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Triglyceride metabolism in human keratinocytes cultured at the air-liquid interface

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Abstract Although epidermis reconstructed in vitro histologically demonstrates the presence of fully differentiated tissue with cornified strata, it does not synthesize or release epidermal barrier lipids in the same proportions as does native skin, causing the barrier function to be impaired. Lipids, the content of which deviates the most, include triglycerides that are present in high amounts and stored as lipid droplets. Our recent studies have revealed that a high triglyceride content may be a reflection of a high synthetic rate and a low turnover. Therefore, the present study was undertaken to examine whether the triglyceride accumulation in the air-exposed cultures may be a result of insufficient supplementation of cells with oxygen, an excessive supplementation of cells with glucose, dysregulation of lipogenesis, or an impaired catabolism of triglycerides caused either by insufficient activity of triglyceride lipase and/or accumulation of free fatty acids due to insufficient activity of β-oxidase. When keratinocytes were cultured at the air-liquid interface in medium containing a standard glucose concentration, both the lactate and triglyceride production was high. Lowering glucose content in the medium resulted in a decrease in both lactate production and triglyceride synthesis. However, even when grown at a low glucose concentration the triglyceride content remained higher than found in vivo and synthesized triglycerides were stored in the cells as a stable pool, suggesting that the catabolism of triglycerides was impaired. Since both lipase and β-oxidase were found to be active in cultured keratinocytes, another factor or other factors are probably implicated in the regulation of triglyceride metabolism.**

Key words Human keratinocytes · Lipids
Triglycerides · Triacylglycerol lipase · Fatty acid oxidation

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Introduction

Continuous efforts in a number of laboratories have focused in recent years upon generating in vitro living skin models that would be suitable for investigations of the processes involved in the regulation of keratinocyte proliferation and differentiation and for toxicology and efficacy testing of cutaneous products. In our laboratory a system introduced by Prunieras et al. [21] has been used in which human keratinocytes are cultured at the air-liquid interface attached to a human de-epidermized dermis (DED) with preserved basement membrane constituents. Previous research has disclosed that although such in vitro reconstructed epidermis (RE-DED) shares many features with native epidermis, its barrier function is defective (reviewed in reference 16). Keratinocytes grown at the air-liquid interface have been found to synthesize all of the same lipid classes as epidermis in vivo, but the relative amounts of some lipid classes are different. A high triglyceride (TG) content and a low content of essential fatty acids (EFA) [14, 19] may account for irregularities in intercorneocyte lipid lamellar structures [3, 4, 6] and may explain why the permeation of a number of compounds through the stratum corneum of RE-DED proceeds at rates three to ten times higher than through native epidermis. The observed deviations in lipid composition are probably caused by different nutritional and environmental conditions, since when native epidermis is incubated under the same conditions as used for the reconstruction of epidermis in vitro, TG accumulation takes place [15]. This hypothesis is further supported by the finding that normalization of lipid composition and barrier function takes place when in vitro RE-DED is grafted onto nude mice [8].

Our recent studies have revealed that a high TG content in RE-DED may be a reflection of a high synthetic rate and a low turnover of TG [15, 17]. One of the environmental conditions that may lead to TG accumulation is insufficient supplementation of cells with oxygen. To examine whether or not the oxygen supplementation is sufficient for keratinocytes cultured at the air-liquid interface, we measured the lactate production in cells grown at the air-liquid interface and compared it with the production in cells grown under conventional, submerged conditions. Since high rates of TG synthesis and lactate production also depend, next to oxygen supply, on glucose supplementation, we further examined whether TG synthesis and lactate production can be affected by lowering the glucose levels in the medium, since the medium used for routine keratinocyte cultures contains relatively high glucose levels (3.8 g/l).

TG accumulation may also be caused, by a high rate of synthesis, as well as by a low rate of TG catabolism, in which the activity of triacylglycerol lipase and β-oxidase play an important role. A decreased activity of lipase, catalysing the hydrolysis of TG into free fatty acids (FFA) and glycerol, may result in the accumulation of TG. However, even when cells possess a high lipase activity, the low rate of fatty acid (FA) metabolism may lead to accumulation of FFA which in their turn may negatively affect the triacylglycerol lipase activity. In order to establish whether the cells are capable of metabolizing TG, the lipase activity and β-oxidase activity in keratinocytes cultured under air-exposed and under submerged conditions was compared with that of native epidermis.

The results of this study show that when keratinocytes were cultured at the air-liquid interface in medium containing a standard glucose concentration, lactate production was high. Lowering the glucose content in the medium resulted in a decrease in lactate production and in a decrease in TG synthesis. Since both lipase and β-oxidase were found to be active in cultured keratinocytes, another factor or other factors are probably implicated in the regulation of TG metabolism.

