Dilutional Effect of Increased Sebaceous Gland Activity on the Proportion of Linoleic Acid in Sebaceous Wax Esters and in Epidermal Acylceramides

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Sebaceous wax esters and epidermal acylceramides were isolated from skin surface lipid obtained from children and from young adults. Fatty acid methyl esters (FAME) were prepared from the esterified fatty acids of these lipid classes and analyzed to ascertain the proportions of methyl linoleate (18:2Δ9,12), methyl sebaleate (18:2Δ5,8), and methyl sapienate (16:1Δ6). On the same subjects, 2 measures of sebum secretion rate were obtained, namely the sustainable wax ester secretion rate (WESR) on the forehead and the ratio of wax esters/(cholesterol + cholesterol esters) [WE/(CH+CE)] in the surface lipid. The proportions of methyl linoleate in FAME from the wax esters decreased, and the proportions of methyl sebaleate increased, with increased rates of sebum secretion. For both methyl linoleate and methyl sebaleate, a better correlation was obtained when the ratio of WE/(CH+CE) was used as a measure of sebum secretion rather than the WESR. The proportions of methyl linoleate in the FAME from the acylceramides were also inversely related to ratios of WE/(CH+CE). In acylceramides, linoleate was replaced by sapienate, a major fatty acid of human sebum. It appears, therefore, that sebum fatty acid composition may change with changes in sebaceous gland activity, and that sebum fatty acids can enter the epidermis and be incorporated into epidermal lipids. *J Invest Dermatol 87:733–736, 1986*

The diunsaturated fatty acids of human sebum were first examined by Nicolaides and Ansari [1], using hydrolyzed scalp lipid from a young man. Of the total fatty acids, only 2–3% were diunsaturated and of this fraction less than 20% was linoleic acid. The remainder consisted of previously unknown fatty acids, the major one being 18:2Δ5,8. This fatty acid was named sebaleic acid because of its occurrence in sebum.

Sebaleic acid apparently is synthesized by a sebaceous Δ6-desaturase acting on 16:0 to produce 16:1Δ6, followed by chain extension to 18 carbons and a second desaturation at the 5,6 position by a different enzyme. The intermediate, 16:1Δ6, is a major fatty acid of human sebum [2], but lacks a trivial name. Since it is characteristic of human sebum we have named it sapienic acid.

Involvement of diunsaturated fatty acids in the pathogenesis of acne has been suspected for some time. In 1972, Kellum and Strangfeld [3] reported that the sebum triglycerides of acne patients contained more of an unidentified 18-carbon fatty acid than was found in control subjects. Shortly thereafter, Krakow et al [4] showed that the fatty acid was sebaleic acid. In 1976 Morello et al [5] reported that individuals with acne had reduced proportions of linoleic acid in their sebum compared with those of normal controls. Together, these reports suggest a reciprocal relationship between linoleic and sebaleic acid concentrations in sebum.

Recently it has been recognized that sebum fatty acid composition is not fixed but changes in relation to sebum secretion rates. Specifically, with increasing sebum secretion, changes appear to occur both in the lipid class composition (more wax esters compared with cholesterol esters [6]), and in the fatty acid composition of the ester lipids (more Δ6 compared with Δ9 fatty acids [7]).

The most reasonable interpretation of these changes seems to be that undifferentiated sebaceous cells in the germinative layer of the gland lack characteristically sebaceous lipids, such as wax esters and Δ6-fatty acids. Instead, the undifferentiated cells synthesize, or obtain from the circulation, cholesterol and Δ9-fatty acids. When differentiation begins, the pathway leading to cholesterol becomes blocked at the step subsequent to squalene [2,8], and a Δ6-desaturase becomes predominant in unsaturated fatty acid biosynthesis [2]. As differentiation proceeds, typically sebaceous lipids would dilute the cells' original endowment of cholesterol and Δ9-type fatty acids. The final degree of dilution in secreted sebum would depend on the average amount of sebum synthesized per sebaceous cell.

If the above mechanism is correct, it would be expected to hold for the relative proportions of linoleic and sebaleic acid in sebum.

