Acta Medica et Biologica
Vol. 38, No. 4, 159-166, 1990

The Characterization by Age of Human Sebaceous Gland Activity

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Received June 8, 1990

Skin surface lipids (SSLs) originate from two sources: most are sebum from sebaceous glands, and the others arise from horny cells of the epidermis (Fig. 1). Since the constituents of the sebum are different from those of the epidermal lipids, one can easily suspect that these lipid classes have different respective roles. In this article, we would like to present the present knowledge regarding characteristic lipid compositions of sebum in normal human individuals.

Measurements and lipid markers of sebaceous gland activity

There are many methods for measuring sebaceous gland activities, most of which involve collections of sebum accumulated on the skin surface for a specified time.[1] In these methods, skin of the forehead is usually utilized, and the accumulated sebum is absorbed into cigarette papers for 3 h. The sebum secretion rate is expressed by the weight of the extracted and dried sebum.[1] Using the adsorption method, we found that the amount of the accumulated sebum gradually increases up to the 20s and is maintained at a high level even after maturity in males, whereas it also increases, reaches a maximum level in the teens, but then decreases with aging in females (Table 1).[3] However, such age-related changes of the sebum weight did not correlate with urinary androgen levels that have been reported to

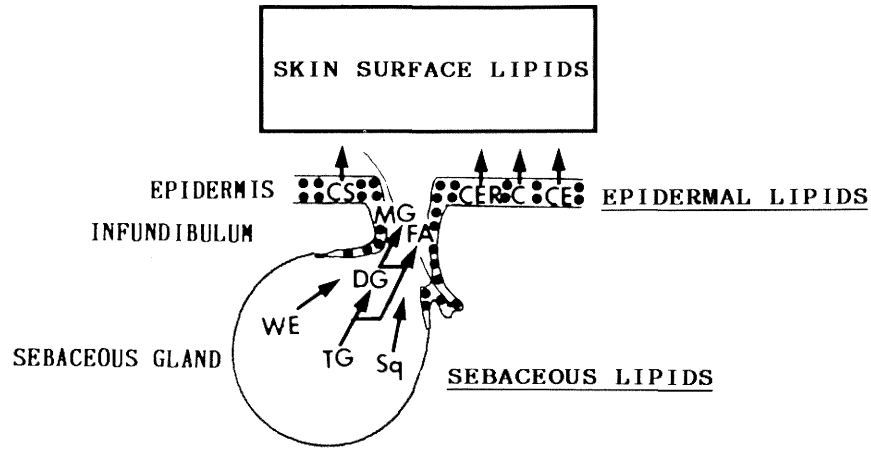

Fig. 1. A diagrammatic representation of skin surface lipids which derive from both sebaceous glands and stratum corneum cells of the epidermis. C, cholesterol; CE, cholesterol esters; CER, ceramides; CS, cholesterol sulfate; DG, diacylglycerol; FA, free fatty acids; MG, monoacylglycerol; Sq, squalene; TG, triacylglycerol; WE, wax esters.

Table 1. Comparison among Different Age Groups of the Amounts of Sebum Accumulated on an Absorbent Paper

Age (years)	Males		Females	
	n	Mean[a] ± SE	n	Mean ± SE
0-9	5	0.55±0.20	4	0.19±0.05
10-19	4	0.98±0.28	10	2.22±0.40
20-29	7	2.37±0.49	3	1.98±0.34
30-49	8	2.99±0.32	5	1.28±0.33
Over 50	4	2.64±0.86	5	0.58±0.19

[a]Milligrams of lipid per 10 cm^2 of skin per 3 h.

Table 2. Sebum Composition in Young Adults aged 20-25

Lipid fraction	Lipid weight (% ± SE)	
	Males (n=5)	Females (n=4)
Wax esters	45.3±12.5	35.9± 9.1
Triacylglycerol	27.8± 4.9	42.4± 7.0
Squalene	16.3± 3.8	11.8± 1.2
Free fatty acids	5.8± 3.0	5.1± 2.4
Cholesterol esters	3.8± 2.2	3.3± 0.3
Cholesterol	0.9± 0.7	0.7± 0.2

show a gradual decreasing tendency during the 30s and the 40s.[4,5] Downing et al.,[2] have argued in this view that the amount of sebum collected by absorbent paper does not represent a true sebum secretion rate, since the collected lipids may contain some extra sebum retained in follicular reservoirs. To avoid contamination from such follicular reservoirs, they have presented the following method to measure the true secretion rate.[2] This method includes depleting reservoirs preliminarily by prolonged absorption into bentonite clay and then followed by measuring the weight of absorbed sebum into freshly prepared bentonite clay for 3 h. The bentonite clay method, however, takes at least 14 h for preliminary absorption. Therefore, a simpler and more specific method to estimate sebaceous gland activity is desirable for routine examinations.

