Effect of cutaneous permeability barrier disruption on HMG-CoA reductase, LDL receptor, and apolipoprotein E mRNA levels in the epidermis of hairless mice

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Abstract Disruption of the permeability barrier results in an increase in cholesterol synthesis in the epidermis. Inhibition of cholesterol synthesis impairs the repair and maintenance of barrier function. The increase in epidermal cholesterol synthesis after barrier disruption is due to an increase in the activity of epidermal HMG-CoA (3-hydroxy-3-methylglutaryl CoA) reductase. To determine the mechanism for this increase in enzyme activity, in the present study we have shown by Western blot analysis that there is a 1.5-fold increase in the mass of HMG-CoA reductase after acute disruption of the barrier with acetone. In a chronic model of barrier disruption, essential fatty acid deficiency, there is a 3-fold increase in the mass of HMG-CoA reductase. Northern blot analysis demonstrated that after acute barrier disruption with acetone or tape-stripping, epidermal HMG-CoA reductase mRNA levels are increased. In essential fatty acid deficiency, epidermal HMG-CoA reductase mRNA levels are increased 3-fold. Thus, both acute and chronic barrier disruption result in increases in epidermal HMG-CoA reductase mRNA levels which could account for the increase in HMG-CoA reductase mass and activity. Additionally, both acute and chronic barrier disruption increase the number of low density lipoprotein (LDL) receptors and LDL receptor mRNA levels in the epidermis. Moreover, epidermal apolipoprotein E mRNA levels are increased by both acute and chronic perturbations in the barrier. Increases in these proteins in response to barrier disruption may allow for increased lipid synthesis and transport between cells and facilitate barrier repair.—**Jackson, S. M., L. C. Wood, S. Lauer, J. M. Taylor, A. D. Cooper, P. M. Elias, and K. R. Feingold.** Effect of cutaneous permeability barrier disruption on HMG-CoA reductase, LDL receptor, and apolipoprotein E mRNA levels in the epidermis of hairless mice. *J. Lipid Res.* 1992. **33:** 1307–1314.

Supplementary key words transepidermal water loss • cholesterol synthesis • apoA

The stratum corneum, the outermost layer of mammalian epidermis, is the site of the permeability barrier that prevents excessive water loss from the body. Although composed primarily of protein, it is the lipid component of the stratum corneum that is crucial for barrier function (1). The three major lipid classes found in the stratum corneum, cholesterol, ceramides, and fatty acids, are all synthesized de novo in the epidermis (2). Disruption of the barrier, acutely either by solvent extraction of stratum corneum lipids or tape-stripping away stratum corneum layers, or chronically by feeding an essential fatty acid-deficient diet, stimulates the de novo synthesis of cholesterol, ceramides, and fatty acids in the epidermis (2–6). Additionally, barrier disruption stimulates epidermal DNA synthesis in these same models (7). Acute disruption of the barrier by either acetone or tape-stripping results in a rapid increase in epidermal lipid synthesis (within 1 to 2 h) while epidermal DNA synthesis is increased 20–24 h later (3, 5–7). While the increase in lipid synthesis could potentially provide lipids required for new cell membrane synthesis, studies by our laboratory have shown that the increase in lipid synthesis is important for barrier repair (8, 9). Inhibition of either epidermal cholesterol or sphingolipid synthesis slows the return of lipids to the stratum corneum, delaying barrier recovery (8, 9).

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; EFAD, essential fatty acid deficiency; PMSF, phenylmethylsulfonyl fluoride; MOPS, 3-[N-morpholino] propane sulfonic acid; LDL, low density lipoprotein; TEWL, transepidermal water loss.

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The stimulation of epidermal cholesterol synthesis after barrier disruption, in both the acute and chronic models, is due to an increase in the activity of HMG-CoA reductase (10), the rate-limiting enzyme in cholesterol synthesis. The activity of HMG-CoA reductase is dependent on two factors: the total quantity of enzyme present and the activation state of the enzyme, which can be modulated by a reversible phosphorylation–dephosphorylation (the dephosphorylated form is active) (11, 12). During the isolation of epidermal microsomes in NaCl buffer, dephosphorylation (activation) of HMG-CoA reductase occurs (10) and therefore HMG-CoA reductase assays performed on these samples are thought to represent an index of the total quantity of enzyme present (11). If microsomes are isolated in an NaF buffer, which blocks dephosphorylation, assays of HMG-CoA reductase activity are thought to be indicative of the active enzyme in situ (11). Our studies have demonstrated that the increase in HMG-CoA reductase activity in the epidermis after barrier disruption is due to both dephosphorylation (activation) of the enzyme and an apparent increase in the quantity of enzyme determined by measuring activity in microsomes isolated in NaCl buffer (10). The increase in the quantity of enzyme based on total activity measurements was maximal at 2.5 h after barrier perturbation (10).

