Influence of Altered Serum Cholesterol Levels and Fasting on Cutaneous Cholesterol Synthesis

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Barrier perturbation stimulates epidermal cholesterol synthesis, which plays an important role in restoring barrier function. In the present study, we examined whether changes in serum cholesterol levels or nutrition regulate epidermal cholesterol synthesis in hairless mice. Serum cholesterol levels were lowered by 50% after injection with 4-aminopyrazolo (3,4-d) pyrimidine and were increased by 51% by feeding an atherogenic diet. In contrast to most other tissues, cholesterol synthesis in the epidermis and dermis was not inhibited by elevations or stimulated by decreases in serum cholesterol levels. Additionally, feeding a high-cholesterol diet did not decrease epidermal or dermal cholesterol synthesis. However, fasting significantly decreased both epidermal (38%) and dermal (34%) cholesterologenesis. Furthermore, barrier recovery after acetone disruption of the barrier was impaired in fasted animals. However, treatment with topical lipids did not restore barrier repair rate to normal, indicating that factors in addition to lipids are necessary to overcome the effects of fasting. These results demonstrate that cholesterol synthesis in the epidermis and dermis is regulated independently of changes in serum cholesterol levels.
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A primary function of the skin is to provide a barrier against excessive water loss. Stratum corneum extracellular lipids, comprised primarily of cholesterol, ceramides, and fatty acids, are essential components of this barrier [1,2]. The epidermis is a very active site of cholesterol synthesis that provides the cholesterol required for the stratum corneum permeability barrier [3,4]. Acute disruption of the barrier stimulates epidermal cholesterol synthesis with cholesterol synthesis returning towards normal in parallel with the restoration of barrier function [5]. Moreover, essential fatty acid deficiency, a chronic model of barrier disruption, also increases epidermal cholesterol synthesis [6,7]. Furthermore, in both acute and chronic models of barrier disruption, occlusion with a water vapor–impermeable membrane artificially corrects barrier function and prevents the increase in cholesterol synthesis [3,7].

Although these data indicate that epidermal cholesterol synthesis is regulated by barrier function, whether epidermal cholesterol synthesis is also regulated by alterations in serum cholesterol levels is unclear. Whereas differentiating keratinocytes in culture display a paucity of low-density lipoprotein (LDL) receptors, proliferating cultured keratinocytes elaborate LDL receptors [8–10] and exhibit feedback regulation of sterologenesis in response to exogenous LDL [10–12]. Thus, these cells are potentially susceptible to changes in circulating cholesterol levels. In many tissues, decreasing serum cholesterol level has been shown to stimulate cholesterol synthesis although, conversely, increasing serum cholesterol level inhibits cholesterol synthesis [13,14]. Both responses appear to be mediated by LDL receptors on the surface of cells in these tissues. However, cholesterol synthesis in some tissues, such as smooth muscle and cardiac muscle, is insensitive to changes in serum cholesterol levels [13,14].

Thus, one goal of this study was to determine the relationship between serum cholesterol levels and the rate of cholesterol synthesis in the epidermis and dermis. Serum cholesterol levels were lowered with 4-aminopyrazolo (3,4-d) pyrimidine (4-APP) [15,16], or elevated by feeding an atherogenic diet [17]. In addition, we determined whether factors other than changes in serum cholesterol levels, such as fasting, regulate epidermal and dermal cholesterol synthesis. Whereas we found that changes in serum cholesterol levels did not regulate epidermal or dermal cholesterol synthesis, fasting decreased cutaneous cholesterol synthesis.

MATERIALS AND METHODS

Materials Male hairless mice (Hr/Hr), 8–12 weeks old, were purchased from Simonsen Laboratories (Gilroy, CA) or Charles River (Cambridge, MA); 4-APP, bovine ceramides type IV, linoleic acid, propylene glycol, and cholic acid were purchased from Sigma Chemicals (St. Louis, MO); lard was purchased from ICN Biochemicals (Cleveland, OH); petroleum ether and acetone were purchased from Fisher Scientific (Fairlawn, NJ); $[^3H]H_2O$ and $[^{14}C]$ cholesterol were purchased from New England Nuclear Research Products (Boston, MA); cholesterol was purchased from Nutritional Biochemicals (Cleveland, OH); and silica G TLC plates were purchased from Brinkmann Instruments (Westbury, NY).