We have tried to find a marker lipid specific for sebaceous gland activity. As shown in Table 2, wax esters, triacylglycerol and squalene are the major components of sebum by the absorption method in young human adults aged 20-25. These lipid classes are considered to originate purely from the sebaceous gland. In contrast, free cholesterol in the sebum is considered to be mainly epidermal in origin, while cholesterol esters may derive from both the epidermis and sebaceous glands.[6] Cholesterol esters may be released into the sebum from membranes broken down of their dying cells, since sebaceous glands are a kind of holocrine gland.[6] A minor component of free fatty acid may come from the hydrolysis of triacylglycerol by lipases in hair canals and on the skin surface.[8] The major lipid classes of wax esters and triterpenoid squalene ($C_{30}H_{50}$) seem to be stable and resistant to skin surface hydrolysis.[8] In addition to wax esters, squalene is also considered specific marker for the sebaceous gland, since sebaceous glands in humans lack the enzyme to catalyze from squalene to cholesterol.[9] These results led us to expect to develop new methods for an index and markers that could estimate actual sebaceous gland activity in just a short time.

$C_{16:1}$ straight chain fatty acid species in wax esters as an index for sebaceous gland activity

Human sebum has long been known to contain unique fatty acids which have unusual positions of unsaturation and branched chains, though detailed data on these had not been available until recently.[10-13] According to Nicolaides,[12] a double bond is always located at the △6 position in the mono-unsaturated fatty acids of wax esters in adults, whereas the △9 unsaturation pattern is common in the mono-unsaturated fatty acids in circulation. Although the differentiated sebaceous cells produce true sebaceous lipids such as △6-type fatty acid, there are two possibilities concerning the origin of △9-monoene fatty acids: △9-monoene may be derived either from the undifferentiated sebaceous cells by de novo synthesis or by uptake from the circulation.[12] The proportions of the △9-fatty acids in wax esters are estimated to be almost the same as in the vernix caseosa, in the sebum of preadolescents and of seniors, but less than these three in the sebum of adolescents.[14]

As for branched chains, Nicolaides et al.,[12] postulated that biosynthesis of the various chain structures could be initiated with acetate, propionate, isobutyrate, isovalerate, or 2-methylbutyrate to produce, respectively, normal even, normal odd, iso even, iso odd, and anteiso odd chain structures when extended in chain length by the conventional addition of 2-carbon units derived from malonyl-CoA.

Our recent report of sebum samples from 55 healthy individuals aged from 3 months to 86 years revealed that the fatty acid composition of wax esters shows coordinated changes with aging, by fused-silica capillary gas chromatographic analyses.[3]

We observed that C_{16} fatty acids were the main component in both saturated and mono-unsaturated fractions of wax esters in all the individuals examined. Straight chain fatty acids always predominated over the corresponding branched chain fatty acids in all age groups.[3,13-15] As shown in Fig. 2, we newly found that the C_{16} straight chain fatty acid shows no notable change with age in relative amounts of saturated fatty acids, while C_{16} iso-branched fatty acid markedly decreases in proportion from infancy through the 20s, and then shows a gradual increase until senescence. Other saturated fatty acids, however, show no statistically significant difference correlated with aging. Fig. 2 also shows that the percentage of $C_{16:1}$ straight chain component (sapienate) increases from infancy through the 20s, correlating with aging, and then decreases until the 50s. In contrast, the $C_{16:1}$ iso-branched fatty acid follows an entirely reversed course, decreasing from infancy through maturity, with a nadir in the 20s, and then increasing until the 50s. Other monoene $C_{14:1}$ straight, $C_{15:1}$ straight, and $C_{18:1}$ straight components show age-related changes respectively, while other straight and terminally branched chain fatty acids such as $C_{15:1}$ iso, $C_{17:1}$ straight, $C_{17:1}$ iso, $C_{17:1}$ anteiso, and $C_{18:1}$ iso, displayed no statistically significant changes in amount in relation to age.[13] Fig. 3 shows typical examples having significant differences in composition of mono-unsaturated fatty acids among three subjects: a 7, a 28 and a 60-year-old male. The most definite difference is seen in the analysis of $C_{16:1}$ fatty acids, showing obvious age-related changes in both straight and iso-branched chains as described above. Thus, it is now clear that not only the quantities of wax esters but also their unique fatty acid species are useful age-related markers of sebaceous gland activity,[3] though some investigators have reported large differences in the fatty acid compositions of wax esters among individuals.[15-17]

WE/(C+CE) ratio as an index for sebaceous gland activity

Downing and his co-workers[6,18,19] state that the ratio of wax esters/[cholesterol + cholesterol esters] (WE/[C+CE]) in the skin surface lipids may be a