Materials and methods

Cell culture

Submerged cultures

Adult human keratinocytes (obtained from healthy patients undergoing surgical correction) were cultured together with irradiated mouse 3T3 fibroblasts in a mixture of DMEM and Ham's F12 (3:1) medium supplemented with 5% HyClone calf serum (Greiner, Solingen, Germany), 0.4 μg/ml hydrocortisone, 5 μg/ml insulin, 1 μ*M* isoproterenol and 10 ng/ml epidermal growth factor (EGF) [22].

Air-exposed cultures

Secondary cultures of adult human keratinocytes were seeded onto DED and cultured under air-exposed conditions [19] in medium of the same composition as above. For most experiments RE-DED of surface area 1 cm^2 was used.

Experimental modification of medium compositon

In experiments in which the effect of glucose was examined the following media were used: (1) a 3:1 mixture of DMEM (4.5 g/l glucose) and of Ham's medium (final glucose concentration 3.8 g/l); (2) a 1:1 mixture of DMEM (4.5 g/l glucose) and of Ham's F12 medium (final glucose concentration 3.15 g/l); (3) a 1:1 mixture of DMEM (1.0 g/l glucose) and of Ham's F12 media (final glucose concentration 1.4 g/l) and (4) a 3:1 mixture of DMEM (1.0 g/l glucose) and of Ham's F12 medium (final glucose concentration 1.2 g/l). All media were supplemented with 5% HyClone calf serum (Greiner), 0.4 μg/ml hydrocortisone, 5 μg/ml insulin, 1 μ*M* isoproterenol an 10 ng/ml EGF. In some experiments, aliquots of stock solution of carnitine (Sigma, St. Louis, Mo., USA) in distilled water (1 mmol/l) were added to the medium to achieve a final concentration of 50 or 100 μ*M*.

Lactate production

Culture media were collected at various time intervals during the whole culture period and the amount of lactate was assayed by measuring the LDH production with an ASA-SX analyser at the Central Clinical Laboratories, University Hospital Leiden.

Lipase assay

Preparation of cell homogenates

Keratinocytes from confluent submerged cultures were harvested by scraping with a rubber policeman into phosphate-buffered saline (PBS), followed by centrifugation for 5 min at 1000 *g*, and stored at –70° C until use. The cells were homogenized in PBS by sonification in ice for six cycles of 10 s, with a 15-s interval between each cycle.

Epidermis from freshly excised skin or from the air-exposed cultures was separated from the underlying dermis by dispase treatment (1.25 IU/ml; Boehringer Mannheim, Mannheim, Germany) for 90 min at 37° C, washed with PBS and stored at –70° until use. The epidermis was then homogenized using a thorax homogenizer in ice for eight cycles of 10 s, with 15-s interval between each cycle.

The homogenate was then centrifuged for 2 min at 10000 × *g*, the pellet was discarded and aliquots of the supernatant were taken for assessment of lipase activity and of protein content [11].

Assay of triacylglycerol lipase

As a measure of lipase activity the liberation of ^{3}H-oleic acid from ^{3}H-triolein was assayed as described previously [9]. Briefly, 15 or 20 μCi of ^{3}H-triolein (glycerol tri[9,10(*n*)-^{3}H]-oleate, 10 Ci/mmol; Radiochemical Centre, Amersham) and 35 mg triolein (Sigma) was dispersed by sonification on ice in 2.5 mg gum acacia solution (pH 7.0, 5% w/v). The substrate was then mixed with 6 ml 10% FA-free bovine serum albumin in various buffer solutions (sodium acetate for pH 4 to 5; sodium phosphate for pH 6.2 to 7.4; Tris-HCl for pH 8 to 8.9 and glycine/NaOH for pH 10.5) and 0.5 ml 5 *M* NaCl. Aliquots (180 μl) of the ^{3}H-triolein emulsions were added to 70 μl cell homogenate and the mixture was then incubated for 30–60 min at 30° C. The reaction was terminated by adding 3.25 ml of a methanol/chloroform/heptane (145/125/100, v/v/v) mixture and the released free acids were extracted as described by Belfrage and Vaughan [1]. The lipase activity was expressed as nanomoles ^{3}H-oleic acid released per milligram protein per hour.

