# Apolipoprotein E Deficiency Leads to Cutaneous Foam Cell Formation in Mice

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**Apolipoprotein E deficiency leads to familial dysbetalipoproteinemia characterized by increases in serum lipid levels, atherosclerosis, and cutaneous xanthoma. Apolipoprotein E is synthesized in many tissues in the body, including the epidermis. In the present study, we determined whether transgenic mice deficient in apolipoprotein E develop cutaneous xanthoma and the effect of dietary fat intake on these lesions. We also determined whether apolipoprotein E–deficient mice have abnormalities in cutaneous barrier function or stratum corneum structure. Homozygous apolipoprotein E–deficient mice (−/−) fed a high-fat diet displayed a diffuse inflammatory infiltrate in the dermis surrounding fat droplets in macrophages. In homozygous mice (−/−) fed a low-fat diet, similar lesions were seen but they tended to be focal and less prominent. In heterozygous mice (+/−) fed the high-fat diet, a few inflammatory cells were present in the dermis but foam cells were not seen. Control mice (+/+) fed a high-fat diet displayed scattered inflammatory cells in the dermis. Heterozygous mice (+/−) fed a low-fat diet were similar to control mice (+/+) fed a low-fat diet. The extent of foam cell formation correlated directly with the degree of atherosclerosis. There were no abnormalities in permeability-barrier function or stratum corneum structure in apolipoprotein E–deficient mice. Thus, the lack of apolipoprotein E production in the epidermis does not appear to lead to any detectable abnormality in structure or function of the stratum corneum. However, lack of apolipoprotein E leads to cutaneous foam cell formation, presumably secondary to disturbances in lipoprotein metabolism.** *Key words: stratum corneum/xanthoma/permeability barrier/transgenic mice. J Invest Dermatol 104:246–250, 1995*

Apolipoprotein E is a 34,000 kd molecular weight glycoprotein that plays a key role in lipoprotein metabolism [1,2]. In humans, apolipoprotein E exists as three major isoforms; the apolipoprotein E2 isoform, which has a single cysteine substitution for arginine at amino acid 158, binds poorly with apolipoprotein E receptors [2]. Apolipoprotein E2 homozygosity is present in approximately 1% of the population, and these individuals have an increased risk of familial dysbetalipoproteinemia (type III hyperlipidemia, broad beta disease) [2]. The combination of apolipoprotein E2 homozygosity and other disorders that affect lipid metabolism, such as hypothyroidism, or environmental factors such as diet, leads to the development of familial dysbetalipoproteinemia [2]. Rare individuals may have the genetic absence of apolipoprotein E and thereby also develop familial dysbetalipoproteinemia [3]. Familial dysbetalipoproteinemia is characterized by increases in serum cholesterol and triglyceride levels due to increased quantities of chylomicron and very-low–density lipoprotein remnants [2]. These alterations in lipoprotein metabolism are associated with an increased risk of not only atherosclerosis, but also cutaneous xanthoma [2]. Palmar xanthoma, xanthelasma, and tuberoeruptive xanthoma are characteristic cutaneous lesions associated with familial dysbetalipoproteinemia [4,5].

Apolipoprotein E is synthesized not only in the liver and intestine, organs that make lipoproteins, but also in most other tissues in the body [6]. In addition to its important role in lipoprotein metabolism, it is likely that apolipoprotein E has other vital functions [7]. For example, very recent studies have suggested that apolipoprotein E produced in the brain may have a role in preventing the development of Alzheimer's disease [8]. The epidermis is also an active site of apolipoprotein E production [9,10]. Furthermore, studies by our laboratory have demonstrated that disruption of the cutaneous permeability barrier results in a rapid increase in epidermal apolipoprotein E mRNA levels [11]. Although we have been able to demonstrate an important role in the maintenance or formation of the cutaneous permeability barrier for many of the proteins that increase in response to barrier disruption, the function of apolipoprotein E in the epidermis is unknown [12].