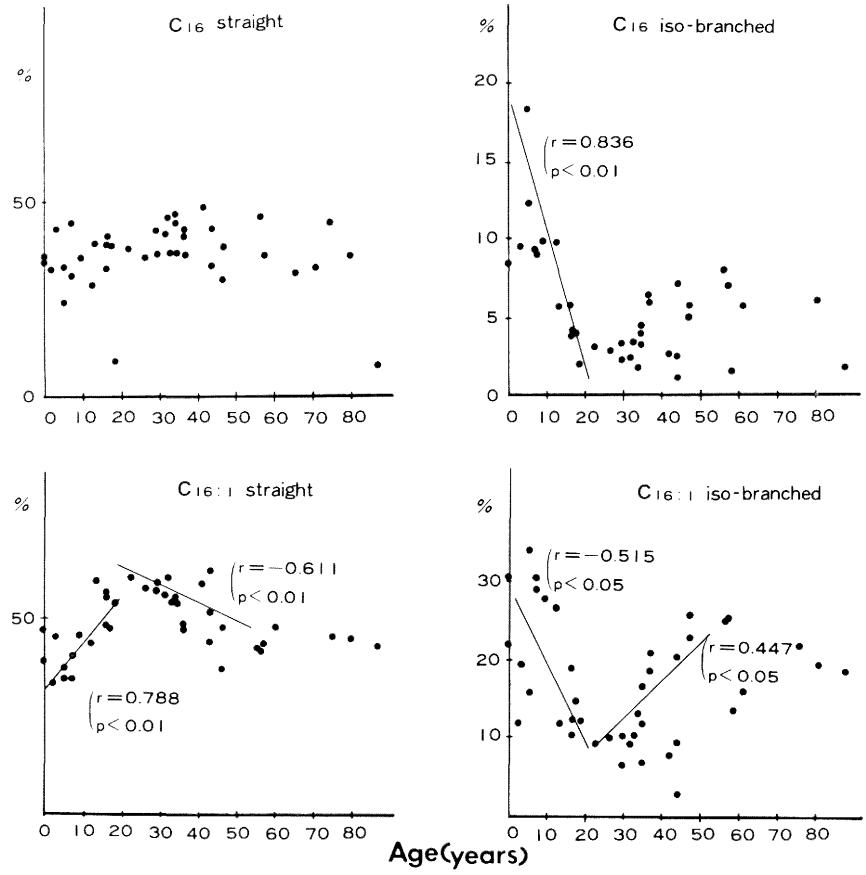

Fig. 2. Composition of C_{16} fatty acid species of sebum wax esters.

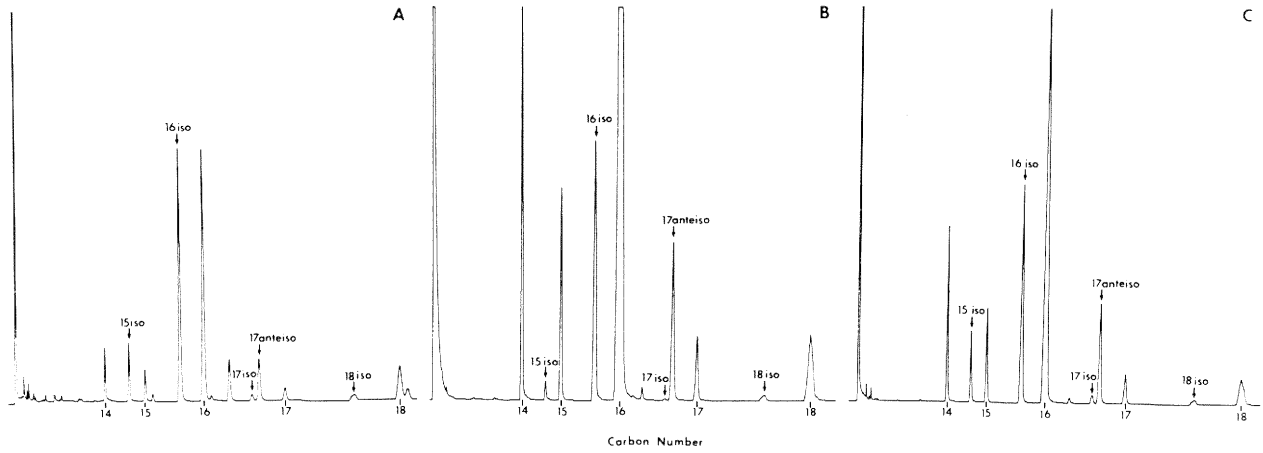

Fig. 3. Fused-silica capillary gas chromatograms of the mono-unsaturated fatty acid methyl esters from the sebum wax esters of 3 subjects, a 7 (A), a 28 (B), and a 60-year-old (C) male.

