

EFFECTS OF ULTRAVIOLET IRRADIATION ON HUMAN SKIN LIPIDS

In Vivo and in Vitro Studies

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Abstract. Human volunteers were irradiated with twice the minimal erythral dose of medium wavelength ultraviolet light. One and two weeks after a single exposure, epidermal lipids increased and sebaceous lipids decreased. Control experiments demonstrated that ultraviolet light was not able to cause chemical hydrolysis of triglycerides or sterol esters, nor did it increase the rate of lipid biosynthesis in homogenates of rat epidermis. The changes noted are considered to be caused by crusts of UV-induced parakeratosis-like changes in the epidermis.

The literature contains conflicting reports on the effects of ultraviolet light (UV) on skin lipids. Several groups reported an increase in cholesterol (2, 5, 10), one (3) reports no significant change, while others (1, 8) report a decrease. The possibility exists that such disparate evidence might result from differing experimental conditions, the use of different species of experimental animal, dietary influence, variations in UV exposure, or different methods of lipid analysis.

This report deals with our attempts to minimize such variations in an attempt to achieve some understanding of the photobiology of the skin. Using single dose UV irradiation on human volunteers, we have observed effects on skin lipids that are in good agreement and have confirmed them with a variety of control experiments.

MATERIALS AND METHODS

In vivo study

Ten healthy male Japanese adults aged between 26 and 37 years were the subjects of this study. Each volunteer received twice the minimal erythral dose (2 MED) of medium wavelength UV on the back in a single exposure. The UV light was emitted by six tubes (Toshiba FL-20SE) which emitted from 260-400 nm, with the maximum energy at 310 nm. Irradiation was given at a distance

of 30 cm for 4.0 min. The energy delivered at the skin surface was determined by a 0.15 M potassium ferrixyalate chemical actinometer to be 1.49×10^4 erg cm⁻² sec⁻¹. To minimize the effects of diet and other environmental factors, different areas of the back of each subject were given a single dose of UV at weekly intervals for 3-4 weeks and lipids were collected from all irradiated areas at the same time. Endogenous lipids were extracted by the cup method (28.26 cm² in area) using 3 ether extractions in a total volume of 60 ml. Replacement lipids were obtained 3 hours afterwards using the same methods.

Lipid analysis

Total lipids were measured gravimetrically, total cholesterol was estimated by the method of Zlatkis et al. (12),

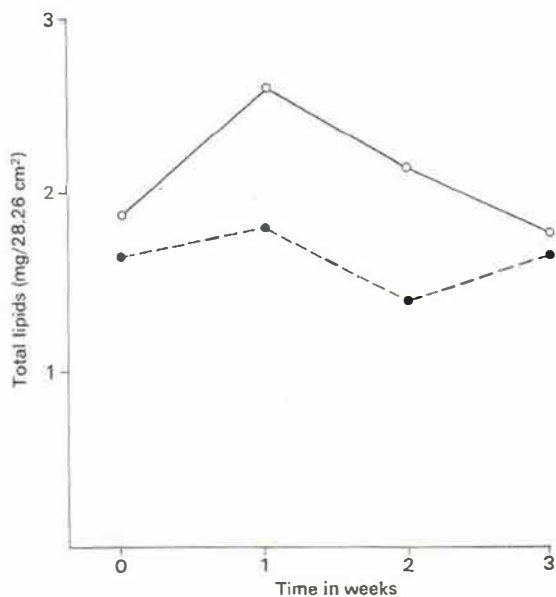


Fig. 1. The total endogenous lipids (—) and the total replacement lipids (---) of skin surface after a single irradiation of medium wave ultraviolet on the backs of volunteers.