The mass of HMG-CoA reductase can be regulated by multiple mechanisms. Studies have shown that the rate of degradation of HMG-CoA reductase protein varies (13–15). Additionally, the rate of transcription of the gene for HMG-CoA reductase (16–18), the degradation of the message (18), and the translation of message (14, 19) can be altered by various manipulations. One purpose of the present study was to assess the mechanism(s) by which disruption of the permeability barrier leads to an increase in the activity of HMG-CoA reductase in the epidermis. We directly measured the quantity of HMG-CoA reductase in the epidermis by immunoblotting, and determined the level of HMG-CoA reductase mRNA after barrier disruption. Additionally, we determined whether the regulation of other proteins that are important in cholesterol homeostasis, such as the LDL receptor and apoE, were altered by barrier disruption.

METHODS

Materials

(α-^{32}P)dCTP (3000 Ci/mmol, 10 mCi/ml) was purchased from NEN Research Products (Boston, MA). The Multiprime DNA Labeling System was purchased from Amersham International (Amersham, UK). Mini-spin columns (G-50) were purchased from Worthington Biochemical Corporation (Freehold, NJ). Molecular grade chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Fairlawn, NJ). Oligo(dT)-cellulose, type 77F, was purchased from Pharmacia LKB Biotechnology AB (Uppsala, Sweden). Chemicals for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Bio-Rad Laboratories. Antibody binding was detected by the Western-Light chemiluminescent detection system (Tropix Inc., Bedford, MA). Nitrocellulose for transfers was purchased from Schleicher and Schuell (Keene, NH). The complimentary DNA for HMG-CoA reductase and for LDL receptor were purchased from the American Type Culture Collection (Rockville, MD): clone name pH Red-102, ATCC No. 57042 and clone name pLDLR$_3$, HTCC No. 57004. Human beta-actin cDNA was obtained from Dr. Peter Gunning of Stanford University. Murine apoE and apoA-I cDNA were prepared as described previously (20). Kodak XAR5 Film was used for autoradiography.

Animals

Hairless male mice (Hr/Hr) were purchased from Simonsen (Gilroy, CA) and were fed a standard mouse diet (Simonsen Labs, Gilroy, CA), and water ad libitum unless stated otherwise.

Acute barrier perturbation

The whole surface area of male hairless mice except for the head, tail, and limbs was treated by gently applying acetone-soaked cotton balls for 5–10 min as described in previous publications (3, 5, 10) or by applying and removing cellophane tape (Scotch^R-type) 6–8 times successively (10). Animals treated with cotton balls soaked in 0.9% sodium chloride or untreated animals (tape-stripped experiments) served as controls. Transepidermal water loss (TEWL) was measured immediately after treatment using a Meeco electrolytic water analyzer, as described previously (3, 5, 10). Animals with TEWL rates greater than 4.0 mg/cm^2 per h (normal $<$0.3 mg/cm^2 per h) after barrier disruption were included in this study.

Chronic barrier perturbation

Hairless mice, 1–2 weeks of age, were maintained on an essential fatty acid-deficient (EFAD) diet until TEWL rates exceeded 4.0 mg/cm^2 per h, which occurred by 2–3 months (4, 21). For control animals the EFAD condition was reversed by feeding with the same diet supplemented with corn oil for 4 days prior to study, by which time TEWL had returned to normal levels.

Isolation of epidermis

At appropriate time points after disruption of the barrier, the animal was killed by cervical dislocation and the skin was excised and placed stratum corneum side downward on a plastic Petri dish. Subcutaneous fat was removed by firm scraping with a scalpel blade. The skin was

then placed dermis side down onto a solution of 10 mM EDTA in calcium- and magnesium-free PBS, pH 7.4, for 35 min at 37°C (3, 5, 10). After incubation the skin samples were again placed dermis side downward on a plastic Petri dish, and the epidermis was isolated by firm scraping with a scalpel blade. The full thickness epidermis was either snap-frozen in liquid nitrogen and stored at −80°C until removal for RNA isolation, or placed into homogenization buffer A (0.25 M sucrose, 15 mM EGTA, 15 mM EDTA, 10 mM Tris-HCl, pH 7.0, PMSF 17 μg/ml, leupeptin 25 μg/ml, and aprotonin 1 μg/ml) and stored for less than 1 h on ice before microsome isolation.

