BRIEF REPORT

Prepubertal Gynecomastia Linked to Lavender and Tea Tree Oils

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SUMMARY

Most cases of male prepubertal gynecomastia are classified as idiopathic. We investigated possible causes of gynecomastia in three prepubertal boys who were otherwise healthy and had normal serum concentrations of endogenous steroids. In all three boys, gynecomastia coincided with the topical application of products that contained lavender and tea tree oils. Gynecomastia resolved in each patient shortly after the use of products containing these oils was discontinued. Furthermore, studies in human cell lines indicated that the two oils had estrogenic and antiandrogenic activities. We conclude that repeated topical exposure to lavender and tea tree oils probably caused prepubertal gynecomastia in these boys.

G YNECOMASTIA IS GENERALLY ATTRIBUTED TO CONDITIONS THAT DISrupt sex-steroid signaling pathways, resulting in increased or unopposed estrogen action on breast tissue.[1] In contrast to gynecomastia in adolescent boys and men, prepubertal gynecomastia is rare and should always be considered pathological, prompting a search for a source of estrogen. Although hyperestrogenemia may be endogenous or exogenous in origin, most persons with prepubertal gynecomastia have normal serum concentrations of sex steroids, and an underlying cause is not identified.[2,3] In such cases, possible exposure to exogenous sources of estrogen should be considered. We investigated the cause of prepubertal gynecomastia in three otherwise healthy boys with normal serum concentrations of endogenous steroids.

CASE REPORTS

Patient 1

A boy who was 4 years 5 months old presented with gynecomastia of apparently 2 to 3 weeks' duration. He had no exposure to any known exogenous form of estrogens (ingestants, salves, or ointments). His height and weight were at the 97th percentile and between the 75th and 90th percentiles, respectively. He had bilateral gynecomastia with firm, nontender breast tissue measuring 2 cm by 2 cm in diameter. His testes were 3 ml in volume and of normal consistency. His genitalia were prepubertal (Tanner stage 1). Laboratory investigation showed normal thyroid function; the follicle-stimulating hormone (FSH) concentration was 1.04 IU per liter (reference range, 0.25 to 1.92), luteinizing hormone 0.47 IU per liter (reference range, 0.02 to 1.03), testosterone 0.08 ng per milliliter (0.27 nmol per liter) (reference range, 0.02 to 0.25 ng per milliliter), estradiol less than 20 pg per milliliter (73 pmol

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per liter) (normal value, <20), dehydroepiandrosterone (DHEA) sulfate less than 5.0 μg per deciliter (0.14 μmol per liter) (reference range, 1 to 40), 17-alpha-hydroxyprogesterone 0.32 μg per liter (0.97 nmol per liter) (reference range, 0.2 to 0.8), and prolactin 8.0 μg per liter per liter (reference range, 2 to 29); the serum biochemistry values, including liver-function tests, were normal. On evaluation 3 months later, the breast buds were tender to palpation and had increased to 2.5 cm by 2.5 cm in diameter with an increased breast mound. The patient's mother reported applying a compounded "healing balm" containing lavender oil to his skin starting shortly before the initial presentation. The gynecomastia partially resolved within 4 months after application of the healing balm was discontinued, at which time the breast buds measured 1.5 cm by 1.5 cm in diameter and were soft in consistency. Several months later, his pediatrician stated that the gynecomastia had resolved completely.

Patient 2

A boy who was 10 years 1 month old presented with a 5-month history of gynecomastia. He and his mother reported that the condition seemed more prominent in the evening and a little less so in the morning. His medical history and family history were unremarkable. His height and weight were above the 97th percentile, and his body-mass index (the weight in kilograms divided by the square of the height in meters) was 21.1. He had firm, tender breast buds, measuring 3.5 cm by 4.0 cm in length and width and approximately 3.5 cm in depth, with stretching of the areolae. His testes were 3 ml in volume and of normal consistency. His pubic hair was Tanner stage 2 (a small amount of long hair at the base of the scrotum), and his genitalia were Tanner stage 1. Laboratory testing showed a testosterone concentration of 0.36 ng per milliliter (1.25 nmol per liter) (normal value, <0.25), free testosterone 0.0066 ng per milliliter (0.0229 nmol per liter) (reference range, 0.0006 to 0.0057), and DHEA sulfate 278 μg per deciliter (7.6 μmol per liter) (normal value, <75). On questioning, it was determined that the patient was not currently using drugs, herbal supplements, or herbal lotions but was applying a styling gel to his hair and scalp every morning and regularly using a shampoo. The labels of both the gel and the shampoo listed *Lavandula angustifolia* (lavender) oil and

