2-Nonenal Newly Found in Human Body Odor Tends to Increase with Aging

Shinichiro Haze, Yoko Gozu, Shoji Nakamura, Yoshiyuki Kohno,* Kiyohito Sawano,† Hideaki Ohta,† and Kazuo Yamazaki†

Shiseido Co. Ltd., Product Development Center, Yokohama, Japan; *Shiseido Co. Ltd., Basic Research Center, Yokohama, Japan; †Takasago International Corporation, Central Research Laboratory, Hiratsuka, Japan

Human body odor consists of various kinds of odor components. Here, we have investigated the changes in body odor associated with aging. The body odor of subjects between the ages of 26 and 75 was analyzed by headspace gas chromatography/mass spectrometry. 2-Nonenal, an unsaturated aldehyde with an unpleasant greasy and grassy odor, was detected only in older subjects (40 y or older). Furthermore, analysis of skin surface lipids revealed that ω7 unsaturated fatty acids and lipid peroxides also increased with aging and that there were positive correlations between the amount of 2-nonenal in body odor and the amount of ω7 unsaturated fatty acids or lipid peroxides in skin surface lipids. 2-Nonenal was generated only when ω7 unsaturated fatty acids were degraded by degradation tests in which some main components of skin surface lipids were oxidatively decomposed using lipid peroxides as initiator of an oxidative chain reaction. The results indicate that 2-nonenal is generated by the oxidative degradation of ω7 unsaturated fatty acids, and suggest that 2-nonenal may be involved in the age-related change of body odor. ***Key words: aldehydes/fatty acids/monounsaturated lipid peroxides/sebum. J Invest Dermatol 116:520–524, 2001***

Human body odor consists of various odor components. Studies examining the volatile components by various methods have detected the presence of alcohols, ketones, aldehydes, esters, ethers, hydrocarbons, and other substances in body odor (Ellin *et al*, 1974; Bernier *et al*, 2000). The key components of body odor present in sweat, the axillary region, or foot have been determined and the mechanisms by which such odors are formed have been examined (Kanda *et al*, 1990; Zeng *et al*, 1991).

Hereditary features (gender, nature, diseases), as well as behavioral habits (drinking, smoking, etc.), are known to affect body odor (Labows, 1979; Senol and Fireman, 1999). Although it has often been indicated empirically that there are characteristic body odors associated with different age groups, such as babies, young people, and senior citizens, there has been little investigation of the relation between body odor and aging.

Here we investigated the changes in body odor as a function of age and found a specific component that is characteristic of the body odor of the middle-aged and the elderly. Furthermore, we present evidence that suggests that both the change of the monounsaturated fatty acid composition of skin surface lipids and the increase of lipid peroxides associated with aging may be involved in the formation of this characteristic odor component.

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Reprint requests to: Dr. Shinichiro Haze, Shiseido Co. Ltd., Product Development Center, 2–2-1, Hayabuchi, Tsuzuki-Ku, Yokohama-Shi, 224–8558, Japan. Email: shinichiro.haze@to.shiseido.co.jp

Abbreviations: DMS, dimethyldisulfide; HPO, hydroperoxide; TBA, 2-thiobarbituric acid.

MATERIALS AND METHODS

Subjects The subjects were 22 healthy individuals, 13 of whom were male and nine female, with ages ranging from 26 to 75 y.

Collection of body odors Each subject was given a shirt to wear for the collection of body odor components. The components that adhere to the fabric were analyzed by the headspace gas chromatography/mass spectrometry (GC/MS) method. Before use, shirts were cleaned in the following manner: cotton-shirts were dry-cleaned and washed by sonication twice, for 20 min, in a 50% ethanol solution. The shirts were then rinsed well with water and air-dried at 75°C. Subsequent headspace GC/MS analysis confirmed that no odor components could be detected in the shirts.

Each subject bathed using odorless soap and shampoo and then put the shirt directly on naked skin for three consecutive nights. During the day, the shirt was tightly sealed in a TEDLA bag (10 liter capacity, GL Science, Tokyo, Japan) and stored at room temperature (23°C) in a dark place.