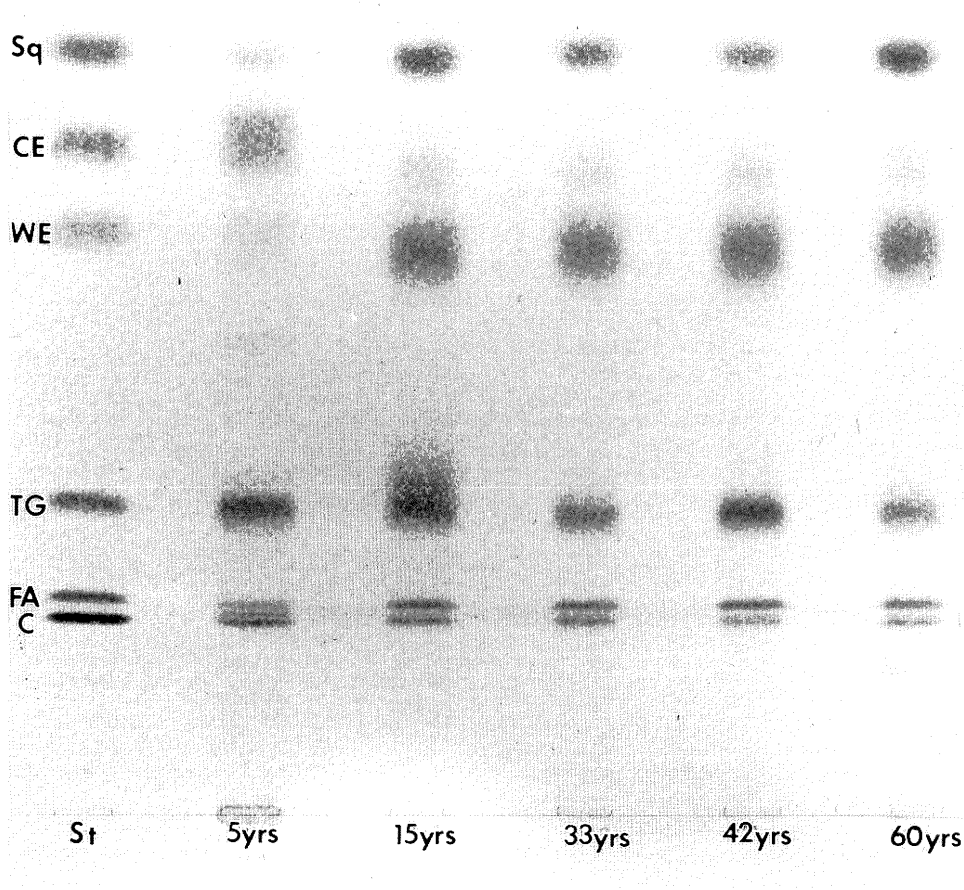

Fig. 4. A typical chromatogram of sebum lipid classes detected on the charred thin-layer plate. The chromatogram was developed with hexane to the top, then benzene to the top, and finally to 10 cm with hexane/ether/acetic acid (70:30:1). Standards: C, cholesterol; CE, cholesterol esters; FA, free fatty acids; Sq, squalene; TG, triacylglycerol; WE, wax esters.

good index for sebaceous gland activity; dying sebaceous cells may supply not only wax esters but also membrane cholesterol, which is subject to esterification, into the sebum. Therefore, it is possible that a high lipid producing activity in sebaceous cells may be reflected as an increase of the WE/[C+CE] ratio by the dilutional effect of wax esters on the proportion of membrane components.[6] We reexamined the ratios of WE/[C+CE] of forehead sebum from healthy individuals using thin-layer chromatography (TLC) (Fig. 4), and found that the ratios changed distinctly in an age-related fashion.[3] As shown in Fig. 5, the curve of the ratios has a peak in the 20s for both males and females. However, there are some discrepancies present when compared with the age-related changes of the WE/[C+CE] ratios (Fig. 5) and those of sebum accumulation by weight (Table 2). In infancy, the amount of accumulated sebum is larger in males, while the WE/[C+CE] ratio is greater in females. During middle age, the amount of sebum in males is maintained at a high level, although the ratio begins to decline after maturity. On the other hand, the WE/[C+CE] ratio correlates positively with the proportion of $C_{16:1}$ straight chain components and negatively with that of $C_{16:1}$ iso-branched chain components.[3] Therefore, not only the high WE/[C+CE] ratio but also the high amount of $C_{16:1}$ straight chain fatty acid that is purely synthesized in the sebaceous cell may reflect the higher rates of sebum secretion, indicating favorable indices for sebaceous gland activity.