In recent years, advances in molecular biology have allowed manipulation of the mouse germ line. Mice that overexpress or carry genetically inactivated genes have been used to gain insight into the functional role of specific proteins. Normal mice do not develop atherosclerosis on a standard diet of low-fat chow and, even on a high-fat atherogenic diet, they develop only minimal atherosclerotic changes **(Table I)**. In contrast, homozygous apoli-

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**Table I. Serum Lipid Levels, Atherosclerosis, and Foam Cell Formation Directly Correlate**[a]

| | Normal (+/+) | | Heterozygous Apo E Def (+/−) | | Homozygous Apo E Def (−/−) | |
|---|---|---|---|---|---|---|
| | Low Fat | High Fat | Low Fat | High Fat | Low Fat | High Fat |
| Serum triglyceride[b] (mg/dl) | 52 ± 2 | 53 ± 12 | 81 ± 5 | 53 ± 5 | 104 ± 8 | 64 ± 11 |
| Serum cholesterol[b] (mg/dl) | 90 ± 8 | 238 ± 23 | 77 ± 4 | 326 ± 44 | 672 ± 90 | 2712 ± 290 |
| Aortic atherosclerosis[c] | 0 | + | 0 | ++ | +++ | ++++ |
| Foam cell formation[c] | 0 | + | 0 | ++ | +++ | ++++ |

[a] Data are presented as mean ± standard error of the mean. Apo, apolipoprotein; def, deficient.
[b] These results have been published previously in detail [15] and are included here to facilitate comparisons.
[c] 0, no atherosclerosis or foam cells; +, minimal atherosclerosis or foam cells; ++++, marked atherosclerosis or foam cells.

poprotein E–deficient mice (−/−) are hyperlipidemic and develop atherosclerosis on a standard diet of low-fat chow **(Table I)** [13,14]. Moreover, feeding a high-fat diet induces marked hyperlipidemia and extensive atherosclerosis [15]. In heterozygous apolipoprotein-E–deficient mice (+/−), a standard diet of low-fat chow results in only minimal atherosclerosis **(Table I)** [15]. However, feeding a high-fat diet increases atherosclerosis [15]. These studies demonstrate that these genetically engineered mice are valuable models for studying the development of atherosclerosis.

The aims of the present study were twofold. First, we determined whether transgenic mice deficient in apolipoprotein E have abnormalities in either cutaneous permeability-barrier function or stratum corneum structure. Second, we assessed whether cutaneous xanthoma occurs in apolipoprotein E–deficient mice and the effect of variations in dietary fat intake on the development of these lesions.

## MATERIALS AND METHODS

Heterozygous and homozygous apolipoprotein E–mutant mice were prepared as described previously [13,15]. These mice have mixed genetic backgrounds derived from two inbred strains, C57Bl/6 and 129. All mice were maintained in a room illuminated from 7 AM to 7 PM. Animals were fed either regular mouse chow (5012, Ralston Purina, St. Louis, MO) which is low in fat (4.5% fat, 0.022% cholesterol), or a high-fat, high-cholesterol diet (TD88051, Teklad Premier, Madison, WI) containing 15.8% fat, 1.25% cholesterol, and 0.5% sodium cholate. Food and water were provided *ad libitum* for 12 weeks. Previous studies have characterized extensively lipoprotein and vessel wall changes in these animals [13,15].

After sacrifice, the abdominal and thoracic cavities were opened and the heart and vascular tree were perfused with buffered paraformaldehyde (4%, pH 7.4) under physiologic pressure. Skin samples from two mice in each treatment group were placed in fresh paraformaldehyde for light microscopic studies. Sections were embedded in paraffin, 5-$\mu$m sections were cut, and the sections were stained with hematoxylin and eosin. Skin samples for electron microscopy were minced to 0.5 $mm^2$, fixed in modified Karnovsky's fixative overnight, washed in 0.2 M cacodylate buffer, and post-fixed in both 1.5% osmium tetroxide containing 0.5% potassium ferrocyanide and 0.1% ruthenium tetroxide containing 0.5% potassium ferrocyanide in 0.1 M cacodylate buffer for 30 min as described [16]. Ultrathin sections 600–800 nM were examined in a Zeiss 10A electron microscope operating at 60 kV.