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Abbreviations:

FAME: fatty acid methyl esters

WE/(CH+CE): ratio of wax esters/(cholesterol + cholesterol esters)

WESR: wax ester secretion rate

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Presumably sebaceous cells can obtain linoleic (an essential fatty acid) acid only while they are in the germinative layer exposed to the circulation. As the cells enlarge during differentiation, they may need additional diunsaturated fatty acids for membrane synthesis and presumably synthesize sebaleic acid as a substitute for unavailable linoleic acid. Details of this scenario have been presented by Downing et al [9].

These authors [9] also suggest that the composition of sebum can affect the composition of the sphingolipids of the follicular epithelium. Specifically, the presence of linoleic acid-deficient sebum in the follicle may cause substitution of sebaceous fatty acids for linoleic acid in the normally linoleate-rich acylceramides. These altered acylceramides might then have impaired function, resulting in a hyperkeratotic follicular epithelium with an incompetent water barrier and possibly other functional defects favorable to the formation of an acne lesion. Such a mechanism might explain the pathogenic significance of low levels of linoleic acid in the sebum of individuals with acne.

In the present study, the feasibility of these hypotheses was examined by observing the effect of sebum secretion rates on the fatty acid composition of sebaceous wax esters and epidermal acylceramides in scalp lipid. The subjects included prepuberal children in the age range when sebum secretion rates begin to rise from the negligible levels of childhood, and young adult subjects whose sebum secretion rates covered a higher range of values.

METHODS

Collection of Scalp Lipid The subjects were 31 children, ages 6–9, and 20 adults, ages 18–23, who were recruited through newspaper advertisements. Informed consent was obtained from the subjects and from a parent in the case of children. The children were asked to refrain from washing their hair for 5 days and the adults for 1 day preceding the collection. Lipids were then obtained from the subjects' hair and scalp by extraction with 95% ethanol [10].

Estimation of Sebum Secretion Rates Sustainable wax ester secretion rates (WESR) were measured on the forehead as previously described [6,11]. Briefly, the method involves a 14-h depletion of accumulated sebum by applications of a gel of bentonite clay to the forehead. Following this depletion step, sebum is collected for 3 h into a fresh application of bentonite gel. Lipid is then extracted from the recovered bentonite and the amount of wax esters is estimated by quantitative thin-layer chromatography.

As a measure of sebum secretion on the scalp, the lipid class composition of the scalp lipid samples was analyzed by thin-layer chromatography [12,13] and the ratio of wax esters/(cholesterol + cholesterol esters) [WE/(CH+CE)] was calculated.

Isolation of Wax Esters and Acylceramides Scalp lipid (50 mg) was applied to 20 × 20 cm thin-layer plates coated with a 1 mm-thick layer of silica gel G. The chromatograms were developed successively in hexane and in toluene, and then sprayed with an ethanolic solution of 8-hydroxyl-1,3,6-pyrenetrisulfonic acid trisodium salt (100 mg/liter).

After being allowed to dry, the chromatograms were inspected under UV light and the position of the band containing wax esters and cholesterol esters was marked. This band was scraped from the plate and the lipid was eluted from the silica gel with ether. A band at the origin containing polar lipids was also scraped from the plate and the lipids were eluted with chloroform:methanol:water, 50:50:1. Wax esters were separated from cholesterol esters by chromatography on $Mg(OH)_2$ as described by Green et al [10]. Acylceramides were separated from the other polar lipids by chromatography on 20 × 20 cm plates coated with a 0.5 mm-thick layer of silica gel H; the chromatograms were developed with chloroform:methanol:acetic acid, 190:9:1 [14]. After detection with the fluorescent indicator as above, the band containing the acylceramides was scraped from the plate, and the lipid was eluted from the silica gel with chloroform:methanol, 2:1.

