Abnormal Lipogenesis in Thyroid Hormone-Deficient Epidermis*

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Striking skin changes can accompany hypothyroidism, among them a scaly ichthyosis. The pathogenesis of these disorders is unknown. Since altered sterol metabolism has been associated with other scaling dermatoses, we tested the hypothesis that altered epidermal lipogenesis may accompany hypothyroidism and induce the observed scaling. Thyroidectomized (TX) rats were sacrificed 53 days postthyroidectomy, and the rate of incorporation of the radiolabeled precursor, [^{14}C]acetate, into epidermal lipids was determined. Approximately 2-fold decreases in incorporation of precursor into both polar and neutral lipids were observed in the TX epidermis; the most significant decreases, however, were in the neutral lipids, particularly the sterol and sterol-ester moieties. Total cholesterol pool size was also reduced in the TX epidermis. Similar decreases in sterol biosynthesis were noted in human keratinocytes cultivated in medium devoid of thyroid hormone. These findings demonstrate that lipid metabolism, and notably sterol synthesis, is altered in epidermal keratinocytes deprived of thyroid hormone, and suggest that, as in other scaling disorders, this abnormal sterologenesis may lead to the clinically observed ichthyosis that can accompany hypothyroidism. *J Invest Dermatol 86:244–248, 1986*

Hypothyroidism is a disease that can be accompanied by a number of striking skin changes, among them eczema, palmoplantar hyperkeratosis, and ichthyosis [1–3]. Although all of these clinical entities have been noted to resolve upon thyroid hormone repletion, the pathogenesis of the skin abnormalities is unknown. There is, however, evidence to suggest that epidermal lipid metabolism, altered by the decrease in thyroid hormone, may play a role in the genesis of the scaly skin that accompanies hypothyroidism. First, hypothyroidism is a disease in which lipid metabolism of many tissues is altered [4–9]; specifically, thyroid hormone modulates regulatory enzymes of sterol and fatty acid metabolism in a number of cells and tissues [5–10]. Second, alterations in epidermal lipogenesis have been associated with a number of scaling disorders. In X-linked recessive and lamellar ichthyosis, Refsum's disease, some cases of harlequin fetus, and some drug-induced ichthyoses, the clinical scaling has been related to quantitative alterations in epidermal lipids [11–15]. The link between altered epidermal lipogenesis and the development of scaling skin in these disease states led to the hypothesis that in hypothyroidism the clinical manifestation of scale may, likewise, result from perturbations in keratinocyte lipid synthesis.

The aim of the current investigation was to determine the effect of thyroid hormone on epidermal lipogenesis in vivo and in cell culture. Epidermal keratinocytes in both an in vivo model of hypothyroidism—thyroidectomized rats—as well as an in vitro model—human keratinocytes cultivated in thyroid-deficient medium—demonstrated profound abnormalities in lipid synthesis. Hence, this study may provide an insight into the mechanism by which thyroid disorders affect the skin.

MATERIALS AND METHODS

In Vivo Studies

Animal Model of Hypothyroidism: Young adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Massachusetts) weighing 175–185 g underwent either total surgical thyroidectomy (TX) or sham thyroidectomy (Sham). Animals were fed ad lib standard laboratory chow (Ralston Purina Co., Missouri) or, in some cases, a diet devoid of animal products which could contain thyroid hormones (Dyets, Inc., Pennsylvania), along with distilled water supplemented with 1% calcium chloride.

Fifty-three days following surgery, dorsal skin was shaved and depilated using Nair (Carter-Wallace, New York). Twelve hours later the animals were anesthetized with diethyl ether and blood obtained by cardiac puncture for quantitation of plasma triiodothyronine (T3), thyroxine (T4), and rat thyrotrophin (r-TSH). Animals were then killed by cervical dislocation, and the skin of the back quickly removed by sharp dissection. A Castroviejo keratome at the 0.2-mm setting was used to take epidermal slices. Random slices as well as a full-thickness biopsy were fixed in formalin for evaluation by light microscopy.