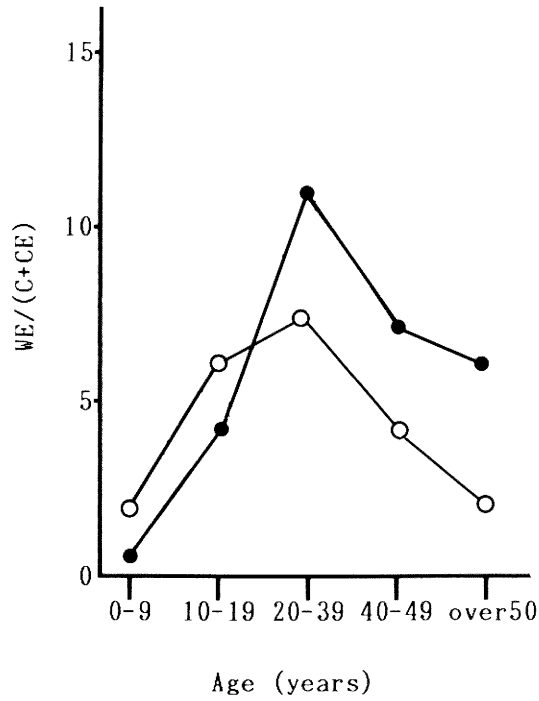

Fig. 5. Age-related changes of the WE/(C+CE) ratio. ○——○, female; ●——●, male.

Correlation between $C_{16:1}$ straight chain index and urinary androgen levels

Sebaceous glands are well known to be under hormonal control. In particular, androgens stimulate sebaceous growth and lipogenesis.[18-21] It is generally accepted that testosterone is an important androgen because of its potentiality to convert to dihydrotestosterone which is considered to be the most active metabolite for stimulating sebum production. Pochi et al.[18] state, however, that sebaceous gland development is evident in prepubertal children, and their sebaceous gland activity increases showing a positive correlation with the increase in urinary excretion levels of 17-ketosteroids (17-KS). These data may suggest an important role for adrenocortical androgens as well as testosterone in controlling sebaceous gland activity.

In a study of sebum and urinary samples from 36 healthy individuals, aged 3 to 59 years, the effects of androgens on the fatty acid compositions of sebum wax esters have been investigated.[22] Fig. 6 shows the concentrations of various straight and terminally branched chain types in the mono-unsaturated fatty acids of wax esters, plotted against the ages of the experimental subjects. The proportions of straight even fatty acids show a curve similar to that of the $C_{16:1}$ straight chain fatty acid, although they are more variable among females than among males. The proportions of iso-even fatty acids follow a reversed course with advancing age, decreasing from infancy through maturity and increasing until the 50s. Straight odd, iso-odd, and anteiso-odd fatty acids do not show any significant change in amount in relation to age. Individual differences among anteiso-odd chain types are quite small, especially in the mono-unsaturated fatty acids (Fig. 6).[22]

Age-related changes in 24-hr urinary excretion of testosterone and total 17-KS are shown in Fig. 7. Urinary testosterone excretion is very low in both males and females under 10 years of age. However, in males it markedly increases in the teens, reaches a peak in the 20s or 30s and is maintained at a high level even during the 40s, while in females it decreases more rapidly than in males after maturity (Fig. 7). All of 17-KS (androsterone, dehydroepian-