Isolation of epidermal microsomes

Microsomes were isolated as previously described (10). Briefly, epidermis (0.05–0.1 g from one flank) was homogenized in 3 ml of buffer A using a glass-Teflon, hand-held homogenizer and subsequently sonicated for two 10-sec bursts at 35% intensity with a 10-sec pause on ice between each sonication. The homogenate was centrifuged at 16,000 *g* for 15 min at 4°C; the supernatant was removed and recentrifuged as above. The final supernatant was centrifuged at 100,000 *g* for 1 h at 4°C to pellet microsomes. The pellet was washed and subsequently resuspended in 50 μl of buffer A. Protein determination (Bio-Rad) gave values of between 0.5 and 2.0 mg/ml of microsomal protein.

Isolation of epidermal mRNA

Total RNA was isolated by a variation of the guanidinium thiocyanate method (22). Briefly, epidermis (0.2–0.4 g from whole area of two mice) was homogenized in 4 ml solution D (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% N-lauroylsarcosine, 0.1 M 2-mercaptoethanol) using a ground-glass hand-held homogenizer and then sonicated twice for 15 sec with a 30-sec intervening pause on ice. After the addition of acid phenol and chloroform–isoamylalcohol 24:1, RNA was precipitated from the aqueous phase with isopropanol and subsequently washed and reprecipitated 3 times. The final pellet was dissolved in RNase-free water (RNA-H_2O) and heated at 65°C for 5 min; oligo(dT)-cellulose (10 mg) was then added and incubation at room temperature was continued for 30 min. The oligo(dT)-cellulose was washed 4 times in 5 ml high salt buffer (0.5 M NaCl, 20 mM Tris, 1 mM EDTA, 0.2% SDS, pH 7.6) and transferred to microfuge tubes with 1 ml of low salt buffer (0.1 M NaCl, 20 mM Tris, 1 mM EDTA, 0.1% SDS, pH 7.6). Poly A(+) RNA was eluted from the oligo(dT)-cellulose with 3 successive washes in RNA-H_2O warmed to 65°C. mRNA in 0.6 ml RNA-H_2O was precipitated from solution by adding 3 M sodium acetate (60 μl) and 1.2 ml 100% ethanol and incubating at −80°C for 1 h. mRNA was resuspended in 50 μl of RNA-H_2O and the absorbance was determined at 260 and 280 nm with yields of 10–30 μg of epidermal poly A(+) RNA obtained from the entire epidermis of two mice.

Western blotting

For the determination of HMG-CoA reductase protein, SDS-PAGE was performed in the presence of urea, as described by Clarke et al. (23) with the following modifications. Microsomal protein (20 μg) was added to an equal volume of buffer (15% SDS, 8 M urea, 10% 2-mercaptoethanol, 62.5 mM Tris-HCl, pH 6.8) along with one-fifth volume of solution B (50% glycerol, 0.01% bromophenol blue). The samples were heated at 37°C for 30–45 min prior to loading on an 8 M urea, 7.5% polyacrylamide gel with a 6 M urea, 5% polyacrylamide stacking gel. For determination of LDL receptor number, SDS-PAGE was carried out in nonreducing conditions as described previously (24). Protein was transferred to nitrocellulose (0.2 μm pore size) and the level of HMG-CoA reductase protein was determined using HMG-CoA reductase antiserum (a gift from Drs. A. Alberts and J. Germershausen of Merck, Sharpe & Dohme, Inc., Rahway, NJ) and the level of LDL receptor protein was determined using LDL receptor antiserum (24). A Tropix Western Light™ chemiluminescent detection system was used. After X-ray film development, band density was measured using an E-C Apparatus Corporation Densitometer (St. Petersburg, FL).

Northern blotting

Aliquots of poly A(+) RNA (4–8 μg) were solubilized in 20 μl sample buffer (48% formamide, 2.1 M formaldehyde, 1.1 × MOPS buffer, 5.3% glycerol, and 0.027% bromophenol blue) and run on agarose/formaldehyde (1%/6.1%) gels for 3 h at 75 V. Ribosomal RNA bands were stained with acridine orange and photographed. RNA was transferred to nitrocellulose (0.45 μm pore size), subsequently fixed by baking at 80°C for 2 h, and stored at −20°C until required for hybridization. [^{32}P]dCTP radiolabeled cDNA probes were prepared by random priming (Amersham Multiprime DNA Labeling System) with incubations at room temperature for 3–4 h. Blots were prehybridized at 42°C for 30 min in buffer F (50% formamide, 5 × SSPE, 1% SDS, 10% dextran sulfate, 100 μg/ml sheared salmon sperm DNA). Hybridization with radiolabeled probes was performed in buffer F overnight at 42°C followed by a 30-min room temperature wash and a 60-min 65°C wash in wash buffer (0.2 × SSC, 0.1% SDS, pH 7.0). Air-dried blots were exposed to X-ray film and bands were quantified by densitometry.