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Abbreviations:

DMEM: Dulbecco's modification of Eagle's medium
EGF: epidermal growth factor
HK: human keratinocyte(s)
HMG CoA: 3-hydroxy-3-methylglutaryl CoA
NL: neutral lipid(s)
PBS: phosphate-buffered saline
PL: polar lipid(s)
r-TSH: rat thyroid-stimulating hormone
Sham: sham thyroidectomy
T3: triiodothyronine
T4: thyroxine
TLC: thin-layer chromatography(graphs)
TX: thyroidectomy, thyroidectomized
TXBS: thyroid hormone-deficient bovine serum

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Incorporation of [U-^{14}C]Acetate into Epidermal Lipids and Lipid Extraction: Epidermal slices were weighed and 50–100 mg portions immediately transferred to incubation vials containing streptomycin (200 μg), penicillin (200 U), and 4.0 μCi (68 nM) [U-^{14}C]Na acetate (sp act 58 mCi/mmol, Amersham Corp., Illinois) in 2 ml of Eagle's minimal essential medium (Flow Laboratories, Virginia) which had been gassed for 1 h with 95% oxygen plus 5% carbon dioxide. In some incubations, nonradioactive Na acetate at a 5 mM final concentration, was added. The mixture was incubated in a Dubnoff shaker at 37°C for 6 h, at which time the skin slices were removed, washed in 3 consecutive baths of phosphate-buffered saline (PBS) at 4°C, blotted lightly on filter paper, and immediately homogenized in chloroform:methanol (2:1) in a Polytron homogenizer at 4°C. Further homogenization was carried out with ground-glass homogenizer tubes. Total lipids were extracted after the method of Bligh and Dyer [16] into the organic phase of chloroform:methanol:0.1 M potassium chloride (2:1:0.2).

Purification of Lipid Extract: Water and tissue debris were removed by filtration through glass wool and anhydrous sodium sulfate. The filtrate was evaporated to dryness and the lipid residue dissolved in chloroform:methanol:water (60:30:4.5). Sephadex G-25, coarse (Pharmacia Fine Chemicals, New Jersey) column chromatography described by Wells and Dittmer [17] removed radioactive acetate and other nonlipid radioactive contaminants. After evaporation of the solvents under nitrogen, the recovered lipid mixture was dissolved in chloroform:methanol (2:1).

Thin-Layer Chromatographic (TLC) Analysis: The ^{14}C-labeled lipids were separated into neutral and polar lipid classes by preparative TLC. Lipid extracts were applied to activated Silica gel G (Merck, F.D.R.) TLC plates and developed in chloroform:methanol:glacial acetic acid:water (180:16:2.0:1.6) to 20 cm. After air drying and exposure to iodine vapor, the origin (containing phospholipids and sphingolipids) and the remainder of the gel (containing neutral lipids and free fatty acids) were separately scraped from the plate and eluted under vacuum with chloroform:methanol (2:1) until no radioactivity could be detected in the wash. The filtrates were then evaporated to dryness and neutral (NL) or polar (PL) lipid residue redissolved in chloroform:methanol (2:1).

Neutral lipid moieties including free sterols, sterol esters, triglycerides, and free fatty acids were separated and identified using Silica gel G plates and development in petroleum ether:diethyl ether:glacial acetic acid (80:220:1). Phospholipid and sphingolipid moieties including phosphatidylcholine, sphingomyelin, phosphatidylethanolamine, phosphatidylserine + phosphatidylinositol, and phosphatidic acid were identified using Silica gel HR ultrapure (Merck) TLC plates and development in chloroform:methanol:glacial acetic acid:water (50:30:0.5:3.0). Aliquots of cell lipid extracts were applied to the plates along with 50 μg each of authentic nonradioactive standards. Lipid moieties were identified after exposure to iodine vapor and comparison of their *Rf*s to those of authentic standards. Radioactivity was counted and radiochromatograms analyzed directly on a Berthold Automatic TLC Linear Analyzer linked to an Apple IIe computer.

