

Measurement of Sebum Secretion Rates in Young Children

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Sustainable rates of lipid secretion were measured on the foreheads of 24 children, ages 6-8. The method of measurement involved absorption of skin surface lipid into bentonite clay and subsequent separation and measurement of the various lipid classes by quantitative thin-layer chromatography. In 9 of the children, sebum secretion appeared to be virtually nonexistent, judging by the low amounts of lipid recovered and the low percentages of wax esters and squalene, which are purely sebaceous lipid classes. An effect of age on sebum secretion rates was observed with median rates of wax ester secretion, being 7, 28, and 48 $\mu\text{g}/10\text{ cm}^2/3\text{ h}$ for 6-, 7-, and 8-year-olds, respectively. These values are well below those of most young adults.

The relation between wax ester secretion rate and skin surface lipid composition on the forehead was investigated and found to conform to the formula: $\mu\text{g wax esters}/10\text{ cm}^2/3\text{ h} = 28 \times \text{wax esters}/(\text{cholesterol} + \text{cholesterol esters})$, with a correlation coefficient (r) of 0.94.

Direct measurement of sebum secretion rates in young children has not been possible previously because gravimetric methods [1,2] are not sensitive enough to measure the small amounts of lipid produced by prepubertal children, and because much of the lipid that is produced may be of epidermal origin. During the past several years, we have been using a new method of measuring sebum secretion in adults [3,4]. The method involves quantitative collection of skin surface lipid by absorption into bentonite clay and subsequent analysis of an aliquot of the collected lipid by thin-layer chromatography. This method has now been adapted for children by applying the entire lipid sample, rather than an aliquot, to the thin-layer plate. The lipid classes are then separated and quantified by photodensitometry. Wax esters (which are a purely sebaceous lipid class) were used as a measure of sebum secretion rate.

We also examined the relation between wax ester secretion rate and the ratio of wax esters/(cholesterol + cholesterol esters) in the lipid samples. This ratio has been used as an indirect measure of sebum secretion in children in the belief that it represents a ratio of a sebaceous lipid class to two epidermal lipid classes [5,6].

MATERIALS AND METHODS

Subjects

The subjects were 12 boys and 12 girls, ages 6-8, who were recruited through a newspaper advertisement. Informed consent for the procedures involved was obtained both from the children and from their parents.

Collection of Lipid

The follicular reservoir of lipid [7] was first depleted during a 14-h continuous absorption into bentonite clay. For this depletion step, the

child's forehead was first washed with soap and water and then dried. An aqueous suspension of bentonite clay containing 0.2% carboxymethylcellulose was then spread in a thin film over the forehead and a 1" \times 2" rectangular piece of nylon cloth was applied over the clay. More clay was then applied over the cloth. The clay and cloth were left in place for 14 h, after which the cloth was removed, bringing the clay with it and leaving a clear area of skin depleted of accumulated lipid [7].

For the quantitative collection step, more bentonite clay was spread on the depleted area and 2 disks of dacron cloth, 1.9 cm in diameter (2.8 cm^2 area), were applied over the clay, equidistant from the midline of the forehead. More clay was applied over and around the disks, which were then left in place for exactly 3 h. When the clay-impregnated disks were removed, they were placed in separate vials containing 5 ml each of ether to extract the collected lipid.

Separation and Measurement of Lipid Classes

The lipid extracts were evaporated to dryness under nitrogen and redissolved in 50 μl of toluene containing 0.1 mg/ml methyl oleate as an internal standard for thin-layer chromatography. Each extract was applied, in its entirety, to a 6-mm lane scored in thin-layer plates coated with a 0.25 mm-thick layer of silica gel G. The chromatograms were first developed with hexane to the top and then with toluene to the top. Prior to a third development, the position to which the methyl oleate standard had been moved was determined. To do this, the chromatograms were sprayed with an ethanolic solution of 8-hydroxy-1,3,6-pyrenetrisulfonic acid trisodium salt (100 mg/liter), allowed to dry, and inspected under UV light. The position of the methyl oleate spots was marked in the margin of the plate, and the lower portion of the plate was then developed in hexane:ether:acetic acid (70:30:1) to a position just below the methyl oleate spots (about 10 cm). The plate was then sprayed with 50% aqueous sulfuric acid and charred by heating slowly to 220°C on a hotplate. After the plate had cooled, the spots were quantitated by photodensitometry [8,9]. The amount of wax esters collected in each disk was calculated by dividing the densitometric area of the wax ester spot by the peak area of the methyl oleate spot and multiplying the result by 5 μg (the amount of methyl oleate added to the sample). An average amount of wax esters in the right and left disks was calculated for each subject and expressed as $\mu\text{g wax esters}/10\text{ cm}^2/3\text{ h}$.