Transepidermal water loss was measured over the ears using a Meeco Electrolytic Water Analyzer (Warrington, PA), as described previously [17].

## RESULTS AND DISCUSSION

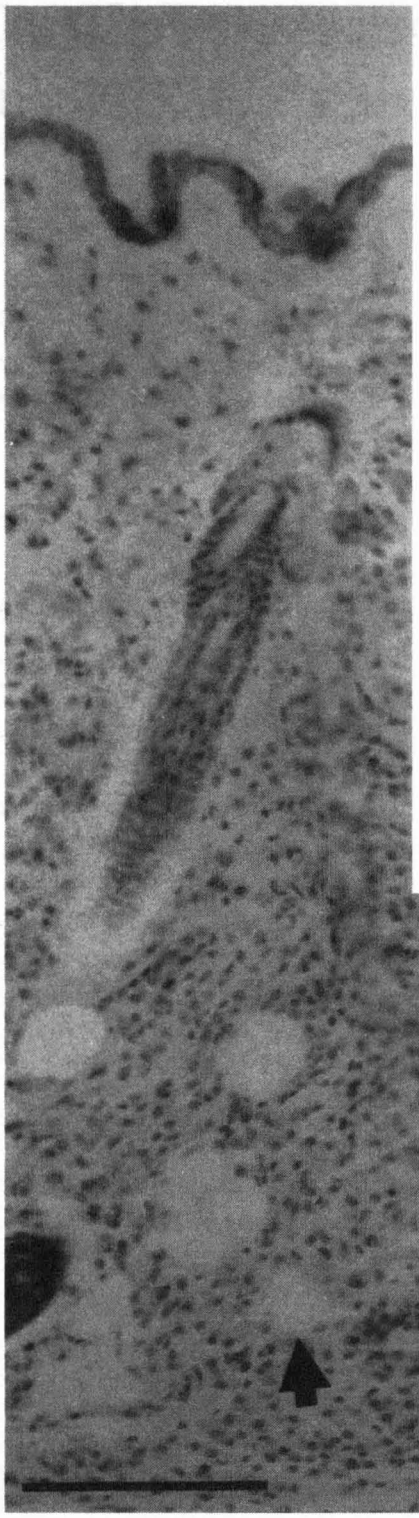
**Apolipoprotein E Deficiency and a High Fat Diet Leads to Cutaneous Foam Cell Formation** On gross inspection, no cutaneous abnormalities were noted in homozygous apolipoprotein-E–deficient mice fed either a low-or high-fat diet, and the skin was indistinguishable from that of controls. However, during dissection, it was very difficult to separate the skin from the carcass in apolipoprotein-E–deficient mice (−/−) fed the high-fat diet. Moreover, on microscopic examination, the homozygous apolipoprotein E–deficient mice fed the high-fat diet displayed a diffuse inflammatory infiltrate in the deeper layers of the dermis, which replaced most of the subjacent subcutaneous fat layer (**Fig 2** as compared to control, **Fig 1**). Interspersed among the inflammatory cells were fat droplets within macrophages, surrounded by an intense foreign-body reaction, consisting of lymphocytes, macrophages, foam cells, and multinucleated giant cells **(Fig 3)**.