Melaleuca alternifolia (tea tree) oil as ingredients. Reevaluation 9 months after use of these products was discontinued showed that his areolar mounds had decreased in depth to approximately 1 cm with almost no palpable glandular tissue.
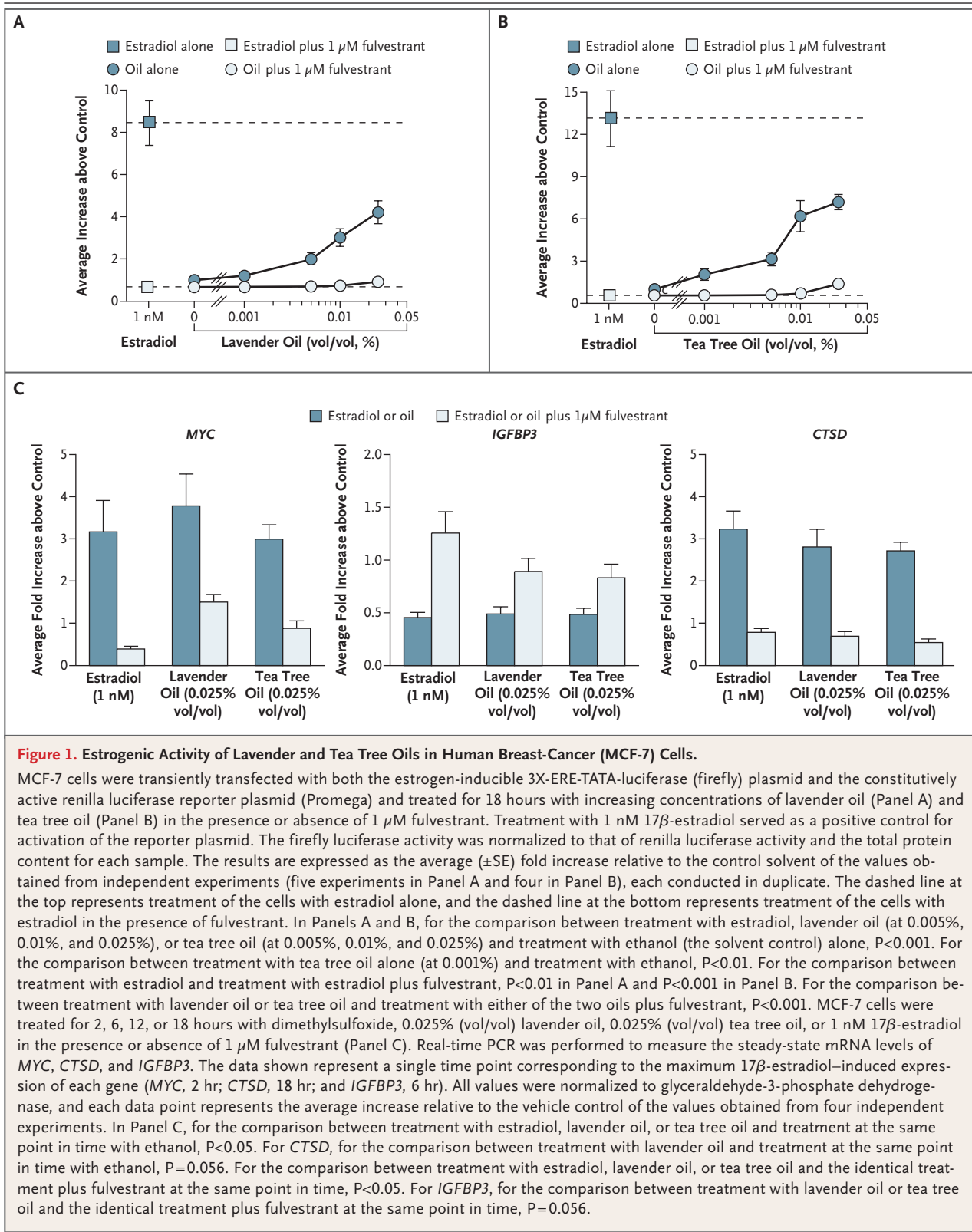
Patient 3

