EFFECT OF ESSENTIAL FATTY ACID DEFICIENCY ON LIPID OF SKIN SURFACE OF RAT

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Summary A study on how EFA deficiency affects lipid metabolism of rat skin, especially lipid on skin surface, was made. The total amount of lipid on skin surface of rat increased due to EFA deficiency, but not significantly. The sterol ester in skin surface lipid was maintained at a normal level in a EFA deficient rat, but the free sterol level was higher than that of the control rat. The glycerides decreased markedly due to EFA deficiency. It was recognized that branched fatty acids increased in each lipid fraction on skin surface.

It was considered that such changes in skin surface lipid were characteristic to skin. It was not predictable whether these changes resulted directly from EFA deficiency or secondary from the occurrence of dermal symptoms.

In a previous paper (1), it was reported that \(\omega\)-linolenic acid could replace linoleic acid for the maintenance of a normal growth and mitochondrial function, but not for the maintenance of normal healthy skin.

It is generally known that the skin has a different metabolism from that of other organs, and it is assumed that a deficiency in the essential fatty acid (EFA) causes a disturbance in skin lipid metabolism which leads to dermatosis (2). In the internal organs, it was reported that the effect on lipid metabolism of \(\alpha\)-linolenic acid was not different from that of linoleic acid (3–5).

These facts allow us to presume that the changes in skin lipid metabolism due to EFA deficiency are characteristic. In the present paper, the effects of EFA deficiency on the skin lipid metabolism were studied.

MATERIALS AND METHODS

I) Animals. Adult female rats of the Wister strain were fed commercial
The female rats were mated with male rats which were also fed commercial chow. During pregnancy, the rats were given a stock diet. Just after attendance, the female rats were divided into two groups. One group designated fat-free group, was fed a fat-free basal diet shown in Table 1. While another group, designated control group, was fed a control diet, which contained an additional 1% of soy bean oil. After weanling, the male pups were separated from the mothers. The male pups received the experimental diet and water ad libitum.

2) Collection of the skin surface lipid. Three-month old males were used for this experiment. The rats were anesthetized with ethyl ether and were reclined on a funnel as shown in Fig. 1. Then the skin was washed with a fine jet of acetone.

![Fig. 1. Scheme of skin surface lipid collection.](image)

Approximately 50 to 80 ml of acetone was used according to body weight of rat. However the head, tail and anal regions were not washed. The first washing was carried out to clean the skin and fur. After this washing procedure, two groups were fed the basal diet for three days, and then the skin surface lipid was collected.
by acetone washing in the same way as described above. The acetone solution was evaporated and to dryness under reduced pressure, and the residue was dissolved in ethyl ether. Each solution was filtered, reevaporated and redissolved in benzene. The benzene solution was stored at $-20^\circ C$ until analyzed.

3) Lipid analysis. The skin surface lipid was separated into hydrocarbon, sterol ester-waxes, triglyceride, free sterol, diglyceride and monoglyceride by column chromatography on Florisil hydrated with 7% water according to the procedure described by Carrol (6). Sterol ester and waxes were recovered together and separated by thin-layer chromatography on alumina by Haahti's method (7). But Haahti's method was used only for measuring fatty acid compositions of sterol ester and waxes because this method was not always satisfactory. The amounts of the total lipid on skin surface and sterol ester-waxes were determined gravimetrically by weighing with a chemical balance. Each glyceride was estimated by the determination of glycerol (8). For glyceride determination, it was saponified and the resulting glycerol was oxidized to formaldehyde, which was then analyzed colorimetrically with chromotropic acid. For sterol determination, the sterol ester-waxes fraction was hydrolyzed, digitonized and analyzed by Zaks's method (9).

Each lipid fraction was hydrolyzed and chromatographed on silica gel G, by Nikkari's method (10). With this procedure, hydroxy fatty acids and unsaponifiable materials could be separated. Fatty acids were methylated by BF$_3$-methanol and hydrogenated on palladium black. Analysis of methyl ester mixture was carried out by using gas chromatography. Throughout the experiment a stainless steel column 3 mm $\times$ 2 m packed with 15% DEGS on Shimalite (60 to 80 mesh) was used. Temperatures were, column, 186$^\circ$C; detector, 250$^\circ$C; and flash heater, 270$^\circ$C. Peak areas were calculated by triangulation and identification was made using an Applied Science Laboratories standard.

