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Presence of wax esters and squalene in human saliva

Andrew J. Brasser, Christopher A. Barwacz, Deborah V. Dawson, Kim A. Brogden, David R. Drake, Philip W. Wertz *

Dows Institute for Dental Research, University of Iowa, N450 DSB, Iowa City, IA 52242, USA

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ABSTRACT

Objective: The purpose of this study was to determine the presence and relative composition of neutral lipids in human saliva.
Design: Whole unstimulated saliva was collected from 12 subjects ranging from 21 to 29 years old. Samples were lyophilized, and lipids were extracted using chloroform–methanol. Lipids were analysed by thin-layer chromatography.
Results: Human saliva contains cholesterol, fatty acids, triglycerides, wax esters, cholesterol esters and squalene. The mean total neutral lipid content was 12.1 ± 6.3 μg/ml.
Conclusions: These lipids in human saliva closely resemble the lipids found on the skin surface. These salivary lipids are most likely produced by the sebaceous follicles in the oral mucosa and sebaceous glands associated with major salivary glands.

1. Introduction

Cholesterol and other neutral lipids, phospholipids and glycolipids have all been reported as lipid components of human saliva[1]; however, the most recently published analysis of human salivary lipids indicated that neutral lipids dominated, with polar components like phospholipids and glycolipids comprising only 1–5% of total lipid mass.[2] The major lipids identified were cholesterol esters, triglycerides, fatty acids and cholesterol.

Sebaceous glands in the oral mucosa and the vermillion border of the lip have often been referred to as ectopic sebaceous glands and considered benign.[3,4] In fact, the vermillion border of the lip and all regions of the oral mucosa contain sebaceous follicles.[5–8] These are sebaceous glands without associated terminal hairs. Although some sebaceous follicles are found in all regions of skin excluding the palmar and plantar regions, these secretory units uniquely surround all orifices of the body, including the vermillion border of the lip. This anatomic distribution suggests a protective function.[9] The sebaceous follicles produce sebaceous lipids with the same composition as the pilosebaceous units of the skin.[10] In addition, sebaceous glands are associated with normal major salivary glands.[11–13] Thus, the secretions of the major salivary glands can be a source of sebaceous lipids. In fact, some of the previous salivary lipid analyses were done using saliva collected from cannulated parotid and submandibular glands.[2] Enlarged sebaceous glands called Fordyce spots, if present on the lips, are sometimes treated for cosmetic reasons.[14]

These observations on sebaceous follicles in the oral cavity suggest that the lipids found in saliva should contain, in addition to the previously noted neutral lipids, wax monoesters and squalene. These two lipids are unique biochemical markers of human sebum. Wax esters are not found anywhere else in the human body. Very minor amounts of squalene are present in most human tissues since it is an intermediate in the biosynthesis of cholesterol.[15] In this context, small amounts of squalene have been detected in mouse salivary glands.[16] Other than in human sebum, significant proportions of squalene have been reported in the skin surface lipids of the otter, kinkajou, beaver and the semiaquatic mole, *Scalopus aquaticus*.[17,18] Squalene is a very effective water proofing agent, and these other mammals that produce squalene all live in wet environments. This suggests that our ancestors may have

* *Corresponding author*. Tel.: +1 319 335 7409; fax: +1 319 335 8895.
E-mail address: philip-wertz@uiowa.edu (P.W. Wertz).
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evolved in or near water. The purpose of the present study was to test the hypothesis that wax monoesters and squalene are present in human saliva.

2. Materials and methods

2.1. Collection of saliva

The study population consisted of twelve subjects aged 21–29 years, five females and seven males. Once informed consent was obtained, subjects were submitted to basic oral exams conducted by Dr. Chris Barwacz, University of Iowa. These exams included hard and soft tissue screening as well as periodontal probing to evaluate oral health. Upon ensuring that subjects were in good oral health, samples of salivary lipids were taken. Subjects were asked to drool into a 50 ml centrifuge tube providing approximately 10 ml of saliva. Saliva samples were placed in a freezer until all samples had been obtained.

2.2. Extraction of lipids

Subsequently, the samples were thawed in a water bath and transferred into 50 ml Erlenmeyer flasks. These flasks were again frozen and then lyophilized. Once lyophilization was completed lipid was extracted from all 24 samples using 5 ml of chloroform:methanol, 2:1 (v/v).[19] The extracts were then filtered into test tubes and dried under nitrogen. These dried extracts were then dissolved in 100 μl of toluene in preparation for thin-layer chromatographic (TLC) analysis.