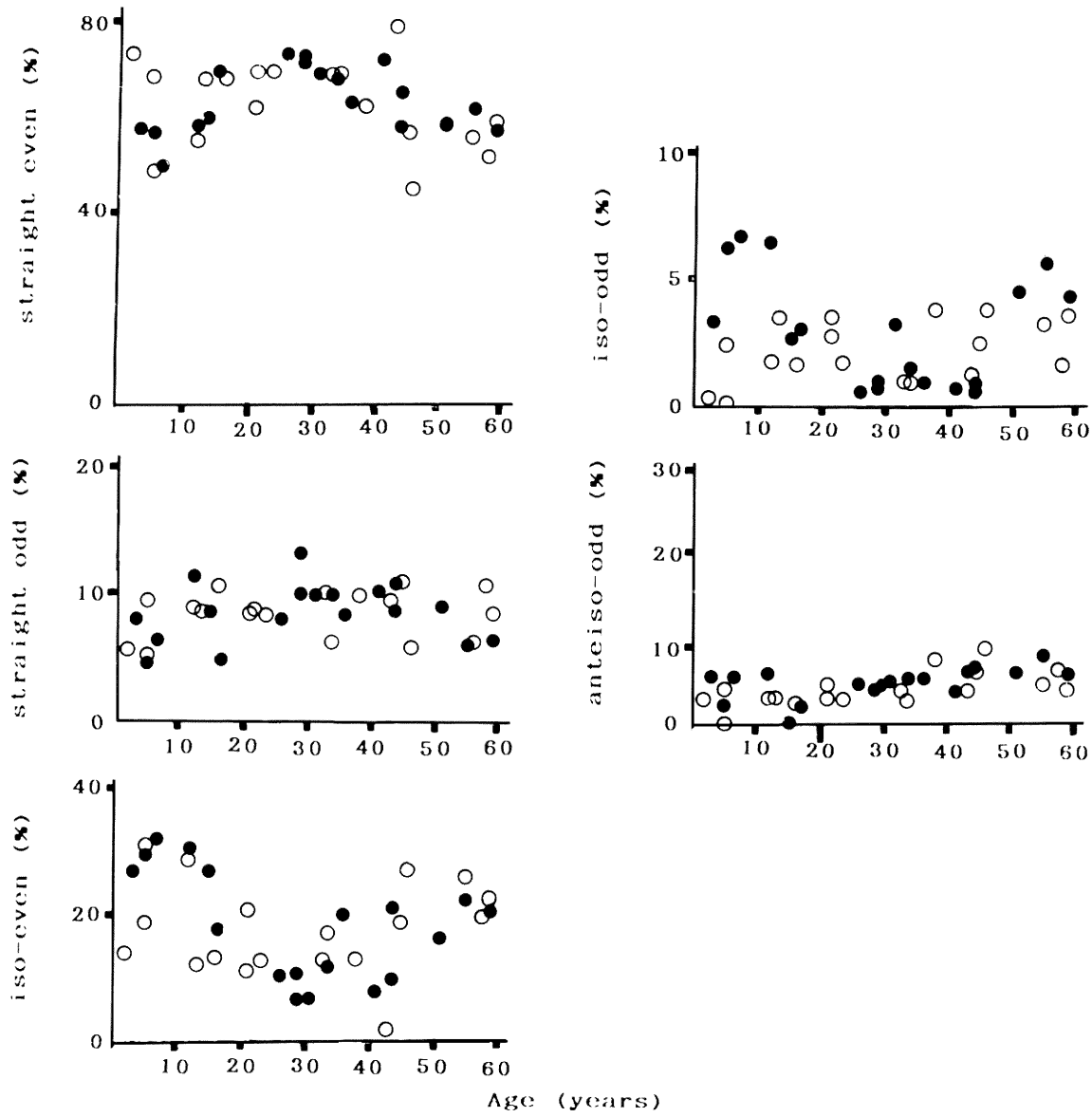

Fig. 6 Variations in the concentrations of various straight and terminally branched fatty acid chain types in mono-unsaturated fatty acids from sebum wax esters of the subjects. ○, female; ●, male.

drosterone, and etiocholanolone) are excreted in larger quantities in girls than in boys under 10 years of age, whereas the excretion peak of each of the 17-KS during maturity is significantly higher in males than in females.[22]

In comparison with the proportions of $C_{16:1}$ straight chain fatty acids in sebum wax esters with urinary androgen levels, $C_{16:1}$ straight chain components show a very fine correlation with testosterone levels in both sexes (Fig. 8) and with the levels of etiocholanolone and total 17-KS in females.[22] In females, the higher sebum secretion rate under 10 years of age (Fig. 5) may be a consequence of elevated levels of 17-KS. It is probable that female sebaceous glands are responsive to 17-KS as well as to testosterone in all age groups.[22] However, there is a large difference in testosterone levels between males and females in spite of the identical proportions of $C_{16:1}$ straight chain fatty acid in both sexes (compare Fig. 7 and Fig. 8). These data suggest that more active sebaceous glands in lipid production excrete sebum with higher proportions of the $C_{16:1}$ straight chain fatty acid, which is considered to be purely endogenous. It appears, therefore, that the proportions of the $C_{16:1}$