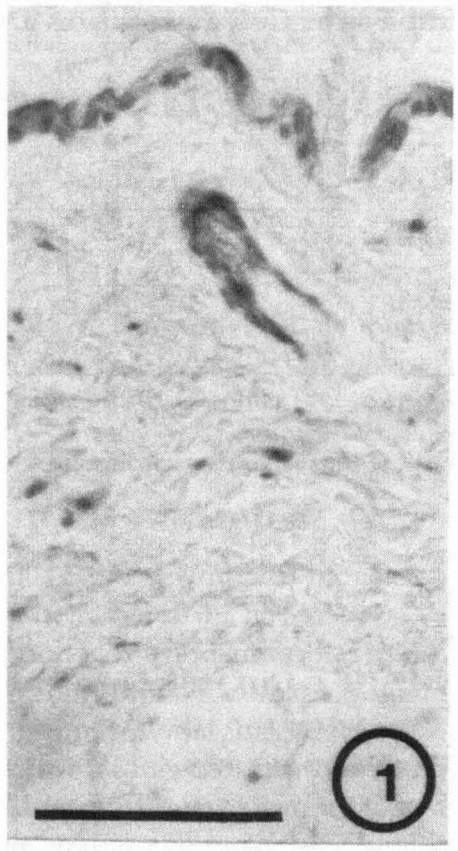
Homozygous apolipoprotein E–deficient mice fed the low-fat diet demonstrated similar lesions, but they tended to be focal, and both fat droplets and foreign-body reaction were far less prominent **(Fig 4)**. In heterozygous apolipoprotein E–deficient mice (+/−) fed the high-fat diet, a few inflammatory cells permeated the dermis, but frank foam cells were absent **(Fig 5)**. Heterozygous apolipoprotein E–deficient mice (+/−) fed a low-fat diet appeared similar to control mice (+/+) fed the same diet (**Fig 6**, compared to control **Fig 1**). Control animals given the high-fat diet displayed scattered inflammatory cells in the dermis.

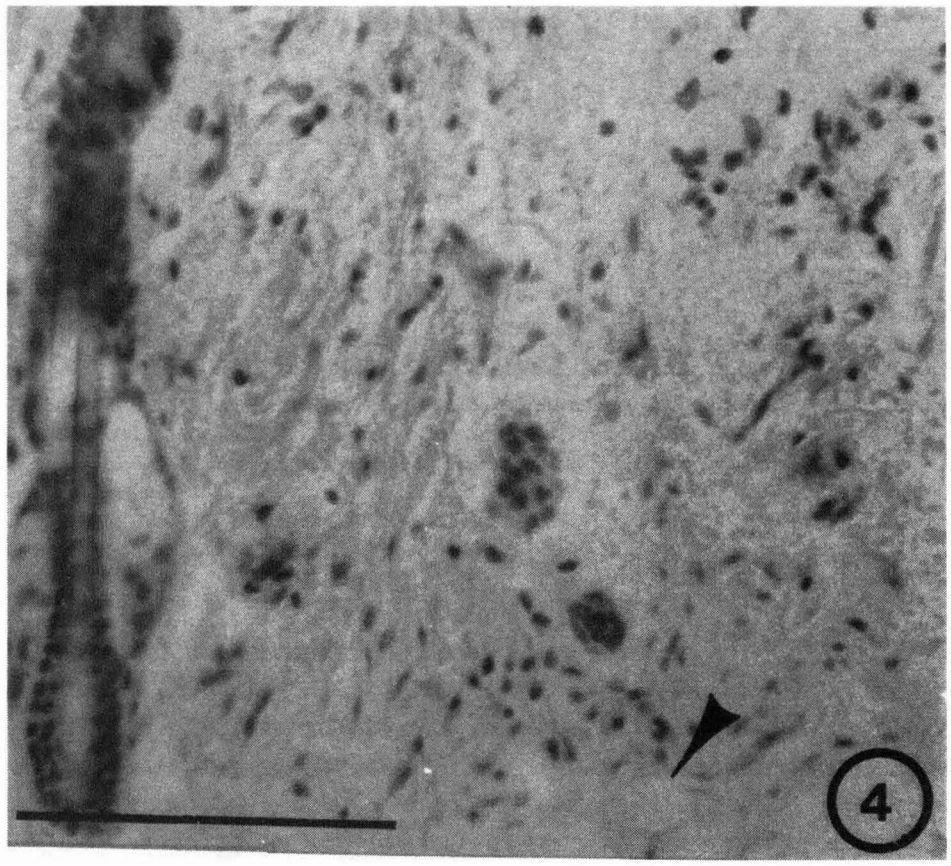
These results demonstrate that genetically engineered mice with apolipoprotein E deficiency develop cutaneous foam cells. The extent of cutaneous foam cell formation correlates directly with the degree of atherosclerosis in the aorta, as reported previously **(Table I)** [15], which suggests that these processes occur by similar mechanisms. In fact, in both the aorta and the dermis, the pathologic lesions consist of macrophages filled with lipid. It is likely that the cellular processes that result in the increased uptake of lipoproteins by macrophages are the same in both tissues. Thus, the cutaneous manifestations of apolipoprotein E deficiency appear to mirror accurately the internal consequences of this metabolic disturbance.