4) Calculation of skin surface area. The calculation of skin surface area was made with the following equation:

$$S=12.54W^{0.8}$$  \hspace{1cm} (II)

$S$: skin surface area (cm$^2$)

$W$: body weight (g)

RESULTS

Three-month old rats fed the basal diet (fat-free group) all showed signs of EFA deficiency. For example, the average body weight of the fat-free group was about half that of the control group as shown in Table 2. The skin surface lipid was collected at this time.

Table 3 shows the amount of each fraction of skin surface lipid. The amount of total lipid per skin surface area showed a tendency to increase due to EFA deficiency. However, EFA deficiency had no influence on the amount of the sterol
ester-waxes fraction. The sterol ester in the fat-free group was maintained at a normal level, but the level of the free sterol was higher than that of the control group. The ratio of sterol ester to total sterol was found to be 0.63 and 0.50 in the control and the fat-free group, respectively. This ratio was lower in the fat-free group than in the control group. The amounts of triglyceride, diglyceride and monoglyceride decreased markedly due to EFA deficiency.

Fatty acid composition of total lipid is given in Table 4. In the case of the

| Lipid fractions         | Cont. (4)a | Fat-free (7) |
|-------------------------|------------|--------------|
| Total lipid (mg)        | 41.0±1.9b  | 50.1±3.8     |
| Waxes+sterol ester (mg) | 23.8±1.7   | 27.9±2.4     |
| Triglyceride (μmoles)   | 1.982±0.320| 0.456±0.067  |
| Diglyceride (μmoles)    | 0.201±0.026| 0.062±0.007  |
| Monoglyceride (μmoles)  | 0.244±0.011| 0.035±0.006  |
| Sterol ester (μg)       | 864±70     | 1,045±102    |
| Free sterol (μg)        | 502±49     | 1,008±81     |

Table 2. Body weight of rats fed fat-free diet for 12 weeks (g).

|                      | Cont. (5)a | Fat-free (10) |
|----------------------|------------|---------------|
| Rat number of determination. | 346±6b     | 183±8         |
| Mean ± SE.           |            |               |

Table 3. Composition of skin surface lipid (per 100 cm² skin surface).

| Fatty acid composition of total lipid (%). |
|-------------------------------------------|
| Number of carbon atoms | Cont. (4)a | Fat-free (5) |
|------------------------|------------|--------------|
|                        | Straight   | Iso branched | Anteiso branched | Straight   | Iso branched | Anteiso branched |
| 14                     | 1.4±0.4b   | 0.9±0.1     | —                | 0.5±0.1   | 0.5±0.1     | —                |
| 15                     | 0.7±0.1    | —           | 1.3±0.2         | 0.4±0.03  | 0.6±0.2     | —                |
| 16                     | 22.3±1.7   | 5.2±1.0     | —                | 18.6±0.7  | 8.0±0.9     | —                |
| 17                     | 1.1±0.1    | —           | 6.0±0.5         | 1.0±0.04  | 4.1±0.2     | —                |
| 18                     | 19.5±1.0   | 2.8±0.3     | —                | 16.4±1.7  | 3.8±0.3     | —                |
| 19                     | trace      | —           | 2.0±0.2         | trace     | —           | 1.3±0.04         |
| 20                     | 16.5±1.5   | 3.5±0.4     | —                | 15.5±0.8  | 7.9±0.6     | —                |
| 21                     | trace      | —           | 2.2±0.3         | trace     | —           | 2.4±0.4          |
| 22                     | 5.4±0.8    | 1.0±0.1     | —                | 6.0±0.6   | 3.6±0.7     | —                |
| 23                     | trace      | —           | 0.7±0.04        | trace     | —           | 1.1±0.2          |
| 24                     | 6.9±2.1    | 1.1±0.4     | —                | 5.8±0.5   | 2.8±0.8     | —                |
| Total                  | 73.3±2.1   | 14.5±1.6    | 12.2±0.3        | 64.9±2.5  | 26.6±2.0    | 9.5±0.3          |