Collection and recovery of headspace components A rectangular piece (20 × 30 cm) was cut from the back of the shirt worn 3 d by the subject and sealed into a TEDLA bag (10 liter capacity). The air inside the bag was then pumped into a TENAX-TA column (GL Science) to collect the headspace odor components. The flow rate of the pump was maintained at 1.8 liters per min while air deodorized with activated carbon was supplied to the bag to keep the capacity at 10 liters. Ten microliters of dimethylanthranilate:ethanol (1:10) solution was applied on the rectangular piece of shirt as a standard calibrator to correct the recovery yield of headspace components. Headspace collection was performed at 23°C for 18 h. After collection, headspace odor components that had adsorbed on the TENAX-TA column were eluted with 10 ml of diethylether.

GC/MS analysis of headspace components A hundred microliters of dihydrojasmone:ethanol (1:2000) solution was added to the eluate as internal standard. The mixture was concentrated to 100 μl under

Table I. Some compounds detected in body odor by GC/MS analysis

Compounds	Detection rate (%)	
	<40 y (n = 9)	⩾40 y (n = 13)
Hydrocarbons		
1-Octene	11	8
Decane	11	23
Undecane	22	23
Dodecane	67	69
Alcohols		
1-Butanol	11	8
1-Hexanol	11	15
2-Ethylhexanol	89	85
Octanol	11	8
1-Decanol	11	15
Amyl alcohol	11	8
Hexadecanol	11	8
Octadecanol	11	8
Acids		
Acetic acid	22	23
Butyric acid	22	15
Ketones		
4-Methyl-2-pentanone	11	8
6-Methyl-5-heptenone	89	77
Aldehydes		
Hexanal	33	23
Heptanal	11	15
Octanal	89	85
Nonanal	89	85
Decanal	89	69
2-Nonenal	0	69

nitrogen gas, and then used as a sample for the GC/MS analysis. The GC/MS analysis was carried out on a G1800A GCD system (Hewlett-Packard, Palo Alto, CA) as follows: column, HP-INNOWAX capillary column (60 m × 0.25 mm × 0.25 μm film thickness, Hewlett-Packard); temperature, 5°C per min (60°C→230°C), 230°C for 30 min; carrier gas, helium 1 ml per min; ionization voltage, 70 eV. Each peak that was detected in the GC/MS analysis was searched and identified using the WILEY 275 mass spectral database and search program software (Hewlett-Packard). 2-Nonenal was quantified using calibration curves that were derived using *trans*-2-nonenal (Wako, Osaka, Japan), dimethylanthranilate, and dihydrojasmone (Wako) as authentic standards. 2-Nonenal per unit area (ng per cm^2) was calculated and then corrected by the amount of skin surface lipids (mg per cm^2) to derive the amount of 2-nonenal (ng per mg skin surface lipids).

Collection of skin surface lipids A piece of cotton gauze (10 × 15 cm) delipidated with hexane was sewn inside the rear of the shirt in order to collect the skin surface lipids. The gauze was recovered from the shirt worn for three nights, and lipids that adhered to it were extracted with hexane. The extract was dehydrated with anhydrous magnesium sulfate, concentrated by evaporation, and dried under nitrogen gas. The yield of the resulting lipids was measured and the amount of skin surface lipids per unit area (mg per cm^2) was calculated.

Determination of TBA reactive substances in skin surface lipids The amount of the substances that can react with 2-thiobarbituric acid (TBA) in skin surface lipids was determined by colorimetry and this TBA value was used as an index of the amount of lipid peroxides in skin surface lipids (Ohkawa *et al*, 1978; 1979). Three milliliters of TBA solution (0.12 g of TBA and 0.5 ml of triethylamine dissolved in 100 ml of 99.7% acetic acid) was added to 2 mg of lipids and the sample was boiled at 95°C for 1 h. After cooling, the absorbance of the sample at 532 nm was measured. For the calibration, fresh palmitoleic acid (Tokyo Kasei, Tokyo, Japan) and oxidized palmitoleic acid were used as the standard for the 0% and 100% TBA values, respectively. The oxidized palmitoleic acid was prepared from fresh palmitoleic acid by auto-oxidation at 37°C for 14 d and was stored at –80°C until just before use.