Fatty acid oxidation

FA oxidation was assessed by measuring the generation of $^{14}CO_2$ from ^{14}C-oleic acid using a combination of the methods of Rodbell

[23] and of Löwik et al. [10]. Briefly, keratinocytes grown either under submerged culture conditions to confluence in six-well plates (Costar) or for 10 days under air-exposed conditions were incubated for various times in HEPES-buffered DMEM supplemented with 0.1% BSA and 10 μ*M* ^{14}C-oleic acid (53.5 mCi/mmol, 0.5 μCi/ml; Radiochemical Centre, Amersham, UK). During the incubation the wells were sealed with a rubber stopper that was placed upside down in the middle of the scintillation vial. To trap the released $^{14}CO_2$ two pieces of Whatman paper no. 3 (12 mm diameter) rinsed with 130 μl of a 50% KOH, 50% glycerol mixture were placed in each vial. The reaction was stopped by injecting 70 μl 2 *N* H_2SO_4 through the rubber stopper and the cultures were incubated for another hour at 37° C to trap all released $^{14}CO_2$. The scintillation vials were then removed and 0.5 ml distilled water was added. After vortexing, 4.5 ml Instagel (Packard Downers Grove, Ill., USA) was added to each vial and the radioactivity counted. In the experiments in which the effect of carnitine on FA oxidation was studied, the cells were incubated for 48 h prior to the experiment in medium containing 50 or 100 μ*M* carnitine.

Lipid extraction and separation

Keratinocytes were cultured on DED in media containing different glucose concentrations (media 1–4). In some experiments, at the time of air exposure (3 days after plating), the cultures were fed with media of the same composition but supplemented with carnitine (50 or 100 μ*M*). The cultures grown for 10 days at the air-liquid interface were then harvested by heating the tissue for 1 min at 40° C, washed with PBS, collected in 2 ml chloroform methanol (1:2) and stored at –20° C until use. Subsequently, the lipids were extracted according to the method of Bligh and Dyer [2], with the addition of 0.25 *M* KCl to ensure extraction of polar species. The extracted lipids were separated by thin-layer chromatography and quantified, as described in detail elsewhere [18].

Lipid metabolism

To assess incorporation of exogenous FA into lipids, 8-day-old air-exposed cultures grown in either medium 1 or 4 were preincubated for 48 h in the presence or absence of 50 μ*M* carnitine and subsequently incubated for 24 h with ^{14}C-oleic acid (1 μCi/ml). The medium was then discarded and the cultures were washed several times with PBS and either harvested directly (to assess the amount of ^{14}C-oleic acid incorporated into the lipids) or harvested after 24–72 h reincubation in medium containing increasing concentrations of carnitine, (0, 50 or 100 μ*M*) to monitor lipid turnover. In parallel cultures both during the labelling and the 24-h chase period, the released $^{14}CO_2$ was also assayed using the same method as described above. After separation of the epidermis from the underlying dermis (by heating the tissue for 1 min at 40° C), the lipids were extracted and fractionated by thin layer chromatography as described elsewhere [18]. In order to compare the distribution of the radiolabel in the various lipid fractions an aliquot of each lipid extract containing the same amount of radioactivity (25000 dpm) was applied to the HPTLC plate. After lipid separation by one-dimensional HPTLC, chromatograms were exposed for 3 days to Fuji X-ray RX film and after development the autoradiograms were scanned using a Desaga photodensitometer.

Results

Lactate production in cultured keratinocytes

Lactate content, as assessed in the media of both air-exposed and submerged cultures grown in medium containing 3.8 g/l glucose (standard culture medium), was high during the whole culture period (Figs. 1a, 2). Under both culture conditions lowering the glucose levels in the culture medium led to a dose-dependent decrease in lactate production (Figs. 1b, 2).

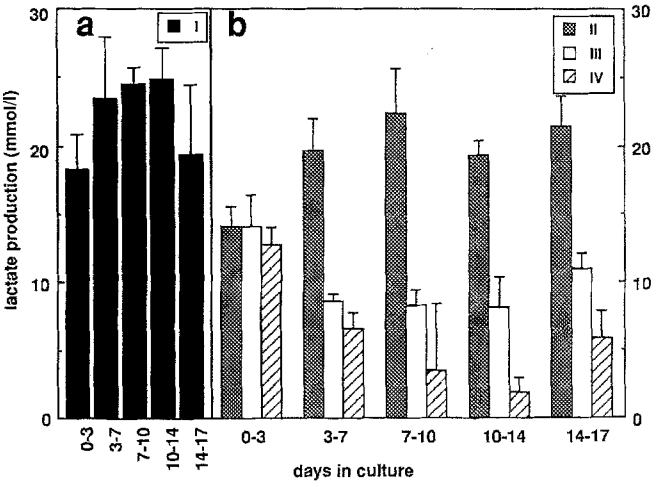


Fig. 1 a, b Lactate production in keratinocyte culture grown at the air-liquid interface in media containing (**a**) 3.8 g/l glucose (■, medium I), or (**b**) 3.15 g/l glucose. (▨, medium II), 1.4 g/l glucose (□, medium III) and 1.2 g/l glucose (▨, medium IV). The media were collected on days 3, 7, 10, 14 and 17, respectively. The results are expressed as nmol/l lactate and are means ± SD ($n = 3$)