Animal Procedures To decrease serum cholesterol levels, mice were injected with 4-APP, which inhibits hepatic secretion of lipoproteins and thereby lowers serum cholesterol concentrations [15,16]. Mice were injected intraperitoneally with 0.8 mg of 4-APP suspended in 0.2 ml phosphate buffer (pH 4). A control group of animals were similarly injected with buffer alone. Both groups of animals were fasted during the study because 4-APP can cause anorexia. To increase serum cholesterol concentrations, mice were fed an atherogenic diet containing 5% lard, 2% cholesterol, and 0.3% cholic acid in powdered rodent chow [17]. Control mice were fed powdered rodent chow alone. The barrier was disrupted by wiping with absolute acetone until transepidermal water loss (TEWL) reached 5–9 mg/cm²/h (normal < 0.15 mg/cm²/h) [5,7]. Barrier function, assessed as TEWL, was determined with

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Abbreviation: 4AAP, 4-aminopyrazolo (3,4-d) pyrimidine.

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Table I. Serum Lipid Concentrations[a]

Treatment	Serum Cholesterol (mg/dl)
Fasting (n = 20)	105 ± 4
Fasting, 4-APP (n = 5)	53 ± 4[b]
Fed (n = 15)	103 ± 5
Fed, atherogenic (n = 10)	156 ± 5[c]

[a] Mice either fasted for 1 or 2 d, or fed *ad libitum*. Because serum cholesterol levels of all groups of fasting mice were similar they were pooled. Fasting, 4-APP-treated mice were injected with 0.8 mg 4-APP in phosphate buffer one day prior to sampling. Fed mice were fed normal rodent chow. Fed, atherogenic mice were fed an atherogenic diet for 7 d. Serum cholesterol levels were measured using Sigma Diagnostic kit for cholesterol (mean ± SEM).
[b] $p < 0.01$ compared to fasting.
[c] $p < 0.01$ compared to fed.

a Meeco Electrolytic Water Analyzer (MEECO, Warrington, PA) [5,7]. Topical lipids were applied to acetone-treated skin (0.5 mg cholesterol, 0.25 mg ceramides, 0.2 mg linoleic acid in polyethylene glycol:ethanol; 7:3 vols; 80 μl/5 cm²), as previously described [18]. This lipid mixture was shown previously to neither accelerate nor delay barrier recovery in acetone-treated hairless mice [18].

Cutaneous Sterologenesis Incorporation of $[^3H]H_2O$ into cutaneous cholesterol was determined as described previously [4-6]. Briefly, each animal was injected with 20 mCi/0.2 ml $[^3H]H_2O$ intraperitoneally. After 2 h, the animals were killed and the skin was removed. Subcutaneous fat was removed, the skin was weighed and then exposed to dry heat at 60°C for 60 seconds, and the epidermic and dermis were isolated by scraping with a number 22 blade. The epidermis and dermis were dissolved separately in a saponification solution (45% KOH, water, 95% ethanol; 4:3:10 vols) by incubating at 60°C for at least 3 h. After an internal standard $[^{14}C]$ cholesterol (approximately 2000 cpms) was added to each sample the cholesterol was extracted with petroleum ether. The extracts were spotted on TLC plates and developed in ethyl acetate:benzene (1:5 vols) for 90 min. Bands corresponding to cholesterol were excised and counted. Because of variations in the rate of cholesterol synthesis from month to month, only animals studied during the same period were compared.

Serum Lipid Levels Serum cholesterol concentrations were measured using the Sigma Diagnostic Kit number 352.

Statistics Statistical significance was determined using the Student test for two-way comparisons. Analysis of variance was used to compare treatments as a function of time. Values of less than 0.05 were considered to be statistically significant.

RESULTS

To determine the effects of lowering serum cholesterol on cutaneous cholesterol synthesis, hairless mice were injected with either buffer or 4-APP to lower serum cholesterol levels, and then studied 1 d later. Because 4-APP causes anorexia, both the 4-APP-treated animals and controls were fasted. Serum cholesterol levels were decreased by a mean of 50% in 4-APP treated mice ($p < 0.01$) (Table I). Neither epidermal nor dermal cholesterol synthesis were altered in 4-APP-treated mice (Fig 1).

To determine the effects of increasing serum cholesterol concentrations on cutaneous cholesterol synthesis, mice were fed an atherogenic diet for 7 d. As shown in Table I, the atherogenic diet increased serum cholesterol concentrations by 51% ($p < 0.01$). However, cholesterol synthesis in both the epidermis and dermis remained unchanged in mice fed the atherogenic diet (Fig 2). Thus, in contrast to most other tissues that are regulated by serum cholesterol levels, alterations in serum cholesterol levels do not regulate either epidermal or dermal cholesterol synthesis.

We next determined the role of acute starvation in regulating cutaneous cholesterol synthesis. Serum cholesterol levels were similar in fed animals and animals fasting for 2 d (Table I). However, epidermal cholesterol (38%) synthesis and dermal cholesterol (34%) synthesis were decreased in fasting mice (Fig 3) ($p < 0.05$). Thus, acute starvation decreases cutaneous cholesterol synthesis.