A boy who was 7 years 10 months old presented with a 1-month history of gynecomastia that had appeared gradually. His height was between the 75th and 90th percentiles, and his weight was at the 50th percentile. He had bilateral gynecomastia with firm, nontender breast tissue that corresponded to Tanner stage 2. His testes were 3 ml in volume and of normal consistency. His genitalia were Tanner stage 1, and there was no pubic hair present. Laboratory testing showed normal thyroid function, FSH 0.49 IU per liter (reference range, 0.25 to 1.92), luteinizing hormone 0.16 IU per liter (reference range, 0.02 to 1.03), estradiol 5 pg per milliliter (18 pmol per liter) (normal value, <10), estriol less than 0.1 ng per milliliter (0.3 nmol per liter) (normal value, <0.1), estrone less than 13 pg per milliliter (48 pmol per liter) (normal value, <13), total estrogens 61 pg per milliliter (225 pmol per liter) (normal value, <130 in adult men), DHEA sulfate 22 μg per deciliter (0.6 μmol per liter) (reference range, 2.5 to 145), 17-alpha-hydroxyprogesterone 0.13 μg per liter (0.39 nmol per liter) (reference range, 15 to 65), and free beta subunit of human chorionic gonadotropin less than 2 mIU per milliliter (normal value, <5); the serum biochemistry values, including liver-function tests, were normal. His history was positive for the use of lavender-scented soap and intermittent use of lavender-scented commercial skin lotions. The gynecomastia resolved completely a few months after use of scented soap and skin lotions was discontinued (personal communication from the patient's family). His fraternal twin used the same skin lotions, but not the lavender-scented soap, and did not have any gynecomastia.

METHODS

MAMMALIAN CELL CULTURE

Human breast-cancer (MCF-7) cells that express estrogen receptors were grown in phenol red-free Dulbecco's modified Eagle's medium containing 10% fetal-calf serum (Atlanta Biologicals), penicillin (100 U per milliliter), and streptomycin (100 μg

Figure 1. Estrogenic Activity of Lavender and Tea Tree Oils in Human Breast-Cancer (MCF-7) Cells.

MCF-7 cells were transiently transfected with both the estrogen-inducible 3X-ERE-TATA-luciferase (firefly) plasmid and the constitutively active renilla luciferase reporter plasmid (Promega) and treated for 18 hours with increasing concentrations of lavender oil (Panel A) and tea tree oil (Panel B) in the presence or absence of 1 μM fulvestrant. Treatment with 1 nM 17β-estradiol served as a positive control for activation of the reporter plasmid. The firefly luciferase activity was normalized to that of renilla luciferase activity and the total protein content for each sample. The results are expressed as the average ($\pm$SE) fold increase relative to the control solvent of the values obtained from independent experiments (five experiments in Panel A and four in Panel B), each conducted in duplicate. The dashed line at the top represents treatment of the cells with estradiol alone, and the dashed line at the bottom represents treatment of the cells with estradiol in the presence of fulvestrant. In Panels A and B, for the comparison between treatment with estradiol, lavender oil (at 0.005%, 0.01%, and 0.025%), or tea tree oil (at 0.005%, 0.01%, and 0.025%) and treatment with ethanol (the solvent control) alone, $P<0.001$. For the comparison between treatment with tea tree oil alone (at 0.001%) and treatment with ethanol, $P<0.01$. For the comparison between treatment with estradiol and treatment with estradiol plus fulvestrant, $P<0.01$ in Panel A and $P<0.001$ in Panel B. For the comparison between treatment with lavender oil or tea tree oil and treatment with either of the two oils plus fulvestrant, $P<0.001$. MCF-7 cells were treated for 2, 6, 12, or 18 hours with dimethylsulfoxide, 0.025% (vol/vol) lavender oil, 0.025% (vol/vol) tea tree oil, or 1 nM 17β-estradiol in the presence or absence of 1 μM fulvestrant (Panel C). Real-time PCR was performed to measure the steady-state mRNA levels of *MYC*, *CTSD*, and *IGFBP3*. The data shown represent a single time point corresponding to the maximum 17β-estradiol–induced expression of each gene (*MYC*, 2 hr; *CTSD*, 18 hr; and *IGFBP3*, 6 hr). All values were normalized to glyceraldehyde-3-phosphate dehydrogenase, and each data point represents the average increase relative to the vehicle control of the values obtained from four independent experiments. In Panel C, for the comparison between treatment with estradiol, lavender oil, or tea tree oil and treatment at the same point in time with ethanol, $P<0.05$. For *CTSD*, for the comparison between treatment with lavender oil and treatment at the same point in time with ethanol, $P=0.056$. For the comparison between treatment with estradiol, lavender oil, or tea tree oil and the identical treatment plus fulvestrant at the same point in time, $P<0.05$. For *IGFBP3*, for the comparison between treatment with lavender oil or tea tree oil and the identical treatment plus fulvestrant at the same point in time, $P=0.056$.