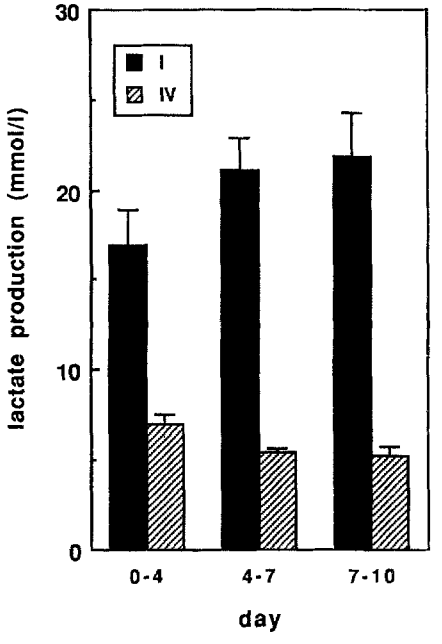


Fig. 2 Lactate production in keratinocyte cultures grown under submerged conditions in media containing 3.8 g/l glucose (■, medium I) and 1.2 g/l glucose (▨, medium IV). The media were collected on days 4, 7 and 10, respectively. The results are expressed as nmol/l lactate and are means ± SD ($n = 4$)

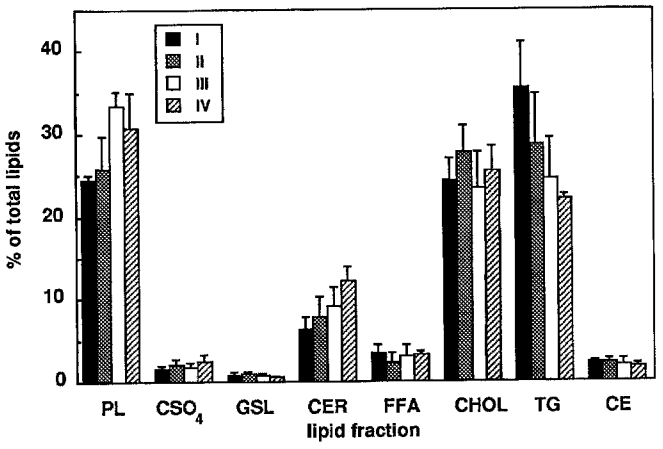


Fig. 3 Effect of extracellular glucose concentration on lipid composition of keratinocytes grown for 10 days in media containing 3.8 g/l glucose (■, medium I), 3.15 g/l glucose (▩, medium II), 1.4 g/l glucose (□, medium III) and 1.2 g/l glucose (▨, medium IV). (*PL* phospholipids, *CSO_4* cholesterol sulfate, *GSL* glucosphingolipids, *CER* ceramides, *FFA* free fatty acids, *CHOL* cholesterol, *TG* triglycerides, *CE* cholesterol esters). The results are expressed as percent of total lipids and are means ± SD (n = 3)

Effect of extracellular glucose on composition of epidermal lipids in keratinocytes cultured at the air-liquid interface

Lowering the glucose level in the culture medium led to a modulation of lipid composition in keratinocytes grown at the air-liquid interface, as demonstrated in Fig. 3. The decrease in glucose level from 3.8 g/l to 1.2 g/l was accompanied by a decrease in the relative amounts of TG from 35% to 22% and by an increase in the relative amounts of phospholipids from 25% to 31% and of ceramides from 6% to 11%.