Previous studies have demonstrated that epidermal cholesterol synthesis plays a crucial role in barrier repair after acute disruption by acetone treatment [3,19]. We next determined whether the re-

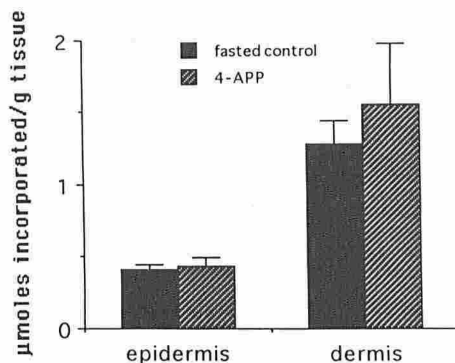

Figure 1. Cholesterol synthesis in 4-APP-treated mice. 4-APP mice were injected with 0.8 mg 4-APP in phosphate buffer and control mice were injected with phosphate buffer alone one day prior to study. Both control and 4-APP animals fasted after 4-APP administration. On the day of the study, the mice were injected with $[^3H]H_2O$ and killed 2 h later, and plasma and skin samples were obtained. The epidermis was separated from the dermis and $[^3H]H_2O$ incorporation into lipid determined (μmoles incorporated/g tissue/2 h) as described in *Materials and Methods.* Values shown are the mean ± SEM (n = 5).

duction in cholesterol synthesis that accompanies fasting might compromise barrier repair. As shown in Fig 4, barrier repair is impaired in fasting versus control animals. Six hours after acetone treatment, TEWL rates are 41% higher in fasting mice ($p < 0.01$), indicating that barrier repair is compromised in fasting animals. By 24 h after barrier disruption, TEWL had returned to normal in both fasting and fed animals (data not shown). Finally, topical application of cholesterol alone or a mixture of stratum corneum lipids (cholesterol, ceramides, and linoleic acid) did not normalize barrier recovery rate in fasting mice (after 6 h, vehicle TEWL = 4.24 ± 1.28; lipid TEWL = 5.00 ± 1.05 mg/cm²/h, n = 5).

DISCUSSION

Permeability barrier function is an important determinant of the rate of epidermal cholesterol synthesis [3,5-7]. The effect of alterations in serum cholesterol levels on epidermal cholesterol synthesis has not been closely examined. Decreased serum cholesterol concentrations stimulate cholesterol synthesis in the rat adrenal gland, liver, lung, kidney, intestine, brain, adipose tissue, and ovary [13,14,20] and in the hamster adrenal cortex [21]. Conversely, increases in serum cholesterol levels inhibit cholesterol synthesis in

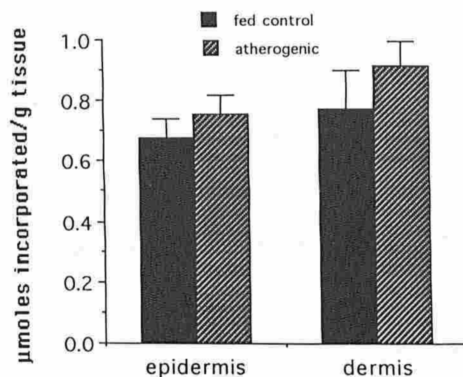

Figure 2. Cholesterol synthesis in mice fed an atherogenic diet. Mice were fed control rodent chow or atherogenic chow *ad libitum* for 7 d. On the day of the study, the mice were injected with $[^3H]H_2O$ and killed 2 h later, and plasma and skin samples were obtained. The epidermis was separated from the dermis, and $[^3H]H_2O$ incorporation into lipids (μmoles incorporated/g tissue/2 h) determined as described in *Materials and Methods.* Values shown are the mean ± SEM (control n = 15; atherogenic n = 10).

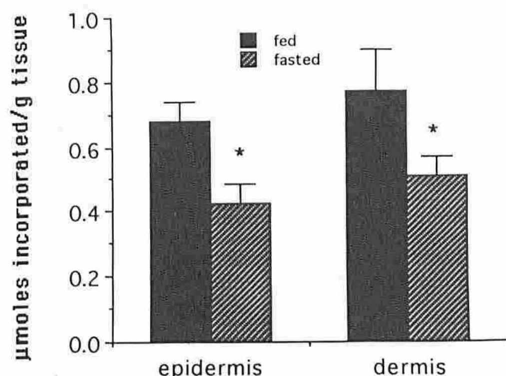

Figure 3. Cholesterol synthesis in fasting and fed mice. Mice were fed normal rodent chow *ad libitum* or fasted for 2 d. On the day of the study, the mice were injected with $[^3H]H_2O$ and killed 2 h later, and plasma and skin samples were obtained. The epidermis was separated from the dermis, and $[^3H]H_2O$ incorporation determined (μmoles incorporated/g tissue/2 h) as described in *Materials and Methods*. *Statistical difference compared to control ($p < 0.05$). Values shown are the mean ± SEM (n = 15).