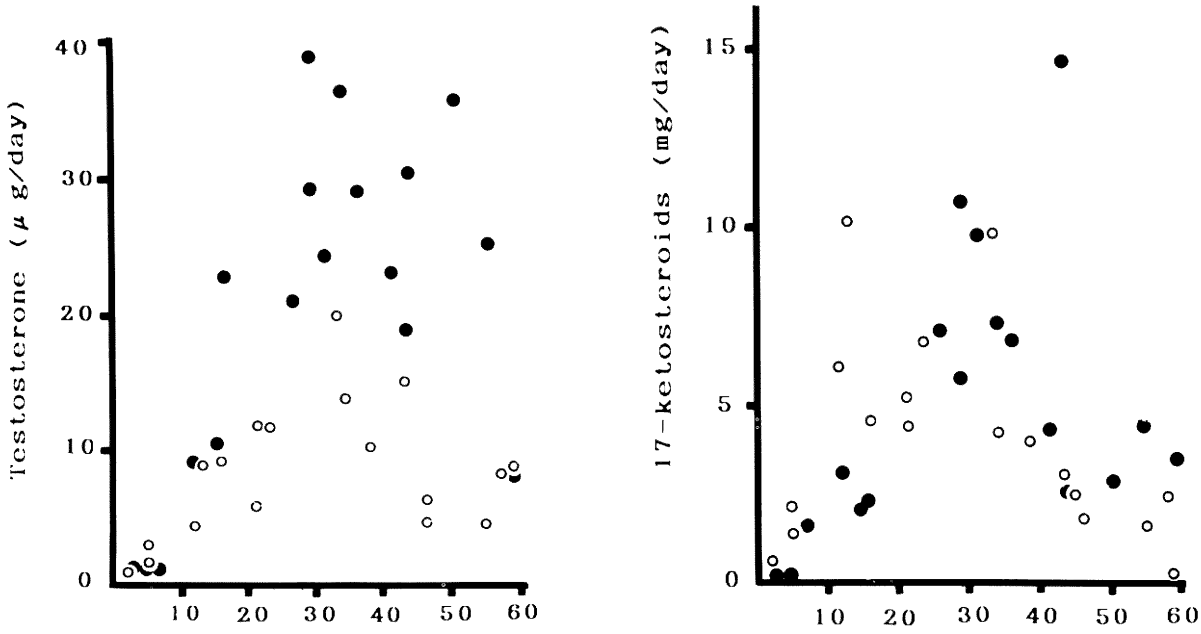

Fig. 7. Variations in 24-hr urinary excretions of testosterone and of 17-ketosteroids with age. ○, female; ●, male.

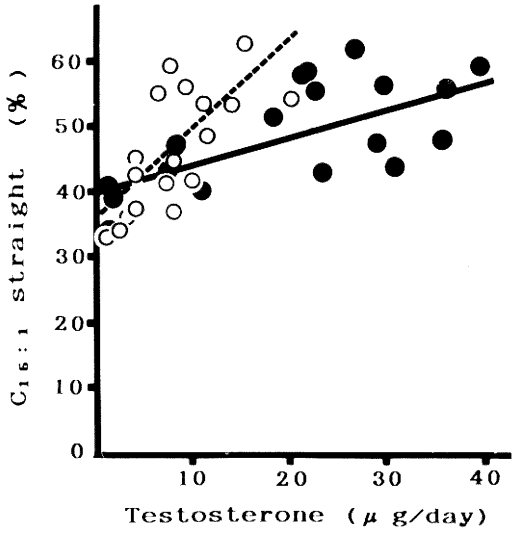

Fig. 8. Correlation between 24-hr urinary testosterone excretion levels and concentrations of $C_{16:1}$ straight chain fatty acid in sebum wax esters. ○, female; ●, male.

straight chain fatty acid in sebum wax esters may indicate the sebaceous gland activity in both sexes. Recently, $C_{16:1}$ have been indicated to play an important role in acne.[23,24] Such reports may be supported by the finding that there are high concentrations of $C_{16:1}$ straight chain fatty acid in maturity.

The straight even fatty acids, including $C_{16:1}$, tend to change in a positive correlation with testosterone levels, in contrast to changes of the iso even fatty acids in both sexes and to those of the iso odd fatty acids in males.[22] The straight odd fatty acids show a similar change to that of the straight even fatty acids in males, while in females there is no significant correlation between the amount of the fatty acids and testosterone levels.[22] Anteiso fatty acids show no notable change correlated with testosterone levels.[22] According to the study concerning age-related changes of the fatty acid compositions of wax esters[3] and the reports by Nicolaides et al.,[10-13] it is believed that active sebaceous glands may have lower concentrations of the terminally branched chain acids than those of the straight chain fatty acids, because of the limited availability for the terminal portions of iso or anteiso fatty acids; namely, the primers of terminally branched chain fatty acids (isobutyrate, isovalerate, and 2-methylbutyrate) are thought to be derived from

the breakdown of cell proteins.[12] However, the subjects in their 20s having high proportions of $C_{16:1}$ straight chain fatty acid show no decrease in levels of anteiso fatty acids (compare Fig. 2 and Fig. 6). Furthermore, it is also difficult to explain the gap in amount between iso even and iso odd fatty acids. Female subjects in their 20s have a relatively high amount of iso odd fatty acids in spite of their low concentrations of iso even fatty acids.[22] These results suggest that there may be another de novo source of terminally branched chain fatty acids in human sebaceous glands.[10,25] On the other hand, Stewart et al.,[16,17] have reported that iso even fatty acids in wax esters are genetically controlled. In this respect the synthesis of iso or anteiso fatty acids may be controlled by complex factors, and the origin and metabolism of these fatty acid types should be elucidated in further studies.[22]