per milliliter). Human breast-cancer (MDA-kb2) cells that express the androgen receptor were maintained as previously described.[4] Cell-culture reagents were obtained from Invitrogen Life Technologies, unless otherwise indicated. For all experiments, the lavender oil (*L. officinalis,* which is a synonym for *L. angustifolia*) and tea tree oil (*M. alternifolia*) (both from Sigma Chemical) were diluted in dimethylsulfoxide before they were added to culture media.

Luciferase Assays and Reverse-Transcriptase and Real-Time Polymerase-Chain-Reaction Analysis

MCF-7 and MDA-kb2 cells were assayed for luciferase activity with the use of the Dual–Luciferase reporter assay system (Promega) and an LMAX II[384] luminometer (Molecular Devices). Total RNA was isolated from MCF-7 cells and MDA-kb2 cells with the use of the RNeasy Mini Kit (Qiagen), according to the manufacturer's protocol. Synthesis of complementary DNA (cDNA) and analyses of gene-specific cDNA concentrations were performed by real-time polymerase chain reaction (PCR), as previously described.[5] The PCR primers were designed with the use of Primer Express software, version 2.0 (Applied Biosystems) (see the Supplementary Appendix, available with the full text of this article at www.nejm.org).

STATISTICAL ANALYSIS

The data were analyzed for statistical significance by the Mann–Whitney nonparametric test.

RESULTS

ESTROGEN-RECEPTOR—DEPENDENT ESTROGENIC ACTIVITY IN VITRO

To determine whether lavender oil and tea tree oil are estrogenic, we performed dose–response experiments in MCF-7 cells that were positive for estrogen receptors and were transiently transfected with an estrogen-inducible luciferase reporter plasmid containing three copies of an estrogen-response element (3X-ERE-TATA-luciferase). Both oils stimulate ERE-dependent luciferase activity in a dose-dependent manner, with the maximum activity observed at 0.025% volume per volume (vol/vol) for each oil, corresponding to approximately 50% of the activity elicited by 1 nM 17β-estradiol (Fig. 1A and 1B). Treatment with higher doses of the oils was cytotoxic. The pure estrogen-recep-

Figure 2 (facing page). Antiandrogenic Activity of Lavender and Tea Tree Oils in Breast-Cancer (MDA-kb2) Cells.