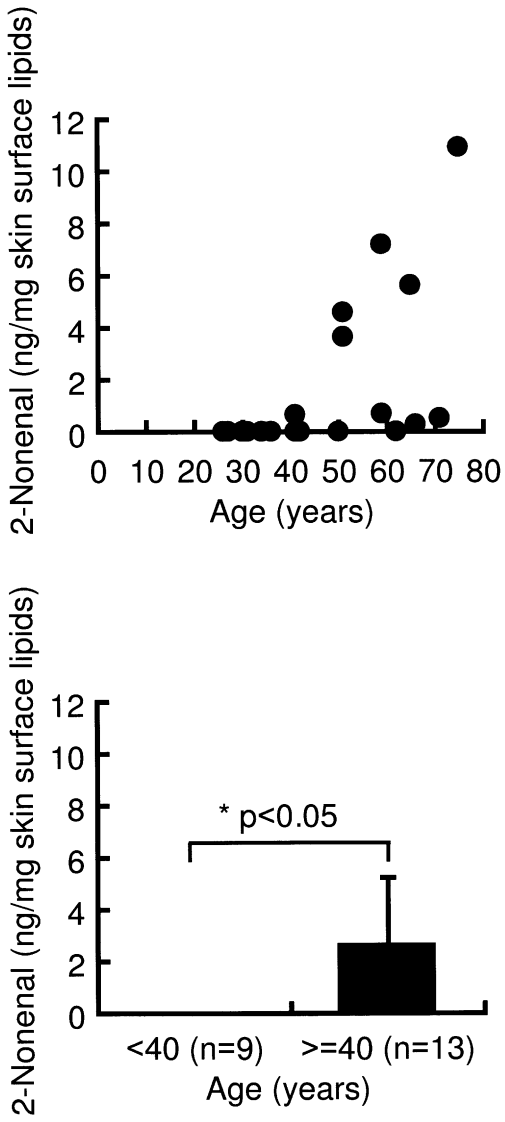

Figure 1. Effect of aging on the amount of 2-nonenal in body odor.

Analysis of the fatty acid composition of skin surface lipids Lipids were heated in 5% hydrogen chloride methanol solution (Wako) for 2 h at 95°C to perform methanolysis of esters and methyl-esterification of free fatty acids in the lipids. After the reaction, fatty acid methyl esters were extracted with hexane and the extract was dehydrated with anhydrous magnesium sulfate, dried under nitrogen gas, and then used for GC/MS analysis. GC/MS analysis was performed with a G1800A GCD system as follows: column, HP-INNOWAX capillary column (60 m × 0.25 mm × 0.25 μm film thickness); temperature, 15°C per min (150°C→200°C), 7°C per min (200°C→250°C), 250°C for 30 min; carrier gas, helium 1 ml per min; ionization voltage, 70 eV. Each peak that was detected in GC/MS analysis was searched and identified using the WILEY 275 mass spectral database and search program software.

Determination of the double bond position in monounsaturated fatty acids of the skin surface lipids Double bonds of monounsaturated fatty acids were cleaved and modified with methyl sulfide groups by the dimethyldisulfide (DMS) method (Francis, 1981). The double bond position was determined from the mass spectral pattern of fragment ions that were formed from each modified compound by GC/MS. 0.5 ml of iodine/DMS solution (13 mg of iodine in 1 ml of DMS) was added to 10 mg of lipids and the mixture was kept at 35°C for 30 min to promote the modification. After incubation, 30% sodium hydrogen sulfate solution was added to the sample until the color of

