of sebaceous glands. The lobules of the sebaceous glands in the control skin (without T or γ-LA treatment) **(Fig 2*A*)** were small and the sebocytes in the lobules were stained poorly with eosin. The flank organs from the T-treated skin **(Fig 2*B*)** contained distinctly large sebaceous lobules, reflecting an increase in both the number and size of eosinophilic sebocytes in each lobule. The T effect was reduced considerably by γ-LA **(Fig 2*C*)** treatment. These γ-LA–induced histologic changes were not observed when γ-LA was replaced by stearic acid.

Effects of Various Fatty Acids on T-stimulated Growth of the Pigmented Macule **Table II** shows the effect of various fatty acids on the T-induced growth of pigmented macules of castrated hamsters. The right flank organs were treated topically with either T (0.5 μg) or T (0.5 μg) and a test fatty acid (1 mg)