MDA-kb2 cells that were stably transfected with the MMTV-luciferase (firefly) plasmid were treated for 24 hours with increasing concentrations of lavender oil (Panel A) or tea tree oil (Panel B) in the presence or absence of DHT. The firefly luciferase activity was normalized to the total protein content for each sample. The data were averaged and plotted as the average (±SE) fold increase above vehicle control of three independent experiments performed in quadruplicate. The upper dashed lines in Panels A and B represent treatment of the cells with DHT alone, and the lower dashed lines represent treatment with DHT in the presence of flutamide. For the comparison between treatment with DHT and treatment with ethanol (the solvent control) alone, $P<0.001$. For the comparison between treatment with either DHT plus flutamide or DHT plus either lavender oil (at 0.001% and 0.005%) or tea tree oil (at 0.005%) and treatment with DHT alone, $P<0.001$. For the comparison between treatment with DHT plus tea tree oil at 0.0005% and treatment with DHT alone, $P<0.05$. For the comparison between treatment with tea tree oil at 0.001% and treatment with DHT alone, $P<0.01$. In Panel C, MDA-kb2 cells were treated with 0.1 nM DHT for 24 hours in the presence or absence of 0.005% (vol/vol) lavender oil, 0.005% (vol/vol) tea tree oil, or 1 μM of flutamide. Real-time PCR was performed to measure the steady-state mRNA concentrations of *CYP4F8*, *C1orf116*, *UGT2B28*, and *SEC14L2*. All values were normalized to $β_2$-microglobulin, and each data point represents the average increase above vehicle control of the values obtained from three independent experiments performed in duplicate.

tor antagonist fulvestrant inhibited transactivation of the 3X-ERE-TATA-luciferase reporter plasmid by both oils, indicating that their activity is estrogen-receptor–dependent (Fig. 1A and 1B). Additional experiments indicated that lavender oil was able to transactivate the estrogen-inducible reporter plasmid in estrogen-receptor–negative SK-BR-3 human breast-cancer cells only after simultaneous transfection with an estrogen-receptor–expression vector (data not shown).

Further experiments in MCF-7 cells indicated that the two oils modulated the expression of the estrogen-regulated endogenous genes *MYC* (also called *C-MYC*),[6] *CTSD*,[7] and *IGFBP3*.[8] Lavender oil and tea tree oil increased the expression of messenger RNA (mRNA) for *MYC* and *CTSD* and decreased the expression of mRNA for *IGFBP3*, as compared with the dimethylsulfoxide controls, in a manner that was similar to the effect of 1 nM 17β-estradiol on the magnitude and timing of the

A

◼ 0.1 nM DHT   ● 0.1 nM DHT plus oil
◻ 0.1 nM DHT plus   ○ Oil alone
  1 μM flutamide

B

◼ 0.1 nM DHT   ● 0.1 nM DHT plus oil
◻ 0.1 nM DHT plus   ○ Oil alone
  1 μM flutamide