Statistics

Statistical significance was determined by Student's *t* test or a paired *t* test.

RESULTS

Effect of barrier disruption on HMG-CoA reductase

Our initial studies addressed whether the acute disruption of the barrier by solvent extraction affected the quantity of HMG-CoA reductase in the epidermis, as measured by Western blots. **Fig. 1** shows the quantitative analysis of HMG-CoA reductase mass in samples from saline-treated control flanks and flanks 2.5 h after barrier disruption by topical treatment with acetone. This time represents the point at which the maximal increase in enzyme activity occurred after barrier disruption with acetone (10). A statistically significant, 1.5-fold increase in the quantity of HMG-CoA reductase was found in the acetone-treated versus control flanks (Fig. 1), an increase in HMG-CoA reductase content that is similar to the increase in total enzyme activity at the same time point (10).

With chronic disruption of the barrier, induced by an EFAD diet, the quantity of HMG-CoA reductase in the epidermis was increased approximately 3-fold (Fig. 1). Again, this increase in enzyme quantity correlates with the increase in total HMG-CoA reductase activity that we observed in prior studies in EFAD animals (10). These results indicate that barrier disruption is associated with a significant increase in the quantity of HMG-CoA reductase in the epidermis.

We next determined whether barrier perturbation influences the levels of HMG-CoA reductase mRNA in epidermis. In order to isolate sufficient mRNA for analysis, we pooled epidermis from two separate animals. When we compared the quantity of epidermal HMG-CoA reductase mRNA 2.5 h after acetone treatment versus control epidermis, there was a 2.0-fold increase in the animals with a disrupted barrier (**Fig. 2**). Similarly, tape-stripping, another acute model of barrier disruption, also resulted in an increase (2.3-fold) in the amount of steady-state epidermal HMG-CoA reductase mRNA levels at 2.5 h after treatment (Fig. 2). Finally, chronic barrier disruption induced by an EFAD diet produced a 3-fold increase in steady-state epidermal HMG-CoA reductase mRNA levels (Fig. 2). Thus, both acute and chronic forms of barrier disruption are associated with increases in steady-state epidermal HMG-CoA reductase mRNA levels that correlate temporally with previously described modulations in enzyme activity (10), cholesterol synthesis (3, 5), and enzyme content (these studies).

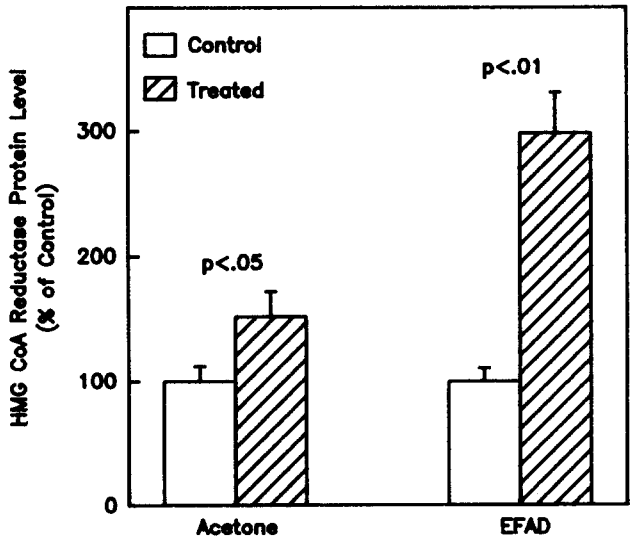

Fig. 1. Effect of acute or chronic barrier disruption on epidermal HMG-CoA reductase protein levels. Mice were treated topically with acetone on one flank or fed an essential fatty acid-deficient diet until TEWL values were > 4 mg/cm² per h. Protein was isolated from the epidermis 2.5 h after acetone treatment. Western blotting was performed as described in Methods. Data are presented as mean ± SEM and expressed as a percentage of the value obtained for the controls. Saline-treated control, n=8; acetone-treated, n=8; EFAD control (fed corn oil for 4 days) n=4; EFAD, n=4.