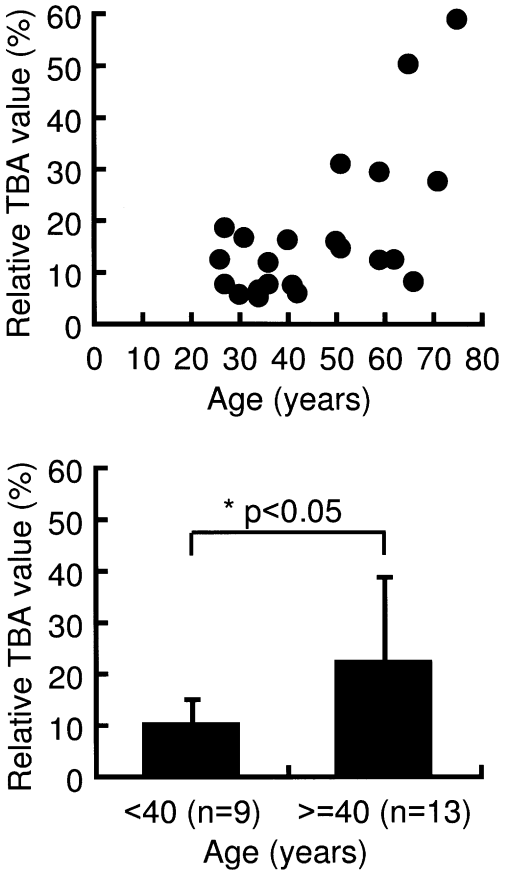

Figure 2. Effect of aging on the amount of lipid peroxides in skin surface lipids.

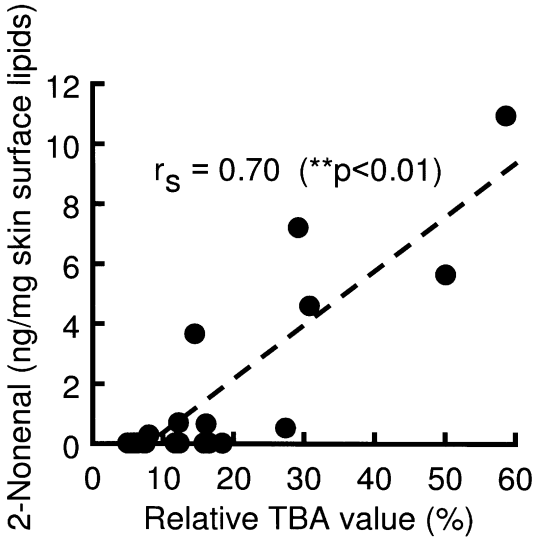

Figure 3. The relationship between the amount of 2-nonenal in body odor and the amount of lipid peroxides in skin surface lipids.

iodine faded away. The sample was extracted with 1 ml of hexane:ether (1:1) and the extract was used for GC/MS analysis.

Demonstration of 2-nonenal formation from the components of skin surface lipids by oxidative degradation Several main components of the skin surface lipids were degraded by oxidation using squalene hydroperoxide (squalene-HPO) as an initiator. The odor components that were formed were subsequently examined by GC/MS analysis and organoleptic evaluation. One milligram of each of cholesterol (Nakarai, Kyoto, Japan), squalene (Nakarai), palmitoleic acid (Tokyo Kasei), oleic acid (Sigma-Aldrich, St. Louis, MO), vaccenic acid (Sigma-Aldrich), or linoleic acid (Sigma-Aldrich) was mixed with 0.1 mg of squalene-HPO separately, and the mixture was incubated to 37°C for 3 d. Squalene-HPO was prepared from squalene by ultraviolet irradiation using methylene blue as the sensitizer, followed by high performance liquid chromatography purification (Kohno *et al*, 1993). The structure of the purified squalene-HPO was confirmed by nuclear magnetic resonance analysis. After the incubation period, the odor components of each sample were checked by GC/MS analysis and organoleptic evaluation.