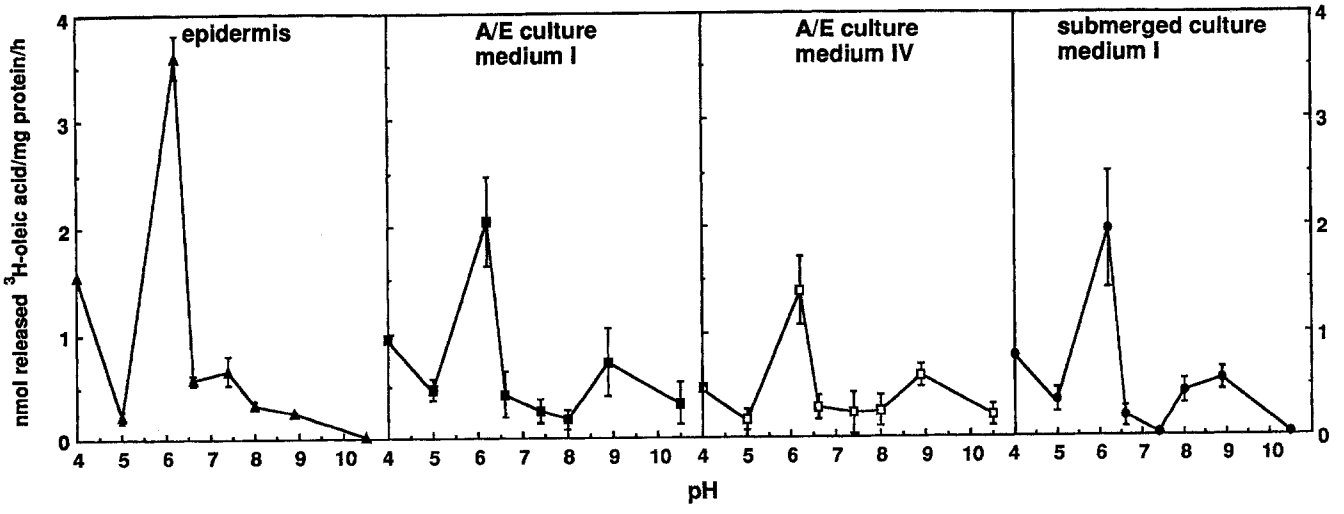
Triacylglycerol lipase activity in cultured keratinocytes

Experiments in which the triacylglycerol lipase activity over the pH range 4.0–10.5 was examined revealed that, as in native epidermis, keratinocytes grown under air-exposed or submerged conditions show triacylglycerol lipase activity with a maximum in the neutral range at pH 6.2 (Fig. 4), at which hepatocytes (that were used as a control) also showed maximal activity (data not shown). Both the lipase profile and the activity were similar in submerged and air-exposed cultures and were not modulated by lowering the glucose content in the culture medium. The neutral lipase activity in native epidermis was about two times higher than that in cultured keratinocytes, irrespective of the culture conditions used. In contrast to native epidermis, cultured keratinocytes also showed some activity of alkaline lipase, with a maximum at pH 8.9. At all pH values no significant changes in lipase activity were observed when the glucose level in the culture medium was lowered from 3.8 g/l to 1.2 g//l.

Fatty acid oxidation

Since the uptake of ^{14}C-oleic acid and the amount of released $^{14}CO_2$ increased linearly with time up to 24 h (data not shown), the β-oxidase activity in submerged cultures was assayed after 6 h and in air-exposed cultures after 18 h incubation time. As shown in Fig. 5, the $^{14}CO_2$ production under both submerged and air-exposed conditions was not modulated by the glucose content in the culture medium. Furthermore, in the presence of 50 μ*M* carnitine, $^{14}CO_2$ production was increased, irrespective of whether

Fig. 4 Triacylglycerol lipase activity in homogenates isolated from the epidermis, air-exposed *(A/E)* keratinocyte cultures grown in medium I (3.8 g/l glucose) or medium IV (1.2 g/l glucose) and submerged keratinocyte cultures grown in medium I (3.8 g/l glucose). The lipase activity was determined by incubation of aliquots of cell homogenates in a buffer with pH values ranging from 4.0 to 10.5 in the presence of ^{3}H-triolein, as described in detail in Materials and methods. The results are expressed as nmol released ^{3}H-oleic acid/mg protein per h and are means ± SD (epidermis, n = 3; *A/E* culture, medium I, n = 4; submerged culture, medium IV, n = 2; submerged culture, medium I, n = 3)