In Vitro Methods

Cell Culture: Primary human epidermal keratinocyte cultures (HK) were prepared from neonatal foreskins using a modification of the method of Rheinwald and Green [18]. Keratinocytes (5×10^5 cells) were plated onto 60-mm dishes (Becton-Dickinson, California) which had been previously seeded with mitomycin-C-treated 3T3 (clone CCL92, American Type Culture Collection, Maryland) feeder cells at 2×10^4 cells/cm^2, following the method of Rheinwald [19]. At confluence, HK were subcultured onto dishes which had been preseeded with feeder 3T3 cells. After allowing 24 hr for attachment of cells, dishes were washed twice, with PBS and experimental medium added. Thyroid hormone-depleted medium (TXBS medium) consisted of 10% bovine serum from a TX animal (Rockland Farms, Pennsylvania), 0.4 μg/ml hydrocortisone (MSD, West Point, Pennsylvania), 10 ng/ml cholera toxin, and 1 ng/ml epidermal growth factor (EGF) (Collaborative Research, Lexington, Massachusetts) in Dulbecco's modification of Eagle's medium (DMEM). Thyroid hormone-repleted medium (TXBS + T3) consisted of TXBS medium to which L-T3 (Sigma, St. Louis, Missouri) at a final concentration of 2×10^{-8} had been added. Cells were fluid changed twice weekly and treated with EDTA at 70% confluence to remove feeder cells. After cultures obtained 70% confluence, EGF was no longer added to culture media.

Incorporation of [U-^{14}C]Acetate into Human Keratinocyte Lipids: For studies of [U-^{14}C]Na acetate incorporation into HK lipid, dishes were washed with PBS and incubated at 37°C in DMEM containing 0.3 μCi/10^6 cells (8.5 nM) [^{14}C]acetate (sp act 58 mCi/mmol, Amersham Corp.) and either L-T3 (2×10^{-8}) or PBS for 18 h. In some incubations, an excess of nonradioactive Na acetate, at a final 5 mM concentration, was added to overcome any contribution by endogenous pool of this substance. After incubation, culture dishes were washed and cells were scraped into ice-cold methanol and homogenized in a glass homogenizer in chloroform:methanol (2:1). Lipids were extracted, purified, separated, and identified by TLC as described above.

Other Determinations Total (free and esterified) cholesterol content of lipid extracts from rat epidermis or HK were determined using the method of Aouidet et al [20]. Lipid phosphorus content was determined from the PL extract of rat epidermis or HK as a measure of phospholipid content by the method of Rouser et al [21] using both phospholipid and inorganic phosphorus standards. Thyroxine and T3 in rat plasma and in experimental culture media were quantitated by radioimmunoassay at the University of California, Davis, Medical Center.† Reagents for measurement of r-TSH by radioimmunoassay were generously provided by the National Hormone and Pituitary Program.‡ An aliquot of cell homogenate prior to lipid extraction was taken for protein assay by the method of Lowry et al [22] using bovine serum albumin as a standard.

Statistical Methods Probabilities were determined using a one-tailed Student's *t*-test with significance assigned at $p < 0.05$.

RESULTS

In Vivo Studies

Thyroid Status: Indices of thyroid function were examined in the TX rats to ensure their hypothyroid status. Analysis of plasma levels of r-TSH demonstrated a 20-fold increase in the TX rats (1433 μU/ml) as compared with Sham controls (67 μU/ml), indicating frank hypothyroidism. In addition, plasma T4 and T3 are sharply reduced 75 and 40%, respectively, in TX animals. Fig 1 illustrates that TX rats suffered dramatic growth failure, another characteristic of hypothyroidism, compared with Sham rats. Thyroidectomized animals had an average 4-fold decrement in weight gain as compared with their Sham counterparts.