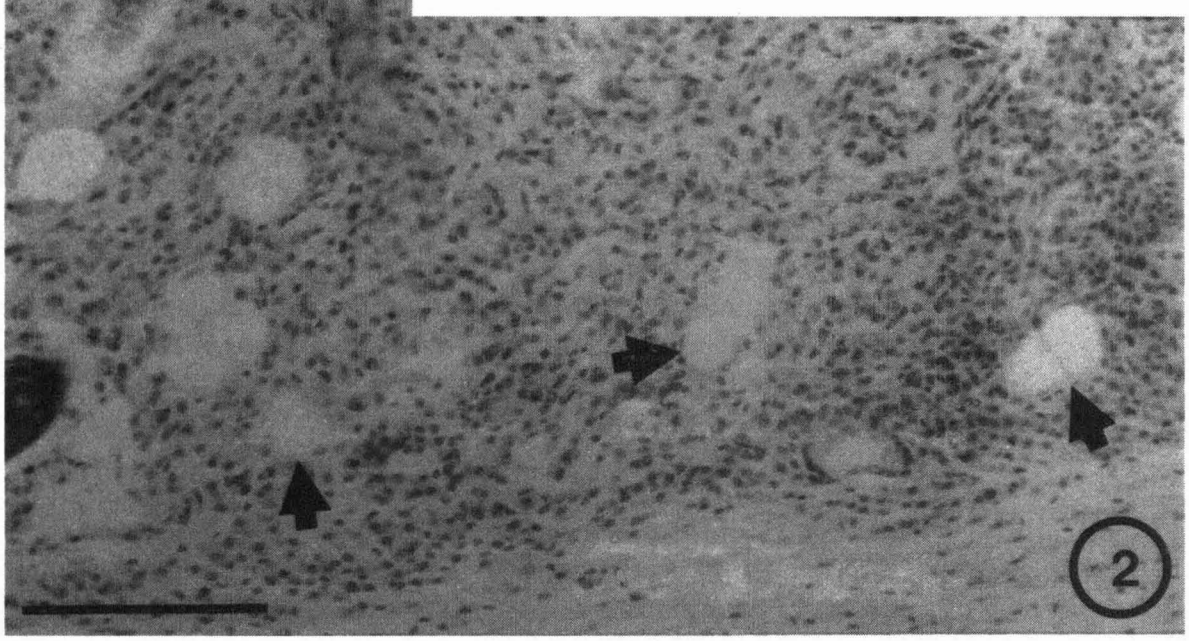
It is interesting that the cutaneous foam cells in this animal model are surrounded by inflammatory cells. These lesions are very similar in histologic appearance to tuberoeruptive xanthoma, which is well recognized to occur in patients with abnormalities in apolipoprotein E metabolism [4,5]. In contrast, animals with homozygous low-density lipoprotein (LDL)–receptor deficiency develop a different type of xanthoma [18–20). In LDL-receptor–deficient animals, xanthomas are located in digital joints, ears, and eyelids, and they lack an inflammatory component. Treatment of LDL-receptor–deficient animals with cholesterol-lowering drugs decreases both aortic atherosclerosis and xanthomas [19]. The lesions seen in LDL-receptor–deficient animals are analogous to the tendinous xanthomas that develop in patients with familial hypercholesterolemia who are also LDL-receptor–deficient [4,5]. Why apolipoprotein E deficiency leads to an inflammatory component is not known, but a likely possibility is that the uptake of chylomicrons and/or very low–density lipoprotein remnants by macrophages leads to the secretion of chemo-attractant cytokines.