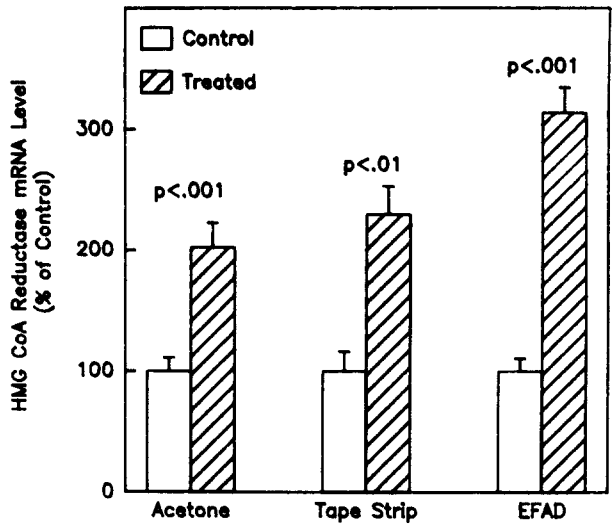

Fig. 2. Effect of acute and chronic barrier disruption on HMG-CoA reductase mRNA levels. Mice were treated topically with acetone or tape-stripped, or fed an essential fatty acid diet-deficient until TEWL values were > 4 mg/cm² per h, as described in Methods. Poly A(+) RNA was isolated from epidermis 2.5 h after acetone treatment or tape-stripping. Northern blotting was performed as described in the Methods. Data are presented as mean ± SEM and expressed as a percentage of the values for the control animals. Saline-treated control, n=12; acetone-treated n=12; untreated control, n=5, tape-stripped, n=5; EFAD control, n=7; EFAD, n=8.

We simultaneously determined the quantity of beta-actin mRNA levels. We observed unexpectedly that acute disruption of the barrier with either acetone treatment or tape-stripping increased epidermal steady-state beta-actin mRNA levels approximately 1.7-fold 2.5 h after treatment (**Fig. 3**). However, chronic barrier disruption did not significantly affect beta-actin mRNA steady-state levels (Fig. 3).

Effect of barrier disruption on the LDL receptor

The effect of barrier disruption on LDL receptor number is shown in **Fig. 4**. In acute models of barrier disrup-

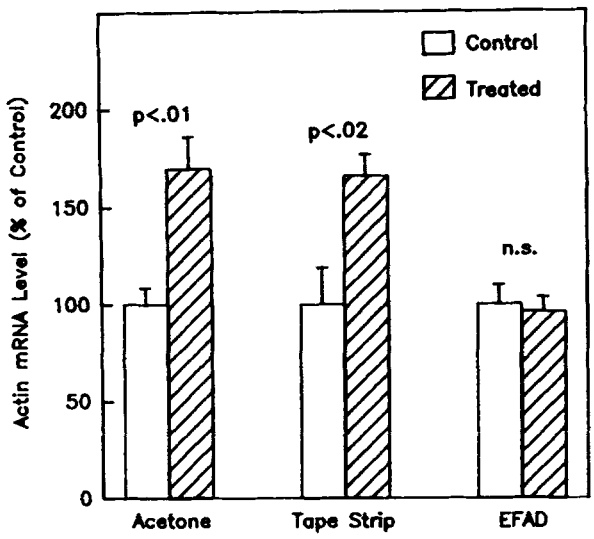

Fig. 3. Effect of acute and chronic barrier disruption on beta-actin mRNA levels. A description of the experimental procedures is given in the legend to Fig. 2. Data are presented as mean ± SEM and expressed as a percentage of the values for the control animals. Saline-treated control, n=12; acetone-treated, n=12; untreated control, n=5; tape-stripped n=5; EFAD control, n=7; EFAD, n=8.

tion, acetone treatment or tape stripping, there was 72% and 59% increase in LDL receptor number, respectively. In EFAD, a chronic model of barrier disruption, a 2.4-fold increase in LDL receptor number was observed. These results indicate that barrier disruption increases the quantity of the LDL receptor in the epidermis.

We next determined the effect of barrier disruption on LDL receptor steady-state mRNA levels in the epidermis. As illustrated in **Fig. 5** both acute and chronic models of barrier disruption increased LDL receptor mRNA levels. Thus, analogous to HMG-CoA reductase, barrier perturbation stimulates both an increase in LDL receptor mass and steady-state mRNA levels in the epidermis.

Effect of barrier disruption on apoproteins

The effect of barrier disruption on apolipoprotein E mRNA levels in the epidermis is shown in **Fig. 6**. Both acetone treatment and EFAD resulted in statistically significant increases in apoE steady-state mRNA levels in the epidermis. In contrast, apoA-I mRNA was not detected in control epidermis or in epidermis after either acute or chronic disruption of the barrier (data not shown).