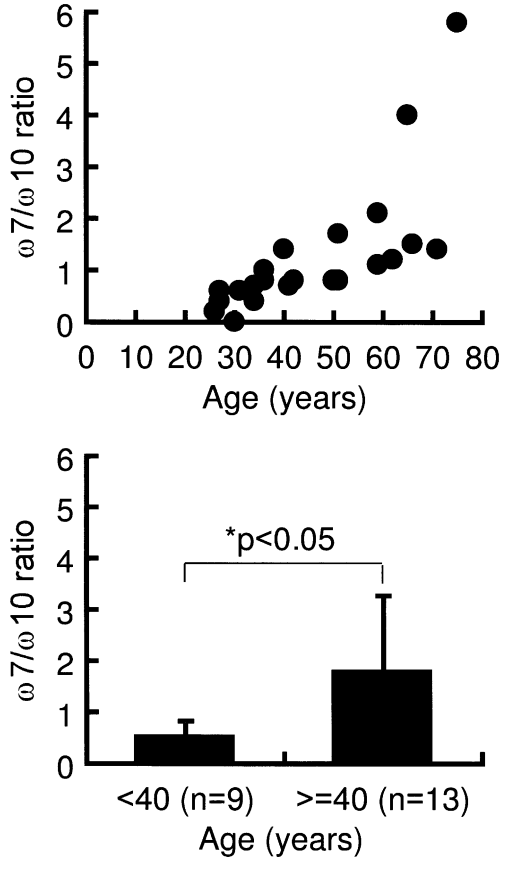

Figure 4. Effect of aging on the quantitative ratio of ω7 monounsaturated fatty acids to ω10 monounsaturated fatty acids.

Statistics The level of significance of the difference was calculated by Student's *t* test and the level of significance of the correlation was calculated by Spearman's correlation coefficient test.

RESULTS

Increase of 2-nonenal in body odor is associated with aging **Table I** indicates some of the body odor components that were detected in subjects by headspace GC/MS analysis. 2-Nonenal was frequently encountered in subjects aged 40 or older (middle-aged and the elderly group), but not in subjects aged under 40 (younger group). In contrast, there was little change in the detection frequency of the other odor components with aging. So we focused attention on 2-nonenal and examined the quantitative change of 2-nonenal in body odor with aging. The result is shown in **Fig 1**. The amount of 2-nonenal showed a tendency to increase with aging, especially in subjects over the age of 40. There was a significant difference in the amount of 2-nonenal between the two subject groups of below or above 40 y old.

Increase of lipid peroxides in skin surface lipids as a function of aging The amount of lipid peroxides in skin surface lipids, measured by the TBA method, also showed a tendency to increase with aging. As shown in **Fig 2**, there was a significant difference in the amount of lipid peroxides between the two subject groups of below or above 40 y old. Furthermore, a positive correlation was observed between the amount of 2-nonenal in body odor and the amount of lipid peroxides in skin surface lipids (**Fig 3**).

Change in monounsaturated fatty acid composition of skin surface lipids associated with aging Members of the ω10 monounsaturated fatty acid family, such as C16:1ω10 (sapienic acid) and C18:1ω10, were found to be the main monounsaturated fatty acid components of the skin surface lipids, in both the young and the middle-aged groups. The amount of such fatty acids changed very little with aging.

In contrast, fatty acids belonging to the ω7 unsaturated fatty acid family, such as C16:1ω7 (palmitoleic acid) and C18:1ω7 (vaccenic acid), were detected in the skin surface lipids of only the middle-aged and/or elderly subjects. Consequently, the quantitative ratio of fatty acids of the ω7 family to ω10 family showed a tendency to increase with aging. The amount of fatty acids belonging to the ω7 family and to the ω10 family was calculated as the sum of C16:1ω7 and C18:1ω7 and the sum of C16:1ω10 and C18:1ω10, respectively. Significant differences in the quantitative ratio of ω7 family to ω10 family were observed between the two subject groups of below or above 40 y old (**Fig 4**). Moreover, there was a positive correlation between the amount of 2-nonenal in body odor and the quantitative ratio of ω7 family to ω10 family in skin surface lipids (**Fig 5**).