most of these same tissues [13,14]. Large increases in serum cholesterol levels produced by lipoprotein infusion decreased cholesterol synthesis in whole skin [13], whereas decreasing serum cholesterol levels had no effect on cutaneous cholesterol synthesis in rats [13,20]. However, because the epidermis comprises no more than 10% of the total skin mass and accounts for a minority of total cutaneous sterologenesis [4], significant changes in epidermal cholesterol synthesis could be overlooked when cholesterol synthesis is measured in whole skin alone. Thus, the aim of the present study was to determine the effect of increases or decreases in serum cholesterol concentrations on epidermal and dermal cholesterol synthesis.

No significant changes were observed in cholesterol synthesis in the epidermis or dermis of mice with either high or low serum cholesterol levels (Figs 1 and 2). Therefore, as in the whole skin, both epidermal and dermal cholesterol synthesis appears to be regulated independent of changes in serum cholesterol levels. Either the absence of significant numbers of functional LDL receptors in the epidermis [22], and/or the inaccessibility of serum lipoproteins to a significant proportion of the cells that synthesize cholesterol, could account for these findings.

In addition, we determined the effect of feeding a high-cholesterol diet on cutaneous cholesterol synthesis. Feeding a diet supplemented with 5% cholesterol did not decrease whole skin sterol synthesis [23]. Likewise, a high-fat, high-cholesterol diet also had no effect on epidermal or dermal cholesterol synthesis (Fig 2).

We next examined the effect of fasting on cutaneous cholesterol synthesis. Fasting decreases cholesterol synthesis in the kidney, liver, and intestine [14,23]. In the skin, others have reported either a decrease [24] or no change [14,23] in cutaneous cholesterol synthesis with starvation. In our studies, fasting decreased both epidermal and dermal cholesterol by 34–38% (Fig 3) ($p < 0.05$). The mechanism by which fasting inhibits cholesterol synthesis in the skin or other organs in unknown but may be related to decreases in ATP levels. Recent studies have shown that decreased ATP levels result in the phosphorylation and inactivation of HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis [25].

Inhibition of cholesterol synthesis due to fasting resulted in an impairment of barrier recovery (Fig 4). These data are consistent with previous studies that demonstrated that inhibition of cholesterol synthesis by lovastatin results in delayed barrier recovery, which is reversed by topical co-application of cholesterol [19]. However, topical cholesterol in this study did not restore barrier recovery, suggesting that the effects of fasting on the epidermis are complex, and that factors in addition to lipids are necessary to overcome the effects of acute starvation. Because fasting induces a catabolic state, many anabolic processes in addition to cholesterol synthesis may be inhibited. Starvation is well known to impair wound healing [26,27], but despite intensive investigation, the reasons for this inhibition are unknown. If the regulation of epidermal cholesterol synthesis is similar in humans, then patients suffering from malnourishment or starvation could display abnormal permeability to drugs or xenobiotics, defective wound healing, and increased susceptibility to the effects of dehydration or skin infections.

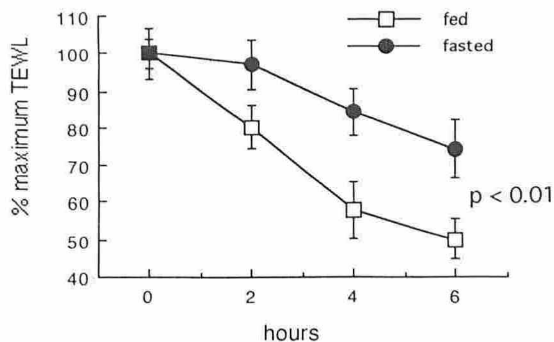

Figure 4. Barrier recovery in fasting mice. Mice were fed rodent chow *ad libitum* or fasted for 2 d and then treated with acetone to disrupt the barrier. TEWL was measured over 6 h. Values shown are the mean percent of maximum TEWL ± SEM (n = 7). Analysis of variance was used to determine statistical difference between groups over time ($p < 0.01$).

A number of pathophysiologic states also affect cholesterol synthesis in the skin. For example, in ketoacidotic diabetic animals and humans, cutaneous cholesterol synthesis decreases in comparison to nondiabetic controls [28,29], and increases with insulin administration [24,30]. In addition, epidermal cholesterol synthesis is decreased in rats that are hypothyroid [31]. Whether barrier homeostasis or repair are impaired in one or more of these disease states remains to be determined.

In summary, the data presented in this study demonstrate that epidermal and dermal cholesterol synthesis are autonomous of changes in serum cholesterol levels and high-cholesterol diets, but are decreased by fasting.

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