Time course of effects of acute barrier disruption on mRNA levels

In order to examine the kinetics of the response to barrier disruption, we next determined the steady-state levels of HMG-CoA reductase, LDL receptor, apoE, and beta-actin mRNAs 30 min, 1, 2.5, 4, and 8 h after disruption of the barrier with acetone. As shown in **Fig. 7**, HMG-CoA reductase and LDL receptor mRNA levels showed a significant increase as early as 30 min after acetone disruption which was sustained for at least 8 h. In contrast, apoE mRNA levels were unchanged at 1 and 2.5 h but were significantly increased at 4 and 8 h (the increase at 8 h was relatively small but, because of very small standard errors, it was statistically significant). At 30 min and 1 h, beta-actin mRNA levels were not increased but at 2.5, 4, and 8 h there was a significant increase in beta-actin mRNA levels. Thus, the timing of the increase in mRNA levels after barrier disruption differs depending upon which mRNA species is analyzed.

DISCUSSION

Lipids in the stratum corneum are essential components of the cutaneous permeability barrier (1, 25). Prior studies have shown that epidermal cholesterol synthesis increases rapidly after barrier disruption (3, 5), and that the magnitude of this increase in synthesis directly correlates with the degree of barrier disruption (3). Moreover, the stimulation of epidermal cholesterol synthesis in response to barrier disruption is due to an increase in HMG-CoA reductase activity in the epidermis (10). Such increases in HMG-CoA reductase activity are observed in both acute (solvent extraction, tape-stripping, detergent

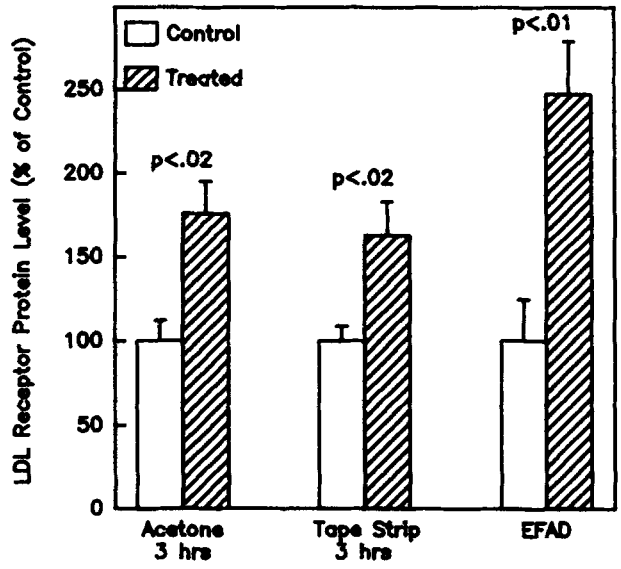

Fig. 4. Effect of acute or chronic barrier disruption on epidermal LDL receptor protein levels. Mice were treated topically with acetone or tape-stripped, or fed an essential fatty acid-deficient diet until TEWL were > 4 mg/cm² per h, as described in Methods. Protein was isolated from the epidermis 2.5 h after acetone treatment or tape-stripping. Western blotting was performed as described in Methods. Data are presented as mean ± SEM and expressed as a percentage of the value obtained for the controls (saline-wiped for acetone, untreated for tape-stripped, fed corn oil for 4 days for EFAD). Acetone control, n=4; acetone-treated, n=4; tape-stripped control, n=8; tape-stripped, n=8; EFAD control, n=4; EFAD, n=4.

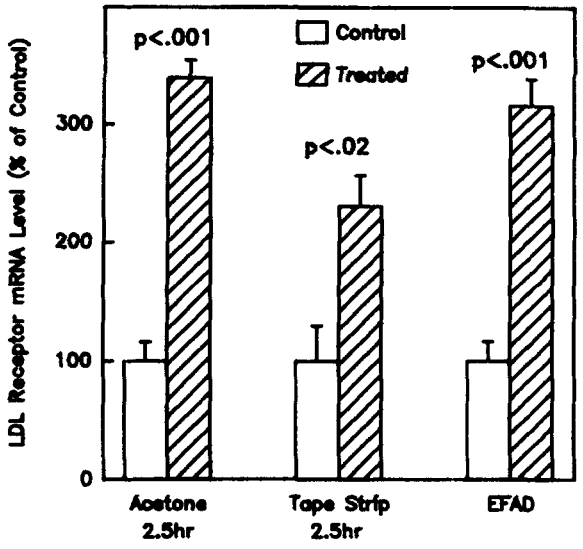

Fig. 5. Effect of acute and chronic barrier disruption on LDL receptor mRNA levels. A description of the experimental procedures is given in the legend to Fig. 2. Data are presented as mean ± SEM and expressed as a percentage of the values for the control animals. Saline-treated control, n=12; acetone-treated, n=12; untreated control, n=5; tape-stripped, n=5; EFAD control, n=7; EFAD, n=8.

