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# Skin Surface Lipid Composition Analysis of Healthy 22-Year-Old Females Utilizing Gas Chromatography-Mass Spectrometry

Poster # 2823

Tiffany N. Oliphant, M.S., Jeff Addy (Floratech, Chandler, AZ), and Robert A. Harper Ph.D. (Harper & Associates, La Jolla, CA)
Email: sales@floratech.com Website: www.floratech.com

## Abstract

The objective of this research was to evaluate the skin surface lipid (SSL) composition of healthy, 22-year-old females. While recent data has been published on the quantity of SSL, little data is available on the variation in composition of SSL when age and sex are controlled. Fifty-nine healthy, 22-year-old females were sampled on the forehead with lipid-free cigarette paper and analyzed by GC/MS for the following lipids: squalene, wax esters, glycerides, free fatty acids, cholesterol, and cholesteryl esters. For the purpose of analysis, glycerides and free fatty acids were combined due to the variation in degree of hydrolysis of triglycerides by bacteria. The variability among subjects for each component was minimal. Correlations among the five components were calculated and found to be statistically or directionally significant with the exception of the following: squalene and wax esters, and cholesteryl esters with squalene or cholesterol. Analysis of subpopulations (even though small in number) indicated that composition of SSL did not appear to be affected by race / ethnicity, self-identified oily skin, or physiological changes produced by birth control. However, SSL composition may be affected by vegetarian or vegan diets. Furthermore, lipid samples taken during various time points during the menstrual cycle (n=9) demonstrated that the changes that occurred throughout the menstrual cycle did not immediately affect the composition of the SSL.

This information provides insight into the variation and complexity of skin surface lipid composition that exists within a well-defined population.


## Introduction / Background

Human skin surface lipids contain a diverse mixture of common lipid components. The biological variation of lipid components that can be found within SSL is complex and broad. Some of the variability in SSL has been attributed to race, sex, environment, and diet.[1,2,3] The purpose of this research is to define the composition of a healthy individual independent of sex and age, and compare the correlations among the different lipid components.

## References


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2. Pappas A, Fantasia J, and Chen T. Age and Ethnic Variations in Sebaceous Lipids. Dermato-Endocrinology 2013; 5(2): 319-24.
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4. Michael-Jubeli R, Bleton J, and Baillet-Guffroy A. High-Temperature Gas Chromatography-Mass Spectrometry for Skin Surface Lipids Profiling. Journal of Lipid Research 2011; 52: 143-51.
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## Materials / Methods

**Subjects:**

The subjects were 59 healthy, 22-year-old females (50 Caucasian, 3 African American, 2 Asian, and 4 of mixed race) who were selected according to the following criteria: no active skin diseases on the face (*e.g.*, psoriasis, atopic dermatitis, eczema, rosacea, skin cancer, acne), no immunological disorders, and not pregnant or nursing. All study procedures were Investigational Review Board (IRB) approved and informed consent was obtained from all subjects.

**Collection:**

Non-invasive sampling of skin surface lipids from the forehead of each subject was taken over the course of two hours. Subjects were instructed to wash their faces approximately 12 hours before sampling utilizing a gentle cleanser to remove all dirt and oil from their faces. Following this 12-hour period, subjects reported to the testing facility where they acclimated in a controlled environment (20-22°C and <50% relative humidity). Two sheets of lipid-free absorbent paper were then placed on top of one another on the center of the forehead and held in position for 30 minutes. The lipid absorption step was then repeated, consecutively, three additional times.

**Materials:**

Solvent-washed cigarette rice papers were used as lipid-free absorbent papers. Of the many papers tested, it was found that rice paper without adhesive contained the least amount of lipids. Absorbent papers were washed at a time with 250 mL of HPLC-grade diethyl ether in an ultrasonic water bath for 15 minutes. After extraction, the solvent was removed and the papers were placed in a rotary evaporator until completely dried. They were then stored in polyethylene jars until further use.

**Extraction:**

Collected samples were stored in cryogenic vials, nitrogen capped, and placed in an ultra-low freezer at -93°C until extraction. Each subject's collection papers were extracted with 25 mL of HPLC-grade diethyl ether in an ultrasonic water bath for ten minutes.

**Analysis:**

Analysis and calibration was completed with reference to the methods described by Michael-Jubeli *et al.*[4] The instrument consisted of an Agilent 6890 GC with an on-column injector coupled to an Agilent 5973N with turbo pump. A 1 µL volume of each derivatized sample was injected a total of three times for repeatability. Qualitative and quantitative analysis was completed by using retention times and identifier ions to isolate the different lipid classes of interest. The fatty acids, where applicable, were found to be between lengths C10 and C22. Varying degrees of saturation, odd chain, and branching chain fatty acids were represented in most lipid classes. The observed separation can be seen in the chromatograms (see Figure 1).

## Conclusions

- The variability among subjects of a controlled (sex and age) population is minimal.
- Generally, there are strong correlations between the skin surface lipids, with the exception of squalene and cholesteryl esters.
- Factors, such as diet, may affect the skin surface lipid composition.

## Results

The composition of the skin surface lipids can be found within Table 1.

For the purpose of analysis, glycerides and free fatty acids were combined due to the variation in the degree of hydrolysis of triglycerides by bacteria[5] (see Table 2). As demonstrated in Figure 2, the variability was minimal between subjects for each component.