a Rat number of determination.
b Means ± SE.
total lipid, iso branched fatty acid C₂₅ increased significantly due to EFA deficiency. However, with the exception of the iso branched fatty acid C₂₅, no great difference was observed in the other fatty acids in a comparison of the two groups. Due to the deficiency each iso branched fatty acid showed tendency to increase, and each anteiso branched fatty acid and each straight fatty acid showed a tendency to decrease. When all iso and anteiso branched fatty acids were added in each group, regardless of the carbon number, the iso branched fatty acid showed an increase because of EFA deficiency ($p<0.01$), while the anteiso branched fatty acid showed a decrease ($p<0.01$) (Table 4). When iso branched fatty acid was added to the anteiso branched fatty acid, the total branched fatty acid was 26.7% in the control group and 36.1% in the fat-free group. The increment of the total branched fatty acid was found in the fat-free group ($p<0.05$). The fatty acid composition in the triglyceride, sterol ester and waxes exhibited the same tendency as that of the total lipid on the skin surface. Iso and anteiso branched fatty acid were contained to a higher degree in sterol ester than in the other lipid fractions (Table 5).

Table 5. Relative amounts of branched fatty acid of various fractions of skin surface lipid (%).

| Fraction    | Branched fatty acid | Cont. | Fat-free |
|-------------|---------------------|-------|----------|
| Triglyceride| iso                 | 15.4  | 26.9     |
|             | anteiso             | 9.5   | 7.9      |
| Sterol ester| iso                 | 25.4  | 35.6     |
|             | anteiso             | 17.1  | 12.2     |
| Waxes       | iso                 | 15.8  | 26.0     |
|             | anteiso             | 10.8  | 8.6      |

**DISCUSSION**

EFA deficient rats show dermal symptoms and hereby a thick epidermis can be observed microscopically. Kingery and Kellum (2) reported that stratum corneum of EFA deficient rats was filled with sudanophiric granules and that these granules were phospholipids. They discussed this phenomenon in terms of an abnormally increased phospholipid synthetic activity due to a suitable biological mechanism which compensates for an epidermal barrier membrane disordered by EFA deficiency. From electronmicroscopical observations of the skin of EFA deficient mice, Menton (12) suggested that the abnormality of the epidermal barrier membrane due to EFA deficiency was the first cause of dermatosis production. However, relatively little information is available on the chemical composition of this barrier, except that protein-lipid complexes appear to be important (13).

Therefore, the changes in metabolism, especially lipid metabolism, in the skin due to an EFA deficiency was studied. It was found that the triglyceride, diglyceride and monoglyceride in the skin surface lipid of rats fed a fat-free diet is re-
markedly decreased. It has been suggested that diglyceride and monoglyceride on the skin surface were products which triglyceride was hydrolyzed by lipase or microorganisms in the sebaceous gland duct or on the skin surface (14). Accordingly, the decrease of all glycerides due to EFA deficiency was caused by a decrease in triglyceride. At present, the cause of this decrease of triglyceride is not known. EFA deficient rats show a fatty liver, and a triglyceride increases in the liver but decreases in the serum (15, 16). Although the triglyceride in the serum and in the skin surface were similarly affected by the EFA deficiency, it is considered that there is no direct relationship between the triglycerides in the serum and in the skin surface. This conclusion was based on the observation that the triglyceride of the skin surface contained branched fatty acids which were not found in the triglyceride of the serum and that the skin surface lipid was synthesized at the sebaceous gland and the epidermis (14).

The most interesting result was obtained with respect to branched fatty acids. Due to an EFA deficiency, the branched fatty acids increased, and they were the iso type and not anteiso type. The branched fatty acids were synthesized from branched amino acids, namely, the iso type from leucine and valine, and the anteiso type from isoleucine (17). The plasma of humans given a low-fat diet contained low amounts of leucine and valine (18). The nitrogen-balance of EFA deficient rats is lower than that of normal rats (19). From these facts, the following hypothesis may be derived:

Due to EFA deficiency, the outflow of valine and leucine from the blood to the skin or the urine increases and the amounts of valine and leucine in the blood decreases. Therefore, the anabolic rate of protein decreases and the nitrogen-balance is lowered.

However, it is not known whether the changes of the fatty acid composition in the skin surface lipid are a characteristic phenomenon in an EFA deficiency or whether they are a common phenomenon of dermal symptoms. Also the reason for an increase of the branched fatty acids is still unsolved.

It has been reported that in human psoriasis the ratio of sterol ester to total sterol is low (20). Therefore, the low ratio found in the present experiment may be an indirect rather than a direct result of the EFA deficiency. The correlations between dermal symptoms and skin lipid metabolism will be studied further.

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