Preparation and Analysis of Fatty Acid Methyl Esters (FAME) Hydrolysis of the wax esters, and of the ester linkages in the acylceramides, was effected by treatment for 1 h at 70°C with 1 N KOH in 95% methanol. The liberated fatty acids were then converted to FAME by addition of an excess of BCl_3-methanol to the reaction mixture and heating to 70°C for an additional 60 min. After cooling, water and either chloroform or hexane were added and the FAME were recovered in the solvent layer. The FAME were then separated from other products by preparative thin-layer chromatography.

The FAME were analyzed by gas-liquid chromatography on a 50 m × 0.2 mm vitreous quartz column wall coated with OV101 (Scientific Glass Engineering, Inc., Austin, Texas) at 160°C. Peak areas and retention times were recorded electronically. Equivalent chain lengths were calculated from retention times according to Green et al [10]. Sebaleate and linoleate had equivalent chain lengths of 17.44 and 17.55, respectively. This was established by cochromatography with authentic methyl linoleate and by analysis of FAME after separation according to degree of unsaturation by thin-layer chromatography on silica gel H containing 10% $AgNO_3$.

Determination of Double Bond Positions Since positional isomers of 16-carbon monounsaturated FAME were not distinguishable by capillary gas chromatography, double bond positions were determined by an oxidative method. For this, FAME from all of the acylceramide samples were pooled and the monounsaturated fraction was isolated by chromatography on silica gel H containing 10% $AgNO_3$, using 2 developments with toluene. The 16-carbon FAME were separated from the total monounsaturated FAME by high-performance liquid chromatography on a μBondapak-C_{18} column (Waters Associates, Inc., Milford, Massachusetts) using methanol as the eluting solvent and UV detection of the peaks. Double bond positions in the 16:1 FAME were then determined by the oxidative method of Downing and Greene [15], except that a 50 m × 0.2 mm BP10 bonded phase vitreous silica capillary column (Scientific Glass Engineering, Inc., Austin Texas) was used to analyze the products.

RESULTS AND DISCUSSION

Sebum Secretion Rates Figure 1 shows the relation between WESR measured on the forehead and WE/(CH+CE) in the scalp lipid. There was a fairly linear relationship in most of the children, but not in the adults. The lack of relationship between the 2 measures in adults may result, in part, from differences in sebum secretion rates between forehead and scalp. However, it is also

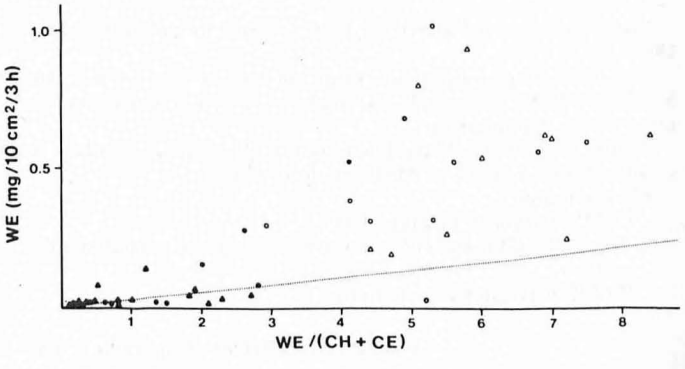

Figure 1. Wax ester secretion rate on the forehead as a function of WE/(CH+CE) in scalp lipid. *Solid symbols* are values for children and *open symbols* are values for adults. *Circles* represent female subjects and *triangles* male subjects. The *dotted line* represents the best fit obtained in a previous study of children aged 6–8.

possible that WESR and WE/(CH + CE) measure different things. The WESR should depend on the percentage of wax esters in sebum (about 25% in adult sebum [16]), on the amount of sebum secreted per follicle, and on the density of follicles per unit area of skin. The WE/(CH + CE), on the other hand, may be a measure of endogenous lipid synthesis per cell since it is a ratio of an endogenously synthesized lipid class (wax esters) to a lipid (cholesterol) which probably is acquired in limited quantity before differentiation begins (see above).