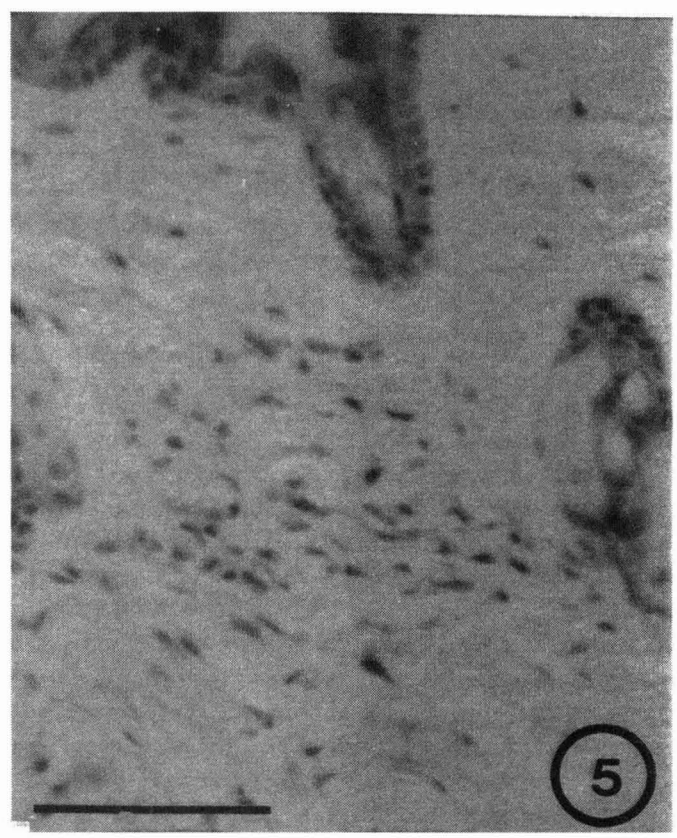
**Apolipoprotein E Deficiency Does Not Affect the Cutaneous Permeability Barrier** There were no differences in permeability-barrier function, as assessed by transepidermal water-loss rates, in control (+/+) or homozygous apolipoprotein-E–deficient mice (−/−). In both groups, water loss was less than 0.15 $mg/cm^2/h$. In addition, we observed no abnormalities in the structure of the lamellar bilayers in the stratum corneum interstices or in the appearance of lamellar bodies within stratum granulosum cells **(Fig 7)**. Although the present study did not delineate the role of apolipoprotein E in the epidermis, the approach of eliminating a