Correlations between five components were calculated (Pearson correlation) and there were statistically significant ($p<0.05$) correlations between all components with the exception of the following: squalene and wax esters, and cholesteryl esters with squalene or cholesterol. Correlation coefficients and p values (correlation coefficient / p value) can be found in Table 3.

Further analysis of subpopulations also revealed that whether or not a subject was on birth control (n=32 and n=27, respectively) did not have any effect on the skin lipid composition. Additionally, the data indicate that subjects with vegan or vegetarian diets have larger levels of glycerides and free fatty acids than subjects with omnivorous diets (n=5 and n=54, respectively); however, due to the small subpopulation size, additional research would be necessary to make conclusive statements.

**Figure 1.** Total-Ion Chromatogram of SSL. This is a full scan TIC of a SSL sample.

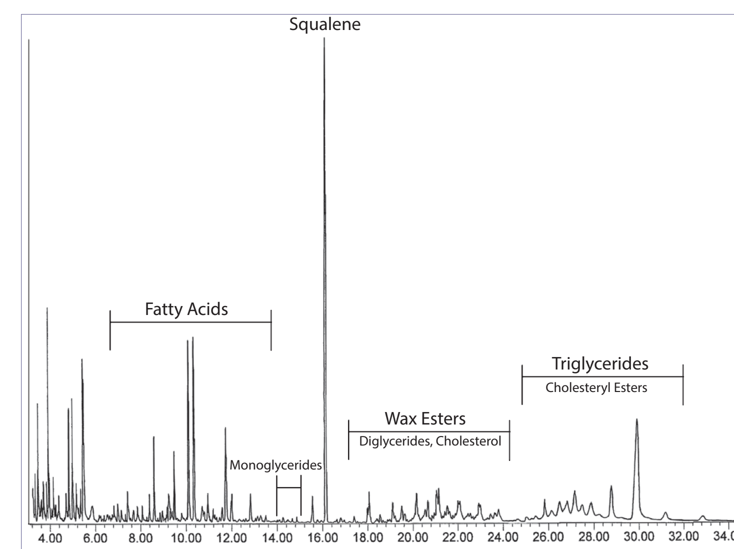


**Figure 2.** Percent Composition of SSL (for each subject).

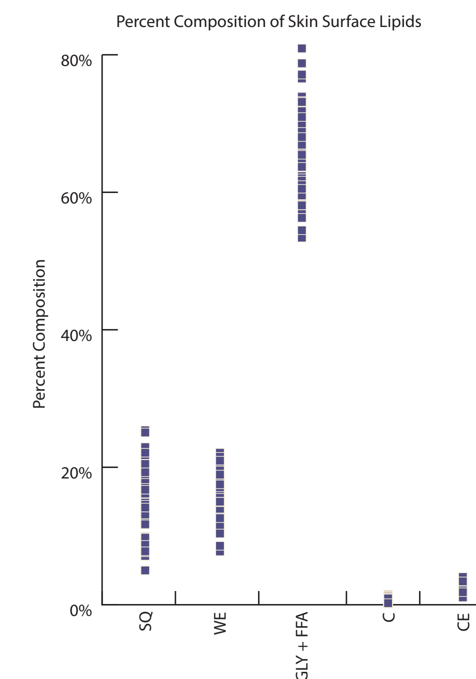


**Table 1.** Percent Composition of the Different SSL Components.

| Component | Percent Composition (Mean ± Standard Deviation) |
|---|---|
| Squalene | 15.6 ± 4.8 |
| Wax Esters | 15.2 ± 3.2 |
| Glycerides | 50.3 ± 12.5 |
| Free Fatty Acids | 16.2 ± 10.2 |
| Cholesterol | 0.6 ± 0.4 |
| Cholesteryl Esters | 2.1 ± 0.6 |

**Table 2.** Percent Composition of the Different SSL Components. Glycerides and fatty acids are grouped.

| Component | Percent Composition (Mean ± Standard Deviation) |
|---|---|
| Squalene (SQ) | 15.6 ± 4.8 |
| Wax Esters (WE) | 15.2 ± 3.2 |
| Glycerides + Free Fatty Acids (GLY + FFA) | 66.5 ± 6.2 |
| Cholesterol (C) | 0.6 ± 0.4 |
| Cholesteryl Esters (CE) | 2.1 ± 0.6 |

**Table 3.** Correlation Coefficients and p Values Between Each of the SSL Components.

| Component | Squalene | Wax Esters | Glycerides + Free Fatty Acids | Cholesterol |
|---|---|---|---|---|
| Wax Esters | 0.112 / p=0.398 | | | |
| Glycerides + Free Fatty Acids | -0.829 / p<0.001 | -0.635 / p<0.001 | | |
| Cholesterol | -0.305 / p=0.019 | -0.258 / p=0.049 | 0.303 / p=0.020 | |
| Cholesteryl Esters | 0.117 / p=0.376 | 0.343 / p=0.008 | -0.382 / p=0.003 | 0.107 / p=0.421 |

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Presented by: Tiffany N. Oliphant, M.S., Jeff Addy, and Robert A. Harper, Ph.D.


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Contact: sales@floratech.com



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