Linoleate and Sebaleate in Wax Esters Figure 2 shows the percentages of linoleate and sebaleate in FAME prepared from wax esters as functions of WE/(CH + CE). Linoleate was inversely related to WE/(CH + CE) while sebaleate was directly related to WE/(CH + CE). Correlation coefficients (r) were 0.824 for linoleate and 0.904 for sebaleate. When percentages of linoleate and sebaleate were plotted vs WESR (not shown), the r values were 0.467 for linoleate and 0.731 for sebaleate.

The results represented in Fig 2*a* support the hypothesis that the proportions of linoleate in sebum are inversely related to the amount of sebum synthesis per cell [9]. The shape of the curve suggests a dilutional mechanism whereby the percent linoleate is reduced by approximately one-half for every doubling of WE/(CH + CE). The relationship is much more evident in the children's portion of the curve, which covers about 15 doublings of WE/(CH + CE), compared with the adult portion, which covers only 1 doubling.

It appears from Fig 2*b* that sebaceous glands synthesize sebaleic acid as needed to maintain a supply of 18-carbon diunsaturated fatty acids, which are used in the synthesis of sebaceous cell membrane phospholipids. In an earlier study [17], it was found that phosphatidylcholine from adult sebaceous glands contained 5.5% sebaleate and only 3.1% linoleate. Before sebaceous cells disintegrate to release sebum, the phospholipids are broken down and the fatty acids released are presumably incorporated into sebum ester lipids (wax esters, triglycerides, and cholesterol esters).

Linoleate in Acylceramides Figure 3*a* shows that the percentages of linoleate in acylceramides were inversely related to WE/(CH + CE). No sebaleate was found in the acylceramides. Linoleate was replaced instead with a 16-carbon monounsaturated fatty acid which was found to be entirely sapienate (16:1Δ6) when its double bond position was analyzed (Fig 3*b*). The results indicate that sebaceous fatty acids can be incorporated into epidermal acylceramides in scalp skin.

It seems likely that sebaceous fatty acids would be incorporated into acylceramides during their synthesis in the living layers of the epidermis, rather than on the skin surface. This is because linoleate in acylceramides is esterified to the hydroxyl group of a long-chain omega-hydroxyacid [18]. This part of the molecule resembles a wax ester, a type of lipid that is not affected by skin surface hydrolases. Moreover, no ceramides with an unesterified omega-hydroxyl group have been found in skin.

Acylceramides are a component of the membranous extracellular matrix in the stratum corneum that constitutes the epidermal water barrier [19]. It seems that the acylceramides must be rich in linoleate to perform their function well. In essential fatty acid-