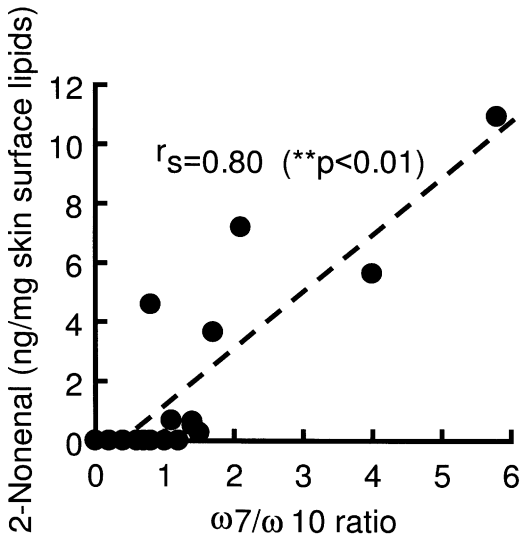

Figure 5. The relationship between the amount of 2-nonenal in body odor and the quantitative ratio of ω7 monounsaturated fatty acids to ω10 monounsaturated fatty acids.

2-Nonenal is formed by the oxidative degradation of ω7 monounsaturated fatty acids Several main components of the skin surface lipids, such as cholesterol, squalene, and unsaturated fatty acids, were oxidatively degraded by radical chain reaction initiated by squalene-HPO, and the resulting odor components were analyzed. In the cases where cholesterol or squalene was used as substrate, 2-nonenal was not detected in the samples, and these samples did not have the characteristic greasy and grassy odor, as assessed by the organoleptic test.

In the cases where unsaturated fatty acids were used as substrates, however, 2-nonenal was detected in those samples in which palmitoleic acid or vaccenic acid was added (**Table II**). The characteristic odor of 2-nonenal was also observed in these samples.

In the samples in which squalene-HPO was not added, no odor was observed after the 3 d incubation period, confirming the

Table II. Aldehydes formed by the oxidative degradation of several sebaceous components

Components tested	Aldehydes detected by GC/MS analysis
Cholesterol	not detected
Squalene	2-methyl-2-butenal
Fatty acids	
Palmitoleic acid	hexanal, heptanal, 2-octenal, 2-nonenal
Vaccenic acid	hexanal, heptanal, 2-octenal, 2-nonenal
Oleic acid	nonanal, pentenal
Linoleic acid	hexanal

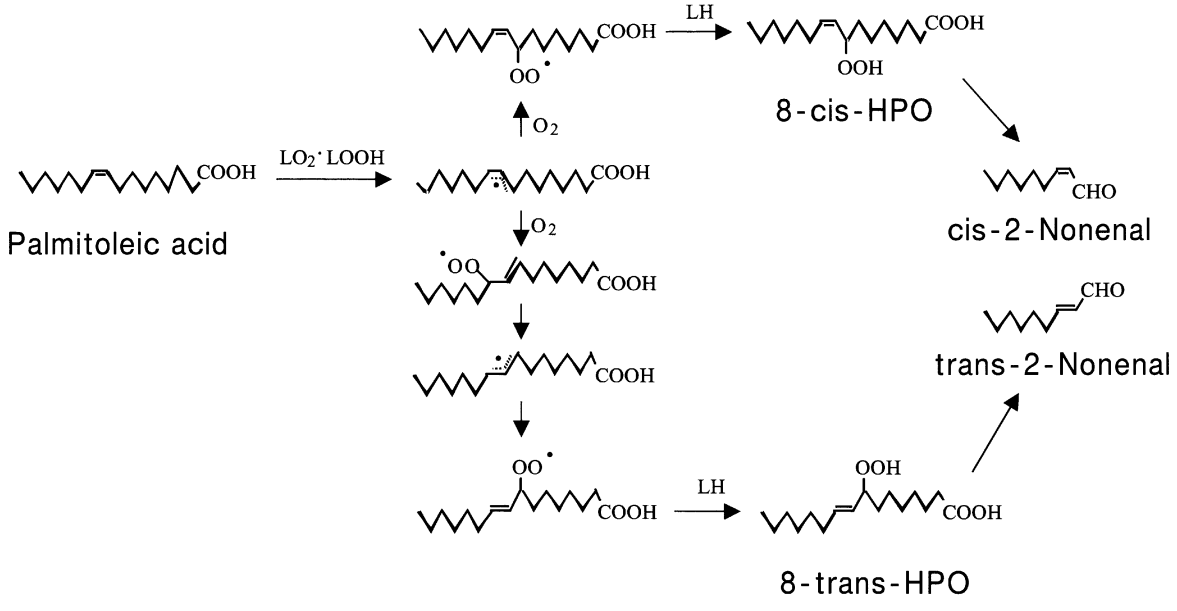

Figure 6. The presumed scheme of oxidative degradation of palmitoleic acid accelerated by lipid peroxides.