C

CYP4F8

UGT2B28

C1orf116

SEC14L2
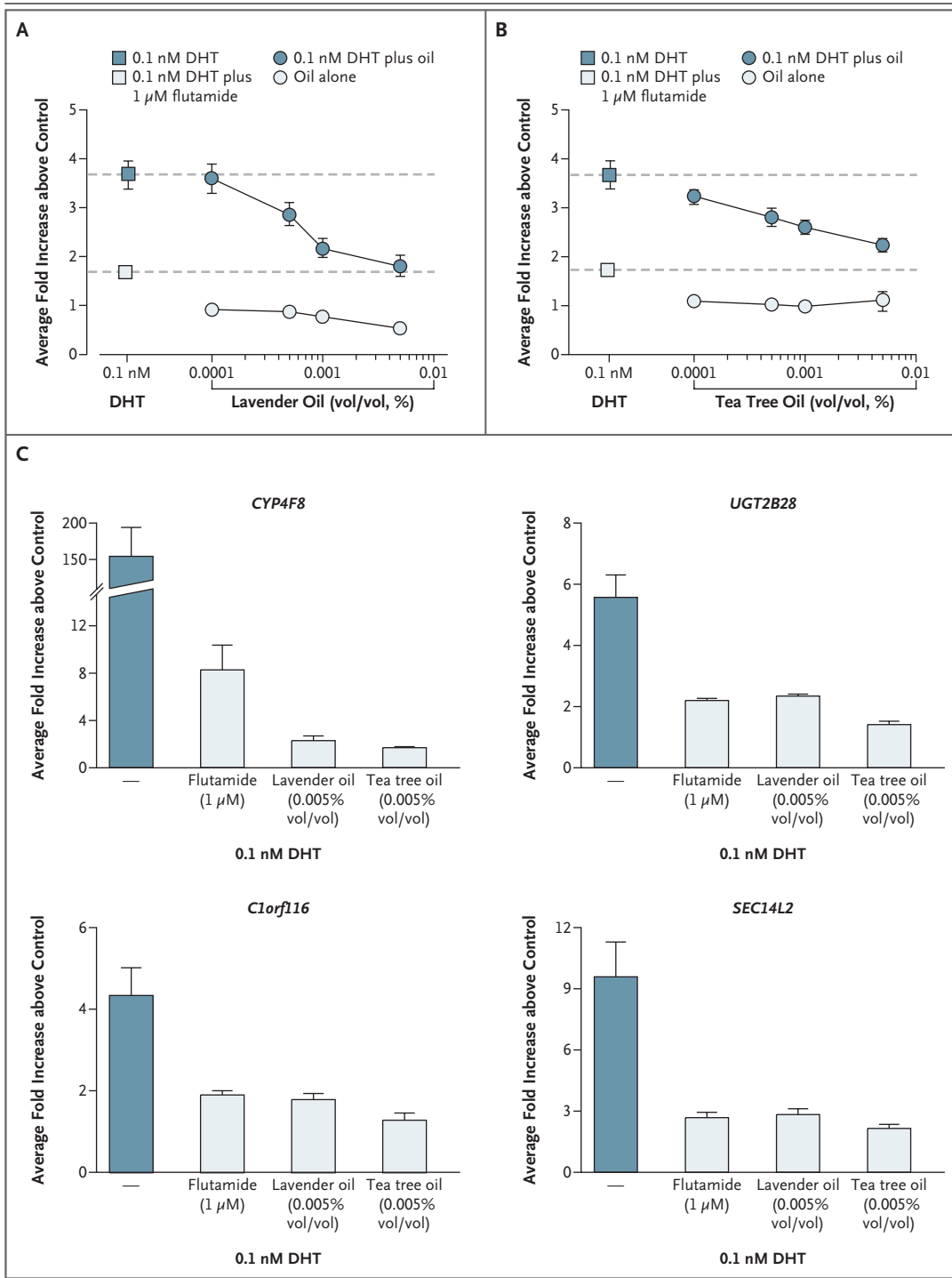
responses (Fig. 1C). These responses were attenuated in the presence of 1 μM fulvestrant (Fig. 1C).

IN VITRO ANTIANDROGENIC ACTIVITY

To evaluate the potential androgenic properties of lavender oil and tea tree oil, we performed dose–response experiments in MDA-kb2 cells, a line of human breast-cancer cells that are positive for the androgen receptor and were stably transfected with an androgen-inducible and glucocorticoid-inducible mouse mammary-tumor virus (MMTV)-luciferase reporter plasmid. Treatment

of MDA-kb2 cells with the androgen-receptor agonist dihydrotestosterone (DHT) at 0.1 nM, the lowest observed effective dose in this cell line,[4] resulted in an increase in luciferase activity that was almost four times higher than that in the dimethylsulfoxide controls (Fig. 2A and 2B). In contrast, neither lavender oil nor tea tree oil transactivated the MMTV-luciferase reporter plasmid at any concentration tested (Fig. 2A and 2B).

The antiandrogenic properties of the two oils were assessed by simultaneously treating the MDA-kb2 cells with DHT and increasing the concentration of lavender oil or tea tree oil. The androgen-receptor antagonist flutamide was also included in these assays, as a positive control for androgen-receptor antagonism. Transactivation of the MMTV-luciferase reporter plasmid by 0.1 nM DHT was inhibited in a concentration-dependent manner by both lavender oil and tea tree oil, as well as by flutamide (Fig. 2A and 2B). Maximum inhibition occurred at 0.005% vol/vol for both lavender oil and tea tree oil, corresponding to a decrease in luciferase activity of 52% and 41%, respectively, in the presence of 0.1 nM DHT. The observed inhibitory effects appear to be specific to the androgen receptor, since neither of the two oils attenuated the glucocorticoid-receptor–mediated transactivation of the MMTV-luciferase reporter plasmid in the presence of 5 nM dexamethasone, the lowest observed effective dose in this cell line[9] (data not shown). Further experiments in MDA-kb2 cells indicated that the antiandrogenic properties of lavender oil and tea tree oil extended to inhibition of DHT-stimulated expression of the androgen-inducible endogenous genes *CYP4F8*, *C1orf116*, *UGT2B28*, and *SEC14L2*[10] (Fig. 2C). The antiandrogenic effects of the two oils are not caused by down-regulation of the expression of the androgen receptor, since neither of the oils altered the amount of androgen-receptor mRNA or protein in these experiments (data not shown).