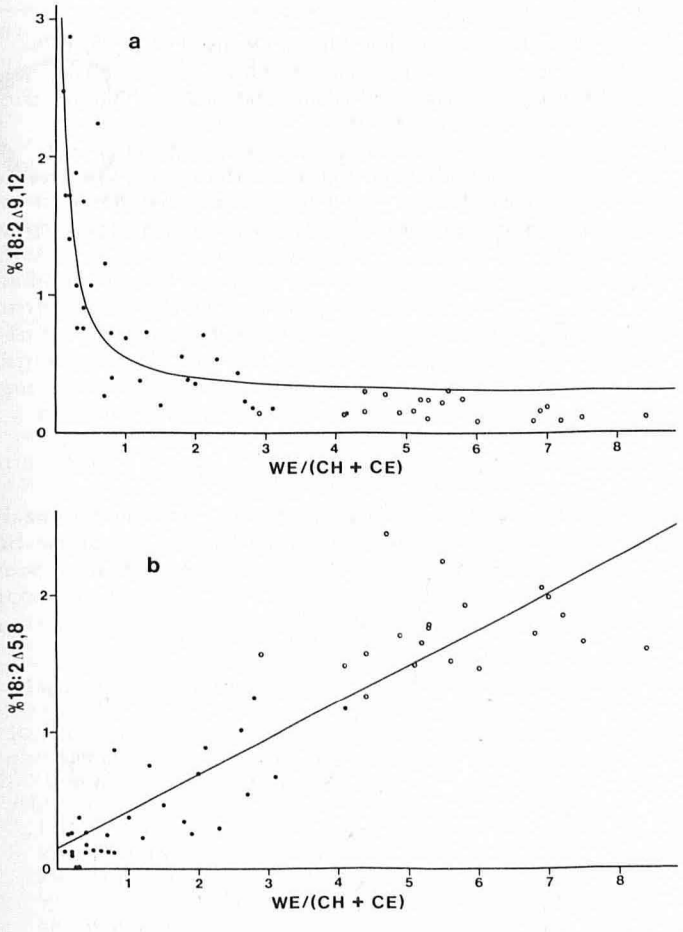

Figure 2. Percentages of (*a*) linoleate (18:2Δ9,12) and (*b*) sebaleate (18:Δ5,8) in FAME from scalp lipid wax esters as a function of WE/(CH + CE) in the scalp lipid. *Solid symbols* represent children and *open symbols* adults. The curve for linoleate vs WE/(CH + CE) was derived by fitting the data to the equation y = a + b/x. The resulting values for the constants were a = 0.25 and b = 0.28. The corrected r value was 0.824 ($p < 0.001$). The line of best fit for sebaleate vs WE/(CH + CE) had a slope of 0.27 and a y-intercept of 0.14. The r value was 0.904 ($p < 0.001$).

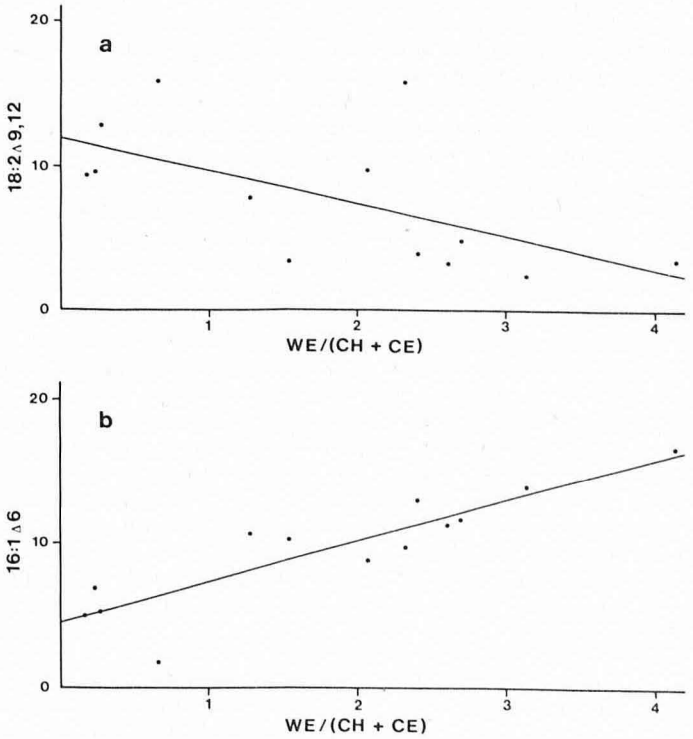

Figure 3. Percentages of (*a*) linoleate (18:2Δ9,12) and (*b*) sapienate (16:1Δ6) in FAME from scalp lipid acylceramides recovered from 13 children. The line of best fit for linoleate vs WE/(CH + CE) had a slope of −2.28 and a y-intercept of 11.98. The r value was −0.597 ($p < 0.05$). For sapienate vs WE/(CH + CE), the slope was 2.89, the y-intercept was 4.33, and the r value was 0.890 ($p < 0.01$).

deficient rats, for example, transepidermal water loss is greatly increased, and it has also been shown that the deficient animals produce acylceramides in which linoleate is largely replaced by oleate (18:1Δ9) [20].

Linoleate and Acne A connection between acne and high rates of sebum secretion is well recognized [21,22]. The results of the present study support the hypothesis of Downing et al [9] that, in anatomic areas where sebaceous glands produce large amounts of sebum, the skin may suffer from a localized essential fatty acid deficiency caused by low proportions of linoleate in the sebum. The follicular epithelium may be particularly vulnerable, and such a localized essential fatty acid deficiency could be responsible for the hyperkeratinization of the follicular epithelium that is seen in acne.

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