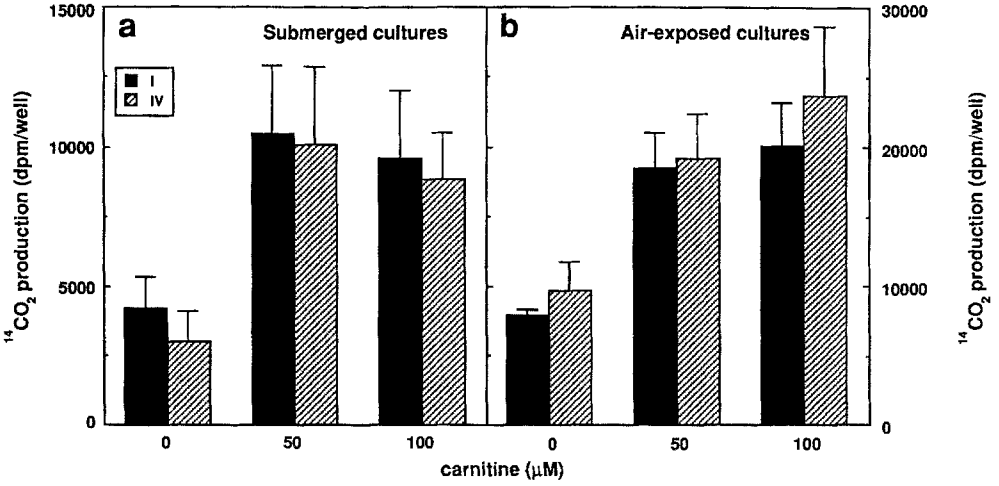


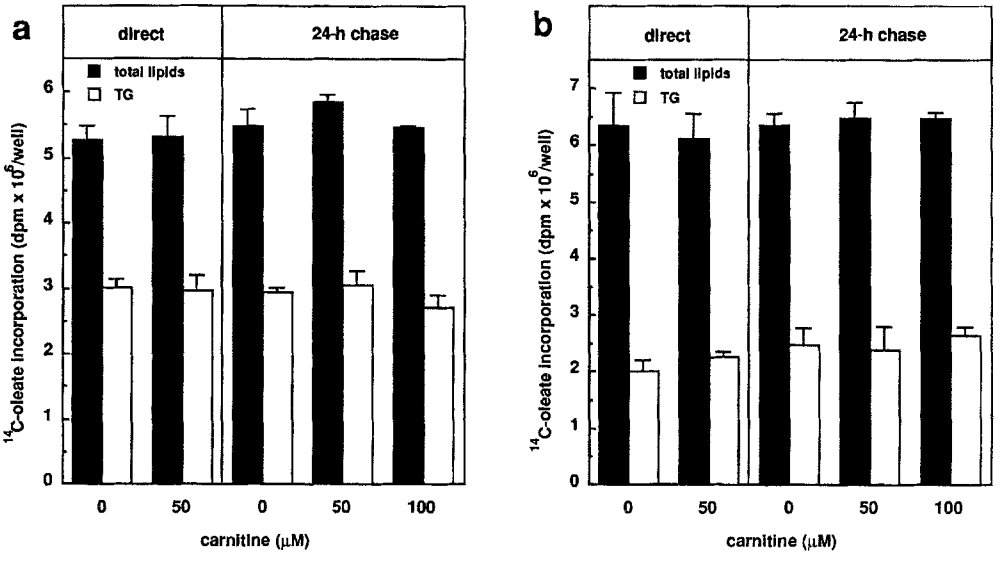
Fig. 5 a, b β-Oxidation of ^{14}C-oleic acid in keratinocytes cultured under submerged (**a**) or air-exposed (**b**) conditions. The cells were cultured for 10 days in medium 1 (3.8 g/l glucose, ■) or medium IV (1.2 g/l glucose, ▨). To study the effect of carnitine, the cells were incubated for 48 h prior to the experiment (from day 8 to 10) in medium supplemented with 50 or 100 μ*M* carnitine. The cultures were then incubated for 6 h (submerged cultures) or 18 h (air-exposed cultures) in the presence of ^{14}C-oleic acid and the released $^{14}CO_2$ determined, as described in Materials and methods. The results are expressed as $^{14}CO_2$ released (dpm/well) and are means ± SD ($n = 4$)

Fig. 6 a, b Incorporation of ^{14}C-oleic acid into total lipids and TG. Air-exposed keratinocyte cultures grown in (**a**) medium I (3.8 g/l glucose) or (**b**) medium IV (1.2 g/l glucose) were preincubated for 48 h in the presence or absence of 50 μ*M* carnitine and then incubated for 24 h with ^{14}C-oleic acid (1 μCi/ml). The medium was then discarded and the cultures were washed several times with PBS and either harvested directly (to assess the amount of ^{14}C-oleic acid incorporated into the lipids) or harvested after a 24-h reincubation in medium containing increasing concentrations of carnitine (0, 50 or 100 μ*M*) (to monitor lipid turnover). The lipids were extracted and fractionated, and the amount of ^{14}C-oleic acid incorporated into the lipids determined, as described in Materials and methods. The results are expressed as the amount of ^{14}C-oleate incorporated into the total lipids (■,) and TG (□) and are means ± SD ($n = 3$)

the cells were grown in low or high glucose media or under submerged or air-exposed conditions. An increase in carnitine concentration to 100 μ*M* did not lead to a further increase in $^{14}CO_2$ production.