Histology of Epidermis: Full-thickness biopsies showed no significant differences on light microscopy. Keratomed slices showed less than 10% dermal contamination by thickness (data not shown).

Rat Epidermal Lipogenesis: Studies of precursor incorporation into epidermal lipids revealed significant differences between TX and Sham rats. Fig 2 demonstrates that TX epidermis incorporated significantly less [^{14}C]acetate into both total PL and NL than Sham epidermis. There was a 2-fold reduction in the phosphorus con-

†Centria Total T3 and Total T4 Radioimmunoassay Test Sets, Ventrex Laboratories, Inc., Portland, Maine.

‡Technical Report No. 29 of the Pituitary Hormones and Antisera Center, National Institutes of Arthritis, Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland.

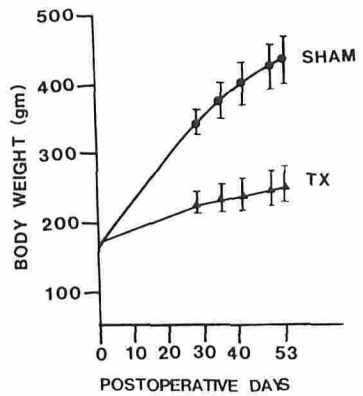

Figure 1. Body weight of TX (●——●) and Sham-operated rats (▲——▲). *Points* represent mean, *bars* represent SD. N = 4 animals in each group.

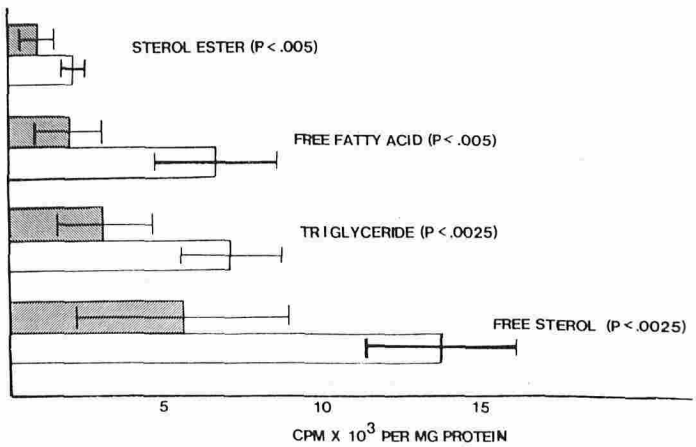

Figure 3. [U-^{14}C]Acetate incorporation into NL of the epidermis of TX (*hatched bars*) (N = 6) and Sham-operated (*clear bars*) (N = 5) rats. Incorporation studies were carried out as detailed in Fig 2. After extraction of lipids, NL were isolated and identified as outlined in *Materials and Methods*. *Bars* represent mean ± SD.

tent of the epidermal phospholipids of the TX epidermis as compared with the Sham epidermis (data not shown), which is in agreement with the observed decreased incorporation of acetate into PL (Fig 2). To rule out possible dilutional effects of pool sizes, similar incorporation studies were performed in the presence of a 5 mM excess of cold sodium acetate. Similar decreases in incorporation into NL and PL were observed under these circumstances. Because the most striking decrease in [^{14}C]acetate incorporation into lipids was noted to be in the NL, this group was further fractionated. Fig 3 demonstrates that although incorporation of precursor into all NL species was reduced in the thyroid-deficient epidermis, the most significant reductions are noted in the sterol and sterol-ester moieties. Additionally, the epidermal cholesterol pool size was examined and noted to be significantly (40%) reduced in the TX animals (data not shown). Therefore, the observed depression of lipogenesis, and specifically of sterol biosynthesis, results in a decreased total cholesterol pool size in TX rat epidermis.