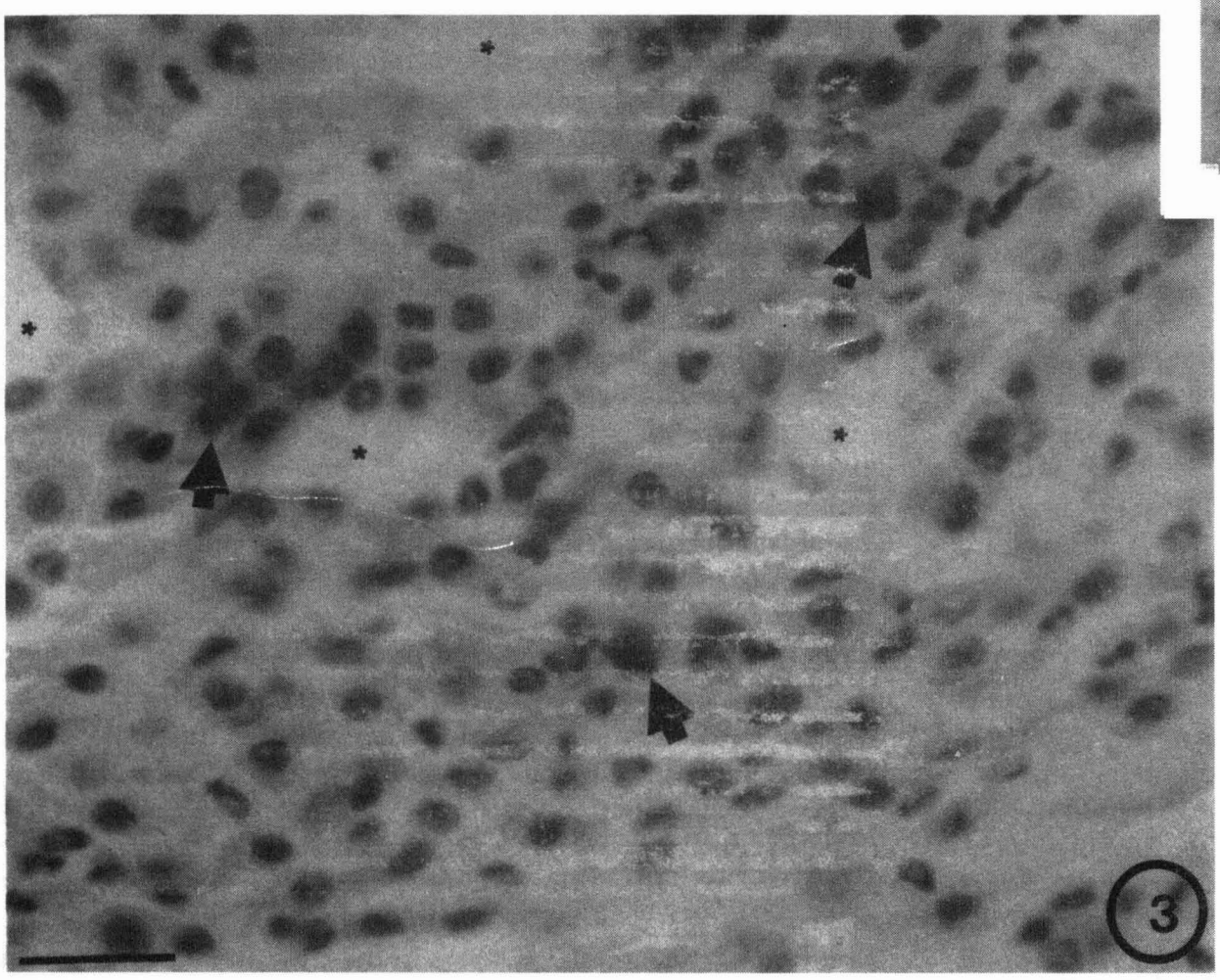












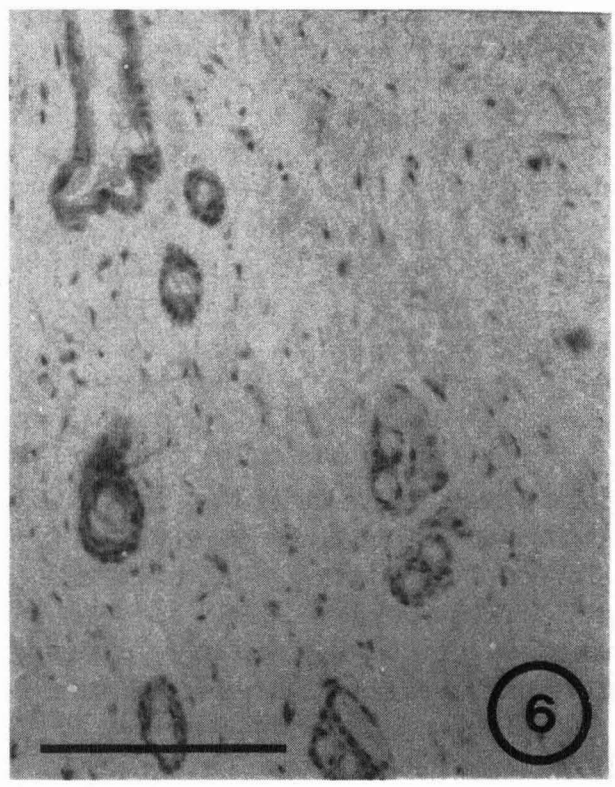


**Figure 7. Stratum corneum membrane structure in homozygous apolipoprotein-E–deficient mice.** In ruthenium tetroxide post-fixed samples, the intercellular spaces are filled with normal-appearing membrane structures. These lamellar bilayers display a normal lamellar bilayer unit structure [16] (*arrows* indicate sites where membrane substructure can be clearly seen). *Bar*, 1 μm.

protein to determine its potential function has limitations. For example, the protein under study may play an important role, but if other proteins can function in a similar manner, they may be able to compensate for the absence of the eliminated protein. In addition, only subtle changes may accompany elimination of a protein under basal or unchallenged conditions. For example, animals homozygous for a deficiency of tumor necrosis factor 55-kd receptor appear normal until infected with *Listeria monocytogenes*, after which they demonstrate an inability to contain the infection [21,22]. Thus, the fact that we did not encounter abnormalities in barrier function or epidermal appearance does not exclude important functions for apolipoprotein E in the epidermis.

In conclusion, our study found that the lack of apolipoprotein E production in the epidermis does not appear to lead to any detectable abnormality in the morphology of lamellar lipid structures or in cutaneous barrier function. On the other hand, the lack of apolipoprotein E leads to cutaneous foam cell formation, presumably secondary to disturbances in lipoprotein metabolism.