Effect of carnitine on lipid synthesis, turnover and composition

The finding that β-oxidase activity was increased by incubation of cells in the presence of carnitine, prompted us to investigate whether the incorporation of ^{14}C-oleic acid into lipids and lipid turnover during the chase (24 to 72 h) in air-exposed cultures could be modulated by carnitine. As shown in Fig. 6, the incorporation of ^{14}C-oleic acid into total lipids was similar regardless of whether the air-exposed cultures were preincubated for 48 h in the presence or absence of 50 μ*M* carnitine. Furthermore, the amount of ^{14}C-oleic acid incorporated into total lipids was not affected by lowering the level of glucose in the culture medium from 3.8 g/l to 1.2 g/l and remained unchanged during the chase period, even when the chase period was prolonged for up to 72 h (data not shown). This occurred



regardless of the presence or absence of carnitine in the culture medium. The amount of ^{14}C-oleic acid incorporated into the TG fraction was higher in cultures grown in medium containing a high glucose concentration (3.8 g/l, medium 1; about 50% of total incorporated radiolabel; Fig. 6a) as compared with cultures grown in low glucose medium (1.2 g/l, medium 4; about 30%, Fig. 6b). Most of the remaining label was incorporated into phospholipids. As with total lipid synthesis, neutral lipid synthesis was also not affected by the presence of carnitine. This finding is not surprising, since the extent of ^{14}C-oleic acid oxidation was very low: in the absence of carnitine about 0.8% of total incorporated ^{14}C-oleic acid was released as $^{14}CO_2$; this amount increased in the presence of 50 or 100 μM carnitine to about 1.7%. Growing air-exposed cultures for 7 days in the presence of 50 μM carnitine did not have any effect on bulk lipid composition (data not shown) even when 1 μM α-tocopherol was added to the medium to prevent the formation of lipid peroxides.

Discussion

Previous studies have revealed (reviewed in references 14 and 17) that cultured keratinocytes are capable of synthesizing all classes of epidermal lipids, but the relative amounts of some of them, for example TG, deviate from those found in native tissue. At present the role TG play in keratinocyte lipid metabolism is not clear, since TG accumulation has been observed both in submerged and in air-exposed keratinocyte cultures. The high rate of TG synthesis may be related to the stage of keratinocyte differentiation since in submerged cultures the TG content increased during the culture time, the TG content in post-confluent cultures being tenfold higher than in preconfluent ones [27]. In addition, the TG content in stratified air-exposed cultures was increased as compared to submerged cultures of normal human keratinocytes [19] and to submerged and air-exposed cultures of squamous carcinoma cells, the latter cells being impaired in their terminal differentiation programme [20]. In cultures of normal keratinocytes TG accumulate in the stratum corneum as non-membrane-bound cytoplasmic droplets which are homogeneous in appearance [6]. In contrast to submerged cultures in which TG to a certain extent provide a FA reservoir for phospholipid synthesis [14], the TG pool in air-exposed cultures is very stable and behaves as stored lipid [15, 17].

The present study was undertaken to examine whether the TG accumulation in the air-exposed cultures may be a result of insufficient supplementation of cells with oxygen, an excessive supplementation of cells with glucose, dysregulation of overall lipogenesis, insufficient activity of TG lipase and/or accumulation of FFA due to insufficient activity of β-oxidase.

It has been reported that hypoxic cultures of fibroblasts accumulate TG and FFA [7] and synthesize lower amounts of cholesterol [13]. Under hypoxic conditions the FFA are not sufficiently utilized due to decreased FFA oxidation in mitochondria. To prevent over-accumulation of FFA the cells esterify and store FA as TG. In addition, hypoxia also induces changes in cellular morphology, in particular mitochondria exhibit an abnormal appearance. At present, it is not clear whether TG accumulation in air-exposed keratinocyte cultures results from insufficient oxygen supply, since even at high lactate production the FFA content remains very low (much lower than in native epidermis [19]) and mitochondria display a normal appearance. Even when the FFA oxidation is increased by the presence of carnitine or when the cultures are maintained in an atmosphere of 40% oxygen/60% air, the TG content remained high (data not shown), suggesting that TG catabolism is not dependent on the rate of FFA oxidation.