treatment) and chronic (EFAD) forms of barrier disruption (10). Furthermore, recent studies have demonstrated that inhibition of cholesterol synthesis with the HMG-CoA reductase inhibitor, lovastatin, delays barrier repair after acute disruption of the barrier (8). Thus, modulations in epidermal cholesterol synthesis play a key role in the maintenance of the cutaneous permeability barrier.

In the present study we have investigated the mechanism by which the activity of HMG-CoA reductase is increased after perturbation of the permeability barrier. We observed a significant increase in the mass of epidermal HMG-CoA reductase in both acute and chronic models of barrier disruption, which corresponded both quantitatively and temporally with prior observations of changes in enzyme activity (10). We next explored the effect of barrier disruption on the steady-state levels of epidermal HMG-CoA reductase mRNA levels. Both acute and chronic forms of barrier disruption significantly increase epidermal HMG-CoA reductase mRNA levels. The increase in steady-state HMG-CoA reductase mRNA levels in the epidermis occurs prior to the increase in both total HMG-CoA reductase activity (10) and quantity, suggesting that an increase in steady-state mRNA levels is one mechanism that accounts for the above-described changes in cholesterol synthesis.

Previous studies have shown that LDL receptors are present in the epidermis (26). In the present study, we now demonstrate that acute and chronic disruption of the barrier increases the number of LDL receptors in the epidermis. Moreover, this increase in LDL receptor number in both acute and chronic models of barrier disruption is associated with an increase in LDL receptor steady-state mRNA levels. The seminal studies of Goldstein and Brown (27) have demonstrated the coordinate regulation of HMG-CoA reductase and LDL receptors in response to intracellular cholesterol levels, and it is possible that the increased requirement for cholesterol induced by barrier disruption stimulates the transcription of both genes. The importance and role of the increase in LDL receptor number in response to barrier perturbation are unknown. One could speculate that an increase in LDL receptors would allow for the increased delivery of lipid from systemic sources to the epidermis and/or for the transport of lipids within the epidermis.

ApoE is produced in many extrahepatic tissues (20) and epidermal cells have been shown to be an abundant source of this apolipoprotein (28, 29). In the present study, we demonstrate that both acute and chronic disruption of the barrier increase apoE steady-state mRNA levels in the epidermis. Of note is that after acute disruption of the barrier the time course for the increase in apoE mRNA levels is delayed in comparison to the rapid increase in HMG-CoA reductase and LDL receptor mRNA levels. Although the role of apoE in the epidermis is unclear, studies in regenerating nerve suggest that apoE may play a role in mediating lipid movement between cells (30-32). In conjunction with the increase in LDL receptors, one could speculate that the increase in apoE represents a response to barrier perturbation that allows for the increased movement of lipid between cells facilitating barrier repair. Additionally, it has been suggested that

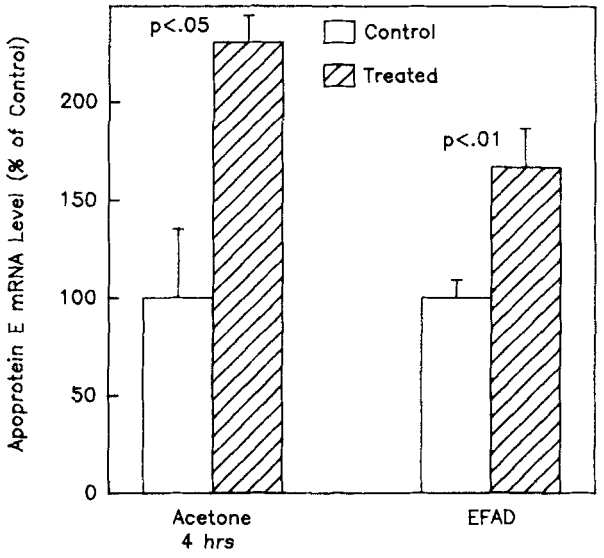

Fig. 6. Effect of acute and chronic barrier disruption on apoE mRNA levels. A description of the experimental procedures is given in the legend to Fig. 2 except that poly A(+) RNA was isolated from epidermis 4 h after acetone treatment. Data are presented as mean ± SEM and expressed as a percentage of the values for the control animals. Saline-treated control, n=12; acetone-treated, n=12; EFAD control, n=7; EFAD, n=8.