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**Figure 1. Normal low-fat diet.** *Bar*, 100 μm. **Figure 2. Homozygous apolipoprotein E–deficient, high-fat diet.** A diffuse inflammatory infiltrate is present in the dermis, which is most intense in the deeper layers. Note the presence of abundant fat droplets (*arrows*), surrounded by a predominantly mononuclear cell infiltrate. The epidermis, appendages, and papillary dermis appear largely unaffected. *Bar*, 100 μm. **Figure 3. Homozygous apolipoprotein E–deficient, high-fat diet.** Upon higher magnification, the dermal inflammatory infiltrate can be seen as enriched in lymphocytes, foam-laden macrophages, and multinucleated giant cells (*arrows*) interspersed among the fat droplets (*asterisks*). *Bar*, 20 μm. **Figure 4. Homozygous apolipoprotein E–deficient, low-fat diet.** Lower fat intake results in a reduction both in the intensity of the inflammatory infiltrate and in the number of lipid droplets (*arrow*) in the reticular dermis. *Bar*, 100 μm. **Figure 5. Heterozygous apolipoprotein-E–deficient, high-fat diet.** The number of inflammatory cells in the dermis is slightly increased, but neither lipid droplets nor foam cells are present. *Bar*, 100 μm. **Figure 6. Heterozygous apolipoprotein-E–deficient, low-fat diet.** A slight increase in the number of inflammatory cells is present, but neither lipid droplets nor foam cells can be discerned. These sections appear indistinguishable from those of control mice (+/+) fed a low-fat diet. *Bar*, 100 μm.

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