Table I. The change in volume of skin surface lipids after a single irradiation of medium wave ultraviolet on the backs of volunteers

Time in weeks	Lipids					
	Total lipids (mg/28.26 cm ²)		Total cholesterol (mg/28.26 cm ²)		Free cholesterol (mg/28.26 cm ²)	
	e.l. ^a	r.l. ^c	e.l.	r.l.	e.l.	r.l.
0	1.85 ± 0.75 ^b	1.59 ± 0.66	0.181 ± 0.062	0.142 ± 0.060	0.045 ± 0.024	0.068 ± 0.077
1	2.58 ± 1.40	1.78 ± 0.90	0.266 ± 0.120	0.231 ± 0.536	0.065 ± 0.047	0.059 ± 0.030
2	2.13 ± 0.79	1.36 ± 0.70	0.230 ± 0.051	0.145 ± 0.142	0.0716 ± 0.064	0.111 ± 0.073
3	1.75 ± 0.21	1.64 ± 0.58	0.196 ± 0.049	0.127 ± 0.170	0.042 ± 0.524	0.093 ± 0.062

^a Endogenous lipids.^b The mean value and the standard deviation of 10 samples.^c Replacement lipids.

free cholesterol by the method of Zak et al. (11), total fatty acids by the method of Drysdale & Billimoria (4) and free fatty acids by the method of Laurell & Tibbling (6). All values are expressed in terms of 28.26 cm² of skin area: total lipid in mg dry weight, mg of total and free cholesterol, free fatty acids as moles $\times 10^{-5}$, and total fatty acids as ml of 2×10^{-3} M NaOH required to titrate to neutrality.

Chemical studies

Cholesterol palmitate was obtained from K and K Laboratories (New York, N.Y., USA), tripalmitin from Sigma (St. Louis, Mo., USA); CHM solution is chloroform/heptane 4:3 (v/v) containing 2% methanol.

UV irradiation procedures were the same as above. Materials were tested by exposure to UV in solution in a Petri dish 57.3 cm² in area and 0.26 cm in depth. All tests and controls were carried out in a darkroom. Fifteen ml of cholesterol palmitate (5×10^{-3} M in CHM solution) was irradiated, evaporated to dryness, and redissolved in 15.0 ml of CHM. A 3.0-ml aliquot was measured for release of free fatty acids by a modification of the method of Laurell & Tibbling. A non-irradiated control was tested in exactly the same manner. Results are expressed as moles of palmitic acid released by 1.5×10^{-5} moles of cholesterol palmitate.

Tripalmitin (1.5×10^{-3} M in CHM solution) was treated in a similar fashion, and the results are expressed as moles of palmitic acid released from 5.3×10^{-5} moles of tripalmitin.

Epidermal homogenate studies

DL-mevalonic acid-2-C¹⁴ (spec. act. 6.33 m Ci/mM) and malonyl Coenzyme A-1,3-C¹⁴ (spec. act. 26.7 m Ci/mM) were purchased from the New England Nuclear Corp. (Boston, Mass., USA). White male rats (ca 150 g) were obtained from the Nakashima Experimental Animal Co.

Rat skin epidermis was obtained as described earlier (9) and homogenized at the rate of 0.050 g in 3.0 ml of 0.01 M phosphate buffer (pH 7.4). Three ml of homogenate was irradiated as described above. Three ml of

irradiated and 3.0 ml of untreated control homogenate was incubated with 1) 2 μ Ci of DL-mevalonic acid -2-C¹⁴, or 2) 0.2 μ Ci of malonyl Coenzyme A-1,3-C¹⁴ for 6.0 hours.

Following the incubation, all 4 mixtures were extracted with Folch solution and the lipids separated on a thin-layer chromatogram (Kieselgel G, 0.25 mm; solvent system *n*-hexane: methyl ether: acetic acid; 180:30:1). The free fatty acid and cholesterol spots were isolated by scraping and counted in a liquid scintillation spectrometer and the results expressed as CPM per gram (wet weight) of epidermal scrapings.

RESULTS

The total endogenous skin surface lipids showed a marked increase during the first week after irradiation (Fig. 1), while replacement lipids were not significantly changed. In a similar fashion (Table I) both total and free cholesterol increased while total fatty acids decreased. Because of the wide standard deviation, we cannot comment on the effects on free fatty acids. The levels of replacement lipids showed no significant changes.