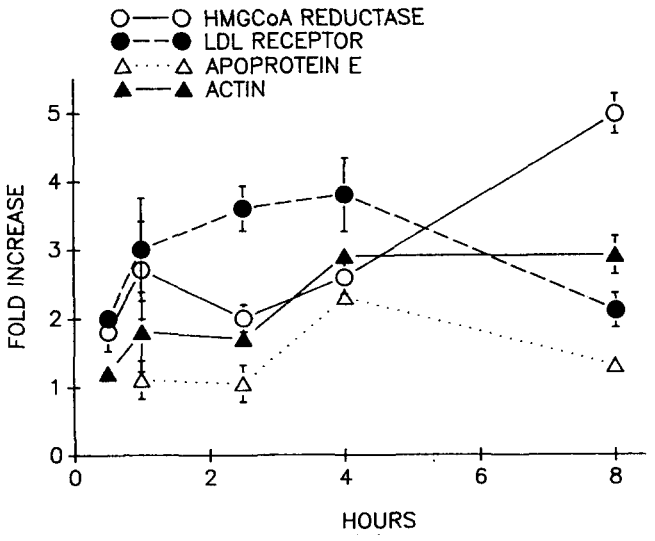

Fig. 7. Time course of changes in HMG-CoA reductase, LDL receptor, apoE and beta-actin mRNA levels after barrier disruption. Mice were treated topically with acetone until TEWL values were > 4 mg/cm² per h. Poly A(+) RNA was isolated from epidermis at 30 min, 1, 2.5, 4, and 8 h after treatment. Northern blotting was performed as described in Methods. Data are presented as mean ± SEM and expressed as a percentage of the values for the control animals, n = 3–12.

apoE may be involved in the formation of the lamellar bilayers present in the intercellular spaces of the stratum corneum (28, 29).

ApoA-I in certain species substitutes for apoE, and recent studies have shown that apoA-I is synthesized in chicken skin (33). In the present study we did not detect the presence of apoA-I mRNA in the epidermis of normal mice or in tissue samples isolated after acute or chronic barrier disruption. This indicates that in this mammalian system apoA-I does not play a role in epidermal biology.

The mechanism(s) accounting for the increases in HMG-CoA reductase, LDL receptor, and apoE mRNA levels that occur after barrier perturbation are unknown. Unfortunately, because of the technical difficulty of isolating sufficient quantities of viable murine epidermis, it is not yet possible to measure gene transcription rates. Similarly, determining the stability of mRNA in the epidermis presents formidable problems, primarily because of the difficulty in delivering inhibitors of RNA synthesis to the epidermis during in vivo studies, as well as the relatively low levels of isolatable mRNA (two adult mice yield 0.2–0.4 g of epidermis, which yields 10–30 μg of mRNA).

In addition to the major findings related to lipid metabolism discussed above, our study also demonstrates that steady-state beta-actin mRNA levels increase in response to barrier perturbation. Actin filaments play a structural role in keratinocytes, and also are involved in the stabilization of secretory organelles (34, 35). As barrier disruption results in the rapid secretion of lipid-enriched lamellar bodies by keratinocytes (36), the increase in steady-state beta-actin mRNA levels may be related to this event or to other changes that occur in response to barrier perturbation. Other investigators have also reported that beta-actin mRNA levels can vary in response to experimental conditions (37, 38). Of note is that chronic barrier disruption induced by feeding an essential fatty acid-deficient diet did not affect beta-actin mRNA levels in the epidermis. This suggests that the increases observed in mRNA levels in response to barrier perturbation are not nonspecific, generalized responses but rather represent a coordinated response. In support of this is the time course data demonstrating that certain mRNA levels increase rapidly after acute barrier disruption (HMG-CoA and LDL) while other mRNA levels are increased only at later time points (apoE).

In summary, the present study indicates that disruption of the cutaneous permeability barrier results in increased steady-state levels of epidermal HMG-CoA reductase mRNA which could account for the increase in HMG-CoA reductase activity and stimulation of epidermal cholesterol synthesis that is required for barrier repair. Moreover, barrier disruption also increases LDL receptor number and mRNA levels and apoE mRNA in the epidermis. Changes in these proteins may allow for increased lipid transport between cells and facilitate barrier repair.

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