In Vitro Studies

Lipogenesis of HK Grown in TXBS or TXBS + T3 Medium: To ascertain that the altered patterns of lipogenesis observed in the rat epidermal slices resulted from epidermal metabolism rather than from the metabolic contribution of the contaminating dermal components, and to determine whether similar metabolic alterations occurred in human cells, similar studies were carried out in an in vitro model of hypothyroidism utilizing HK cultivated in thyroid hormone-deficient culture medium. Radioimmunoassay of experimental growth medium used in these studies revealed TXBS medium and TXB serum to have undetectable levels of both T3 and T4. The TXBS + T3 medium had a level of T4 that was below the limits of detection and a T3 level of 1.2–1.4 ng/ml. Repeated treatment of these cultures with 0.02% EDTA freed the plate of any contaminating fibroblasts, yielding pure keratinocyte cultures.

Studies of lipogenesis and cholesterol pool size of HK cultivated in thyroid-deficient medium gave results similar to those obtained with the in vivo rat studies. Specifically, a decrease in incorporation of [U-^{14}C]Na acetate into neutral lipid moieties was observed. To obviate the problem of dilutional effect due to differences in substrate pool size, a 5 mM excess of cold substrate (Na acetate) was added to the incubation mixture. Similar to the findings noted in the in vivo rat studies, decreased incorporation into all neutral lipids was noted (Fig 4). To determine whether,

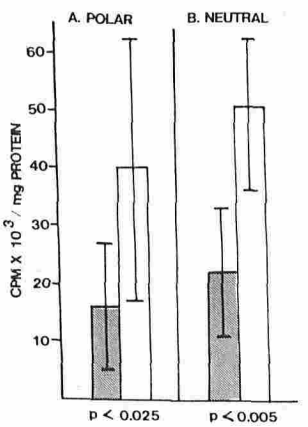

Figure 2. [U-^{14}C]Acetate incorporation into epidermal lipids of TX (*shaded bars*, N = 6) and Sham-operated (*clear bars*, N = 5) rats. [U-^{14}C]Na acetate (4.0 μCi) was incubated with 100 mg of keratomed rat epidermal slices in 2 ml of Eagle's minimal essential medium for 1 h at 37°C. Lipids were then extracted and fractionated as outlined in *Materials and Methods*. *A*, Polar lipids. *B*, Neutral lipids. *Bars* represent mean ± SD.

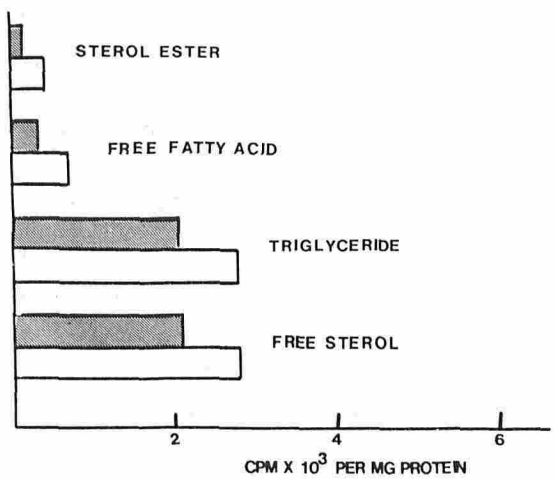

Figure 4. [U-^{14}C]Acetate incorporation into cellular lipids of HK (strain HEp 101 pl) cultivated in T3-containing (*clear bars*) and T3-deficient (*hatched bars*) medium in the presence of 5 mM cold Na acetate, as outlined in *Materials and Methods*. Data represent determinations from 2 separate experiments.

as in the in vivo experiments, pool size of cholesterol was decreased in the thyroid-deficient cultures, cholesterol content was quantitated in one experiment and an approximate 25% reduction of total sterol pool was noted in the TXBS cultures (data not shown). Similarly, polar lipid phosphorus content of the HK cultured in T3-deficient medium was also noted to be decreased (data not shown).