acceleration effect of squalene-HPO on the oxidative degradation of fatty acids.

DISCUSSION

In this report, the body odor components that adhered to the subjects' shirts were analyzed by GC/MS. It was demonstrated that 2-nonenal, which had not been reported previously as a human body odor, was indeed present in increasing amounts in the body odors of persons of 40 y or older.

2-Nonenal is an unsaturated aldehyde that has a characteristically unpleasant greasy and grassy odor, and a threshold value for detection of about 3–4 ppm in paraffin oil solution (Stahl, 1973). In our experiments, the mean amount of 2-nonenal that was detected in the older group of subjects (40–70 y) was 2.6 ± 3.5 ppm (mean ± SD), in terms of concentration in skin surface lipids. It is conceivable that this level of 2-nonenal may be a major cause of the deterioration in body odor that has been observed with aging.

It is presumed that *cis*-2-nonenal and *trans*-2-nonenal are formed from the oxidative degradation of palmitoleic acid in the manner shown in **Fig 6** (Porter, 1986), although it was not possible to determine the stereochemical structure of 2-nonenal from the data obtained by GC/MS analysis. It will be important to determine the absolute structure of 2-nonenal present in body odor by appropriate instrumental analyses in the future.

It has been reported that large amounts of ω10 monounsaturated fatty acids are contained in human skin surface lipids, and that the combination of C16:1ω10 and C18:1ω10 could reach about 30% of the total amount of fatty acids in skin surface lipids (Nicolaides, 1974; Nazzaro-Porro *et al*, 1979; Green *et al*, 1984). On the other hand, ω7 monounsaturated fatty acids such as C16:1ω7 and C18:1ω7 have been reported to constitute only 0.5% or less of the fatty acids in adult skin surface lipids (Nicolaides, 1974). It has also been reported that the amount of ω7 monounsaturated fatty acids in skin surface lipids changes with aging (Nazzaro-Porro *et al*, 1979). Our results show that the proportion of ω7 monounsaturated fatty acids in skin surface lipids increases up to 6-fold dramatically with age.

The cause of the increase in ω7 monounsaturated fatty acids is not yet clear. It is conceivable that either a change in the activity of the enzyme (desaturase) that introduces double bonds into fatty acids, and/or quantitative changes in the sebaceous and epidermal lipids of which skin surface lipids are composed, may be contributing to this increase (Nazzaro-Porro *et al*, 1979; Stewart, 1992).

Hydroperoxides, such as squalene-HPO, are known to be among the lipid peroxides of skin surface lipids (Ohkido *et al*, 1980; Picardo *et al*, 1991). It has been reported that the amount of lipid peroxides in skin surface lipids increases with aging (Kohno and Takahashi, 1995) and our results support this observation.

Our experiments also demonstrate that 2-nonenal can be formed by the oxidative degradation of ω7 monounsaturated fatty acids such as palmitoleic acid and vaccenic acid, and that the formation of 2-nonenal can be accelerated by lipid peroxides such as squalene-HPO. These results raise the possibility that the same reaction can occur with skin surface lipids and with the lipids adhering on clothes.

In conclusion, our results indicate that the amount of 2-nonenal in body odor, and the amount of ω7 monounsaturated fatty acids and lipid peroxides in the skin surface lipids, tend to increase with age. These findings suggest that the oxidative degradation of ω7 monounsaturated fatty acids, accelerated by lipid peroxides, may be involved in the formation of 2-nonenal, resulting in deterioration of the body odors for the middle-aged and the elderly.

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