DISCUSSION

In contrast to gynecomastia, which occurs in more than 60% of boys during puberty, prepubertal gynecomastia is extremely uncommon. Since there is no known physiologic cause of prepubertal gynecomastia, pathologic causes should be considered. However, a specific cause is rarely identified, and in 90% of patients, prepubertal gynecomastia is labeled idiopathic.[2,3] In such patients, the condition may be caused by exposure to an environmental chemical that disrupts the endocrine system and leads to disproportionate estrogen and androgen pathway signaling, a finding reported in a limited number of adults with gynecomastia.[11,12]

In this report, we describe three otherwise healthy boys with prepubertal gynecomastia, all of whom had normal serum concentrations of endogenous steroids and none of whom had been exposed to any known exogenous endocrine disruptor such as medications, oral contraceptives, marijuana, or soy products.[1] The repeated topical application of one or more over-the-counter personal care products that contained lavender oil or lavender oil and tea tree oil was documented for all three patients. Case 1 provided the clinical clue to lavender oil as a potential source, because it was the only topically applied agent used by that child. Use of lavender oil was considered trivial by the child's mother, who acknowledged its use only after repeated questioning. In Case 2, the boy had biochemical evidence of physiologic adrenarche, but the evidence was unrelated to his gynecomastia, which resolved after discontinuation of the use of products containing lavender oil and tea tree oil, despite the persistence of adrenarche. The daily temporal fluctuation in the severity of the gynecomastia reported by the patient's mother might have been caused by the transdermal absorption kinetics of the oils after application each morning. In Case 3, the patient was exposed intermittently to various over-the-counter personal-care products containing lavender oil. His twin brother used the same lotions but not the scented soap, and gynecomastia did not develop in him.

The common use of products containing lavender oil, tea tree oil, or both by the three boys and the resolution of their gynecomastia within months after ceasing use of those products suggest that these oils may possess endocrine-disrupting activity that causes an imbalance in estrogen and androgen pathway signaling. Other components in these products may also possess endocrine-disrupting activity that contributed to the gynecomastia, but those components were not tested because we chose to evaluate only the component that was found in all the products used by the patients (lavender oil) and a chemically similar component that was found in some of the products (tea tree oil).

Our in vitro studies confirm that lavender oil and tea tree oil possess weak estrogenic and antiandrogenic activities that may contribute to an imbalance in estrogen and androgen pathway signaling. Estrogenic or antiandrogenic activities have been reported for other essential oils and some of their monoterpene constituents.[13-18] On the basis of the three case reports and the in vitro studies, we suspect that repeated topical application of over-the-counter products containing lavender oil or tea tree oil was the cause of gynecomastia in the three patients.

This report raises an issue of concern, since lavender oil and tea tree oil are sold over the counter in their "pure" form and are present in an increasing number of commercial products, including shampoos, hair gels, soaps, and body lotions. Whether the oils elicit similar endocrine-disrupting effects in prepubertal girls, adolescent girls, or women is unknown. Since gynecomastia is labeled idiopathic in approximately 10% of men, one might speculate that unidentified exogenous sources of endocrine-disrupting chemicals may contribute to the onset or progression of the condition, or both, in such patients.[1] The results of our in vitro studies indicate a dose–response relationship in the estrogenic and antiandrogenic activities of lavender oil and tea tree oil, suggesting that susceptibility to gynecomastia or other manifestations of endocrine disruption may require exposure to a threshold dose of these oils. The threshold might depend on several undefined factors, including the concentration of the oil in a product; the duration, frequency, and quantity of use of the product; and the genetic characteristics of persons exposed. Until epidemiologic studies are performed to determine the prevalence of gynecomastia associated with exposure to lavender oil and tea tree oil, we suggest that the medical community should be aware of the possibility of endocrine disruption and should caution patients about repeated exposure to any products containing these oils.

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