To obtain the percentage composition of the skin surface lipid, the densitometric area for each spot (excluding that of methyl oleate) was corrected for the carbon content of the original lipid [8] and converted to a percentage of total corrected peak areas.

RESULTS

Fig 1 shows the wax ester secretion rates of the children. The median rates of wax ester secretion were 7, 28, and 48 $\mu\text{g}/10\text{ cm}^2/3\text{ h}$ for 6-, 7-, and 8-year-olds respectively.

Fig 2 shows densitometric scans of two representative thin-layer chromatographic analyses of lipid from children with quite different rates of sebum production. The internal standard (MO) peaks are about the same size in both chromatograms and represent the same amount of lipid (5 μg). Therefore, the chromatograms can be compared in terms of absolute, as well as relative, amounts of the various lipid classes. The child with the higher rate of sebum production (scan B) had much more triglycerides, wax esters, and squalene relative to the internal standard than did the child with the very low rate of sebum secretion (scan A). The amounts of cholesterol and cholesterol esters appear to be about the same in both children. In fact, the rates of cholesterol and cholesterol ester secretion were about the same in all 24 children, the averages and

Manuscript received March 12, 1984; accepted for publication June 19, 1984

Supported in part by U.S. Public Health Service Grant AM22083.

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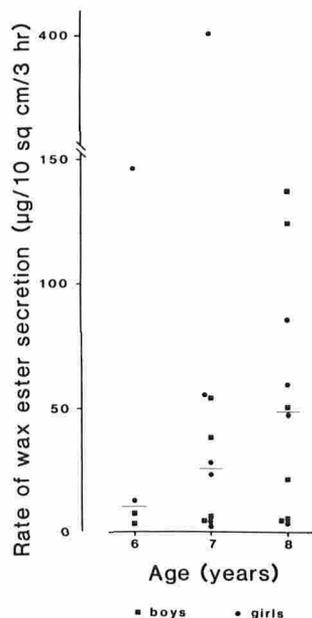


FIG 1. Rates of forehead wax ester secretion in boys and girls aged 6-8 years.

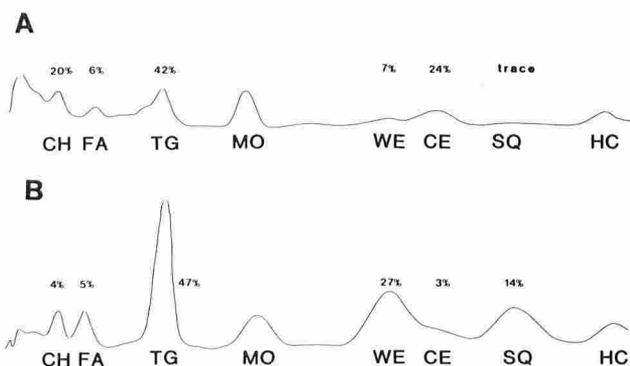


FIG 2. Densitometric scans of charred thin-layer chromatograms of skin surface lipids from two children, one (A) with a very low rate of sebum secretion and another (B) with a higher rate of sebum secretion. CH, cholesterol; FA, free fatty acids; TG, triglycerides; MO, methyl oleate (added as an internal standard); WE, wax esters; CE, cholesterol esters; SQ, squalene; HC, hydrocarbon contaminants.

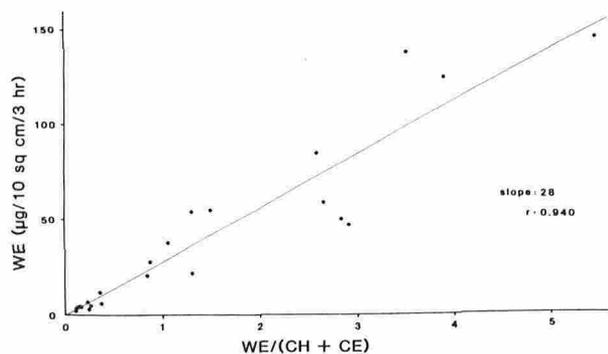


FIG 3. Relation between rates of wax ester secretion on the foreheads of young children and the ratio of wax esters/cholesterol + cholesterol esters in forehead skin surface lipid. WE, wax esters; CH, cholesterol; CE, cholesterol esters.

standard deviations being 11.0 ± 4.5 and 16.6 ± 8.7 $\mu\text{g}/10$ $\text{cm}^2/3$ h for cholesterol and cholesterol esters, respectively.