REFERENCES

1) Strauss JS, Pochi PE: The quantitative gravimetric determination of sebum production. *J Invest Dermatol* 36: 293-298, 1961.
2) Dowing DT, Stranieri AM, Strauss JS: The effect of accumulated lipids on measurements of sebum secretion in human skin. *J Invest Dermatol* 79: 226-228, 1982.
3) Yamamoto, A, Serizawa S, Ito M, Sato Y: Effect of aging on sebaceous gland activity and on the fatty acid composition of wax esters. *J Invest Dermatol* 89: 507-512, 1987.
4) Keutmann EH, Mason WB: Individual urinary 17-ketosteroids of healthy persons determined by gas chromatography: biochemical and clinical considerations. *J Clin Endocrinol* 27: 406-420, 1967.
5) Kato, K, Ibayashi H: Aging and Steroid hormons. *Jap J Clin Med* 42: 2494-2502, 1984. (in Japanense)
6) Stewart ME, Downing DT: Measurement of sebum secretion rates in young children. *J Invest Dermatol* 84: 59-61, 1985.
7) Jacobsen E, Billings JK, Frantz RA, Kinney CK, Stewart ME, Downing DT: Age-related changes in sebaceous wax ester secretion rates in men and women. *J Invest Dermatol* 85: 483-485, 1985.
8) Nicolaides N, Foster RC Jr: Esters in human hair fat. *J Amer Oil Chemists Soc* 33: 404-409, 1956.
9) Griesemer RD, Thomas RW: Lipogenesis in human skin. III. Variation with body site. *J Invest Dermatol* 41: 235-238, 1963.
10) Nicolaides N: The structures of the branched fatty acids in the wax esters of vernix caseosa. *Lipids* 6: 901-905, 1971.
11) Nicolaides N, Fu HC, Ansari MNA, Rice GR: The fatty acids of wax esters and sterol esters from vernix caseosa and from human skin surface lipid. *Lipids* 7: 506-517, 1972.
12) Nicolaides N: Skin lipids: Their biochemical uniqueness. *Science* 186: 19-26, 1974.
13) Nicolaides, N, Apon JMB: The saturated methyl branched fatty acids of adult human skin surfacce lipid. *Biomed Mass Spectrom* 4: 337-347, 1977.
14) Nazzaro-Poro M, Passi S, Boniforti L, Belsito F: Effects of aging on fatty acids in skin surface lipids. *J Invest Dermatol* 73: 112-117, 1979.
15) Green SC, Stewart ME, Downing DT: Variation in sebum fatty acid composition among adult humans. *J Invest Dermatol* 83: 114-117, 1984.
16) Stewart ME, Downing DT: Proportions of various straight and branched fatty acid chain types in the sebaceous wax esters of young children. *J Invest Dermatol* 84: 501-503, 1985.
17) Stewart ME, McDonnel MW, Downing DT: Possible genetic control of the proportions of branched-chain fatty acids in human sebaceous wax esters. *J Invest Dermatol* 86: 706-708, 1986.
18) Pochi PE, Strauss JS, Downing DT: Skin surface lipid composition, acne, pubertal development, and urinary excretion of testosterone and 17-ketosteroids in children. *J Invest Dermatol* 69: 485-489, 1977.
19) Pochi PE, Strauss JS, Downing DT: Age-related changes in sebaceous gland activity. *J Invest Dermatol* 73: 108-111, 1979.
20) Ebling FJ: Hormonal control and methods of measuring sebaceous gland. *J Invest Dermatol* 62: 161-171, 1974.
21) Pochi PE, Strauss JS: Endocrinologic control of the development and activity of the human sebaceous gland. *J Invest Dermatol* 62: 191-201, 1974.
22) Yamamoto A, Serizawa S, Ito M, Sato Y: Fatty acid composition of sebum wax esters and urinary androgen level in normal human individuals. *J Dermatol Sci.* (in press)
23) Stewart ME, Grahek MO, Cambier LS, Werts PW, Downing DT:Dilutional effect of increased sebaceous gland activity on the proportion of linoleic acid in sebaceous wax esters and in epidermal acylceramides. *J Invest Dermatol* 87: 733-736, 1986.
24) Melnik B, Plewig G: Neue lipidbiochemische Aspekte in der Pathogenese der follikulären Verhornungsstörung bei Acne vulgaris. *Z Hautkr* 63: 591-596, 1988.
25) Stewart ME, Steele WA, Downing DT: Changes in the relative amounts of endogenous and exogenous fatty acids in sebaceous lipids during early adolescence. *J Invest Dermatol* 92: 371-378, 1989.