DISCUSSION

In humans, hypothyroidism can be accompanied by a scaling ichthyosis, the pathogenesis of which is not well understood. In an effort to elucidate the molecular basis of the observed scaling, we evaluated epidermal keratinocytes in both an in vivo and in vitro model of hypothyroidism. A generalized decrease in the rate of synthesis of neutral and phospholipids was observed in both systems of thyroid hormone deficiency. However, the rate of synthesis of the neutral lipids, especially of cholesterol and cholesterol esters, was most markedly affected in the hypothyroid epidermis. These findings were accompanied by a decrease in total pool size of cholesterol in both the hypothyroid rat epidermis as well as keratinocytes cultured in a thyroid hormone-deficient milieu. These observations, therefore, document profound alterations in epidermal lipogenesis in the hypothyroid condition and provide an insight into the mechanism by which hypothyroidism may clinically affect the skin.

The epidermal lipid abnormalities noted in these models of hypothyroidism, especially reduction of sterologenesis and fatty acid synthesis, might be expected, since hypothyroidism leads to abnormalities in the lipids of so many tissues including serum, dermis, adipose tissue, and liver [5,8–10]. The key enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase, is induced in the presence of thyroid hormone and suppressed in its absence [5,10], and reduced cholesterol synthesis has been observed in hepatocytes isolated from hypothyroid animals [23]. In light of the recent finding of Ponec et al [24] that HMG CoA reductase is also the rate-limiting enzyme for sterol biosynthesis in HK, it is not surprising that the data presented here show total cholesterol content as well as the rate of sterol biosynthesis to be reduced in both TX rat epidermis and in HK grown in thyroid hormone-deficient medium. Acetyl CoA carboxylase, the rate-limiting enzyme in fatty acid biosynthesis, is also regulated by thyroid hormone in animal liver, kidney, adipose tissue, and in human diploid fibroblasts [6–9]. Our findings of reduced levels of fatty acids and phospholipids in both hypothyroid models probably result from the thyroid hormone-mediated suppression of acetyl CoA carboxylase and resultant decreased availability of constituent fatty acids. The generalized retardation in lipid biosynthesis reported here might be due to a reduction in metabolic rate, but the reduced total pools of cholesterol and phospholipid indicate actual suppression of epidermal lipid biosynthesis. Therefore, these changes demonstrate that the hypothyroid state is associated with abnormal epidermal lipogenesis and result in decreased levels of fatty acids, phospholipids, and sterols in the affected epidermis.

It is tempting to speculate that the altered epidermal sterologenesis noted in these 2 models of hypothyroidism may contribute to the clinical observation of an ichthyotic-like scale in myxedema. There is, in fact, much evidence to link scaling disorders with aberrations in keratinocyte sterologenesis. Examples of such abnormalities associated with clinical scaling disorders include increased sterol esterification in essential fatty-acid deficiency [25], decreased sterol esters, as well as increased phospholipid and cholesterol in both psoriasis and lamellar ichthyosis [11,26,27], and increased cholesterol sulfate with decreased free sterols in X-linked recessive ichthyosis [11]. Thus, there is much evidence to substantiate the link between altered stratum corneum cholesterol and alterations in the process of desquamation. Although the mechanism by which altered sterol synthesis leads to scale formation is unclear, Elias [28] and coworkers have suggested that the intercellular lipids secreted by the lamellar bodies are responsible for the orderly dyshesion of corneocytes. Additionally, the adhesive properties of some epithelial cells have been noted to be dependent on cholesterol biosynthesis [29]. Hence, the abnormalities in epidermal free sterols, as were observed in both models of the hypothyroid epidermis examined in this study, might be expected to lead to disordered stratum corneum retention, which, in turn, could lead to clinical ichthyosis. Confirmation of this hypothesis will require further examination of human skin of myxedematous patients. The present study suggests, however, that altered keratinocyte sterologenesis may be a major pathogenic factor in the origin of scale in hypothyroidism.

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