Epidermal lipids increased during the first and second weeks after irradiation and then decreased. Since total and free fatty acids are considered to be sebaceous in origin, and these decreased after irradiation, the possibility exists that the excursion of sebum might be limited by keratotic plugs. Other evidence for this is the fact that after removal of endogenous lipids, there was no change in the amount of total fatty acid in replacement lipids.

The irradiation of cholesterol palmitate or tripalmitin produced no release of fatty acid, as

Total fatty acids consumption volume of (2×10^{-2} N NaOH/28.26 cm ²)		Free fatty acids (10^{-8} M/28.26 cm ²)	
e.l.	r.l.	e.l.	e.l.
0.61 ± 0.46	0.61 ± 0.59	10.25 ± 41.94	5.94 ± 5.79
0.56 ± 0.56	0.52 ± 0.97	13.83 ± 75.31	7.19 ± 8.53
0.55 ± 0.53	0.52 ± 0.45	10.04 ± 52.28	4.58 ± 5.10
0.74 ± 2.04	0.54 ± 0.32	9.07 ± 34.28	4.88 ± 4.48

shown in Table II. Cholesterol formation from labelled mevalonic acid was identical (2×10^3 CPM/g wet weight) in irradiated and control samples.

DISCUSSION

There are many conflicting reports on the effects of UV radiation on skin lipids. Rauschkolb et al. (8) observed a decrease in the amount of free extractable cholesterol in human skin (in vitro) after UV irradiation. They conclude (1) that this light-susceptible site of sterol synthesis was between acetate and mevalonate. On the other hand, Wells & Baumann (10) and Horlick (5) report an increase in sterol synthesis in rat skin after UV irradiation. Black & Anglin (2) also report increased cholesterol synthesis in guinea pig skin after UV irradiation.

Downing et al. (3) measured surface lipids on the forehead of adult males for periods of up to 14 months and found them to be relatively constant. This suggests an absence of seasonal variation; i.e., no effect of seasonal variations of sunlight.

We suggest that such differences and the results of differences following UV exposure, are func-

tions of the differing species of experimental animals used, methodology of lipid measurement, and the fact that diet has a profound effect on skin surface lipids.

In our experiments we used a single irradiation per skin site on skin sites which were changed weekly for 3-4 weeks, and all lipids were collected at the same time, minimizing the possible effects of dietary differences and external contamination.

Moreover, chemical methods, rather than thin-layer chromatography, were used to quantitate lipids. For these reasons we felt confident in reporting that epidermal lipids increased during the first and second week after irradiation, while sebaceous lipids decreased.

It has already been reported (7) that a dosage of 2 MED of UV to human skin causes increased scaling by the 10th day postexposure. Microscopic examination shows that these scales are caused by parakeratosis-like crusts. It is likely therefore, that increased epidermal lipids arise from these crusts, while decreased sebaceous lipids might result from a parakeratotic plugging of sebaceous glands. The chemical and in vitro studies rule out the possibility of any direct effect of UV on cholesterol esters or triglyceride hydrolysis, or on the biosynthesis of cholesterol or fatty acids.

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Table II. The effect of ultraviolet on releasing free fatty acids from cholesterol ester and triglyceride

Released palmitic acid from 1.5×10^{-8} M cholesterol palmitate		Released palmitic acid from 5.3×10^{-8} M tripalmitin	
Irradiated ^a	Non-irradiated	Irradiated	Non-irradiated
$(4.4 \pm 0.63) \times 10^{-8}$ M	$(4.5 \pm 0.37) \times 10^{-8}$ M	$(7.4 \pm 0.82) \times 10^{-9}$ M	$(7.2 \pm 0.74) \times 10^{-9}$ M

^a The conditions and dosage of ultraviolet irradiation are described in the text.

^b Mean value and the standard deviation of 10 samples.

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