The two lipid samples represented in Fig 2 differ considerably in percentage composition of the various lipid classes. Wax

esters and squalene are present in much lower percentages, while cholesterol and cholesterol esters are found in much higher percentages in the low sebum producer compared to the higher sebum producer. There were 9 subjects in the study who had wax ester secretion rates of less than 10 $\mu\text{g}/10$ $\text{cm}^2/3$ h and all of these showed a percentage composition resembling that of scan A. None of the 9 had more than 8% wax esters, and 4 of the 9 had no measurable squalene.

Fig 3 shows the relation between wax ester secretion rates and the ratio of wax esters/(cholesterol + cholesterol esters) in the lipid collected in the disks. The line of best fit gives the relation: μg wax esters/ 10 $\text{cm}^2/3$ h = $28 \times$ wax esters/(cholesterol + cholesterol esters).

DISCUSSION

Wax Ester Secretion Rates in Young Children

The present study shows that wax ester secretion rates in young children can range from almost nothing to, in one case, the surprisingly high value of 400 $\mu\text{g}/10$ $\text{cm}^2/3$ h. In comparison, young adults without acne were found to have sustainable rates of wax ester secretion ranging from 70 – 804 $\mu\text{g}/10$ $\text{cm}^2/3$ h [3]. Individuals with acne can have wax ester secretion rates of well over 1 $\text{mg}/10$ $\text{cm}^2/3$ h [3,4]. Most of the children in the present study, therefore, were below the young adult level.

An age-related increase in median wax ester secretion rates can be seen in Fig 1. This increase is most probably a consequence of increases in adrenal secretion which begin at about age 7. Pochi et al [6] showed that there was a significant positive correlation between urinary excretion of several adrenal androgens and ratios of wax esters/(cholesterol + cholesterol esters) in the skin surface lipid of children aged 5-10.

Sebum Secretion Rates and Skin Surface Lipid Composition

The simplest interpretation of the variation in lipid class compositions among the subjects is that this variation is solely the result of variation in the ratio of sebaceous to epidermal lipid. In this interpretation, cholesterol and cholesterol esters are considered to be mainly of epidermal origin and to be released at rates that vary little among individuals. Therefore, at higher rates of sebum production, cholesterol and cholesterol esters become diluted by sebum. The data of the present study are not incompatible with this interpretation. However, in an earlier study in which acne patients were treated with 13-*cis*-retinoic acid, the rate of cholesterol ester secretion was suppressed in proportion to the rate of squalene secretion [10], and it was concluded that cholesterol esters are of mostly sebaceous origin. If this is the case, an alternative explanation is required for the present data on cholesterol ester variation. The explanation might be that low sebum producers secrete sebum with a high percentage of cholesterol esters. A possible mechanism for this may be explained as follows.

Sebaceous glands are holocrine organs and all of the constituents of a dying cell must be disposed of somewhere. A cell would have a certain amount of membrane cholesterol to secrete regardless of whether it had synthesized much sebum. Since the dying sebaceous cell breaks down its membrane phospholipids, fatty acyl chains would be available for esterification to the cholesterol that also was being released by membrane breakdown. In a cell that had produced little sebum, these cholesterol esters might constitute a fairly large proportion of the total lipid secretory product, while in a cell that had produced a large amount of sebum, the cholesterol esters resulting from membrane breakdown would be diluted. If this mechanism is correct, wax ester secretion rates may not be proportional to total sebum secretion at low levels of sebum secretion. Unfortunately, however, total sebum is not measurable because human sebum contains lipids, such as triglycerides, which probably are also present in epidermal lipid.

Ratios of Wax Esters/(Cholesterol + Cholesterol Esters) and Sebum Secretion Rates

The effects of sebum secretion rate on skin surface lipid composition are reflected in the ratio of wax esters/(cholesterol + cholesterol esters). In 23 of the 24 children in the present study, there appeared to be a linear relation between this ratio and the rate of secretion of wax esters (the exception being the child with the highest rate of wax ester secretion). This observation should be useful in situations where direct measurement of lipid secretion is difficult as, for example, when lipid is collected from the scalp. The linear relation found here probably does not hold at the higher rates of sebum secretion found in young adults. Rather, it would be expected that the ratio of wax esters/(cholesterol + cholesterol esters) would rise less steeply at higher levels of sebum secretion and tend to approach the ratio of these components in adult sebum, i.e., about 6 to 8 [10,11]. Another situation where the relation found here may not hold is in subjects whose sebum secretion has been suppressed by 13-*cis*-retinoic acid. Percentages of cholesterol esters in freshly secreted skin surface lipid do not become elevated in patients on the drug [10]. Moreover, the effect, if any, of 13-*cis*-retinoic acid on the rate of epidermal lipid production is unknown.

We wish to thank Allison M. Benoit for her expert technical assistance.

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