2.3. Thin-layer chromatography[20]

Twenty centimetre by twenty centimetre glass plates coated with 0.25-mm-thick silica gel G (Adsorbosil-plus-1; Alltech Associates; Deerfield, IL) were washed with chloroform:methanol, 2:1, activated in a 110 °C oven, and the adsorbent was scored into 6-mm-wide lanes. Calibrated glass capillaries were used to apply 5 μl samples 2 cm from the bottom edge of the plate. The chromatogram was developed to 20 cm with hexane, followed by toluene to 20 cm, followed by hexane:ethyl ether:acetic acid, 70:30:1, to 12 cm.

After development, chromatograms were air dried, sprayed with 50% sulphuric acid, and slowly heated to 220 °C on an aluminium slab on a hot plate. After 2 h, charring was complete and a digital image of the chromatograms was captured using a UMAX flatbed scanner with MagicScan software. The TIF images were analysed using TNIMAGE (Thomas Nelson, Bethesda, MD).

2.4. Identification of squalene and wax monoesters

The lipid that matched the squalene standard and a fraction containing a mixture of cholesterol esters and wax esters were isolated by preparative TLC. Lipid was applied as a streak to an undivided silica gel TLC plate. After development as described above, the plate was sprayed with 0.02% 2′,7′-dichloro-fluorescene in 95% ethanol. After drying, the plate was viewed under UV light. The silica gel containing the bands of interest were scraped from the plate and placed in small glass columns. Lipids were eluted with chloroform:methanol, 2:1. The samples were dried under nitrogen.

The isolated squalene was dissolved in n-hexane prior to analysis by gas–liquid chromatography. This was done with a Shimadzu GC-14A equipped with a flame ionization detector. A 30 m EC-WAX quartz capillary column was operated isothermally at 175 °C.

The chain length distributions within the cholesterol ester and wax esters are such that the individual lipid types cannot be individually isolated. The mixed cholesterol ester/wax ester fraction was treated with 2 ml of 1 M NaOH in methanol at 50 °C for 1 h. After cooling to room temperature, 2 ml of 1 N HCl (aq) and 10 ml of chloroform were added and mixed. The chloroform layer was transferred to a clean tube and dried under nitrogen. The residue was dissolved in 100 μl of chloroform:methanol, 2:1, and 10 μl of this was applied to a lane on a thin-layer plate about 2 cm from the bottom edge. The chromatogram was developed to 20 cm with a mobile phase of hexane:ethyl ether:acetic acid, 70:30:1. After air drying, the plate was sprayed with 50% sulphuric acid (aq) and slowly heated to 220 °C to induce charring. Stearic acid, stearyl alcohol and cholesterol standards were included on separate lanes.

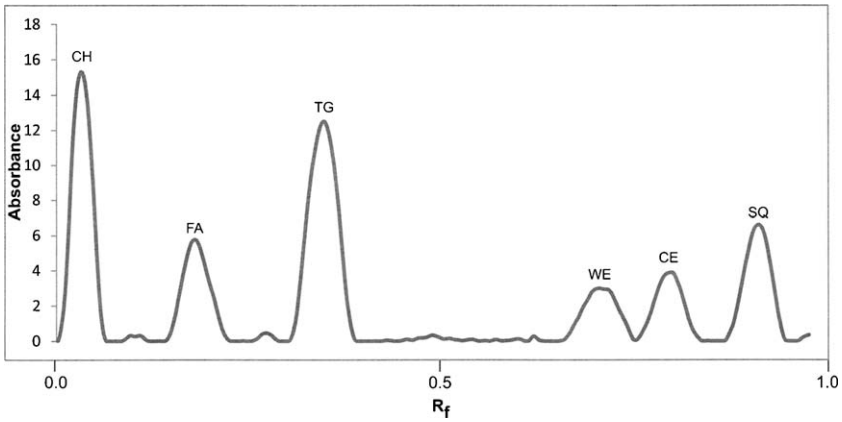

Fig. 1 – Carbon density profile of charred lipids after separation by TLC. CH, cholesterol; FA, fatty acids; TG, triglycerides; WE, wax esters; CE, cholesterol esters; SQ, squalene.