Since in various cell types the rate of lactate production and of TG synthesis have also been shown to be dependent on supplementation of the cells with glucose, we examined whether the modulation of glucose concentration in the culture medium affects lactate production and TG synthesis. These experiments revealed that the lowering of glucose levels was associated with a decrease in both the TG content and lactate production. A reduction in the TG content was also accompanied by a marked reduction in the number of lipid droplets, as revealed by transmission electron microscopy (data not shown). In spite of the improvement in the profile of epidermal lipids, as judged from the lower TG and higher ceramide contents, the stratum corneum barrier function still remained impaired. Recent studies have disclosed that in cultures grown in low-glucose media the lipid ordering and structure, as revealed by X-ray diffraction [4] and transmission electron microscopic studies (M. Fartasch, unpublished data), still deviate from that in native epidermis. In addition, it should be noted that FFA levels in RE-DED are much lower than those found in native epidermis. As is the case in vivo, the levels of FFAs in the stratum corneum in vitro are also higher than in the viable epidermal layers. The increase in FFA levels is a result of phospholipid degradation within the stratum corneum which occurs both in vivo and in vitro. Low FFA levels are maintained in viable epidermal cell layers both in vivo and in vitro to prevent toxic effect of FFA on living cells. In stratum corneum the presence of FFAs is required for the proper organization of lipid bilayers, since it has been demonstrated that an altered FFA/cholesterol/ceramide ratios coincide with the appearance of abnormal barrier function [12]. The impaired organization of stratum corneum lipids in RE-DED may therefore arise from inappropriate relative amounts of FFA, cholesterol and ceramides.

When the TG synthesis and turnover was monitored in chase experiments (by determining the incorporation of ^{14}C-oleic acid into lipids) it appeared that the rate of TG catabolism was very low. The high rate of TG synthesis and the presence of a stable TG pool suggests that the degradation of endocellularly synthesized TG was reduced. This may be due to a specific defect in the activity of cytoplasmic triacylglycerol lipases. Examination of tri-

acylglycerol lipase activity over the pH range 4.0–10.5 in the homogenates prepared from cultured and native epidermis revealed that the profiles of lipase activities were similar, but in cultured keratinocytes the lipase activity was lower (by a factor of about 2) than in native epidermis. In spite of this finding, the differences in the lipase activities cannot explain why the TG catabolism in cultured keratinocytes was so low. Since no deficiency of lipase activity in cell homogenates could be demonstrated, a functional lipase deficiency may have existed within intact cells, as may arise if the enzyme, enzyme activator or cofactor or even substrate are mislocalized to the wrong subcellular compartment. Impaired TG catabolism may also arise from a low rate of FA oxidation, since lipase activity is inhibited when the levels of FFA are high. This possibility is very unlikely, since an analysis of epidermal lipids has revealed that the content of FFA in RE-DED is very low [13] and that keratinocytes can oxidize exogenously administered FA. In addition, the turnover of TG remains low even when the FFA oxidation is enhanced by carnitine supplementation. Furthermore, recent pulse label experiments have revealed [15, 17] that while radiolabel incorporated into TG remains high during the chase period of 21 days, the radioactivity associated with FFA remains low, indicating that newly synthesized FFAs are rapidly esterified and that the TG pool is very stable. If cultured cells synthesize excessive quantities of FA, in a vain effort, for example, to correct EFA deficiency by synthetizing unsaturated FA (reviewed in reference 17), the excess FA so produced would be stored in TG. The significant reduction in TG content with a reduction in glucose argues that excessive FA synthesis, beyond that required for phsopholipid synthesis or energy production (oxidation), results in an accumulation of TG.

Another explanation for TG accumulation may be sought in the dysregulation of overall lipogenesis. The results of recent studies by Williams et al. [29] suggest that FA and TG synthesis are in some manner linked to cholesterol synthesis. Thus, when the synthesis of cholesterol is inhibited in submerged keratinocyte cultures by treatment with HMGCoA-reductase inhibitors [29], the synthesis of both FA and TG is enhanced, resulting in accumulation of lipid droplets within the cell. Since, in the air-exposed keratinocyte cultures, the rate of cholesterol synthesis was high, it is unlikely that the TG accumulation is related to disturbances in cholesterologenesis.

In conclusion, keratinocytes grown at the air-liquid interface synthesize abundant amounts of TG which accumulate within the cells, the extent of TG accumulation being dependent on the concentration of glucose in the culture medium: the lower the glucose concentration, the lower the TG content. However, even when the TG content is reduced the accumulated TG form a stable pool which is utilized neither as a source of energy nor as a pool for synthesis of other lipid species. The findings that active triacylglycerol lipase (when examined over a wide range of pH values) was present in the homogenates, and the cells were capable of oxidizing FFA, suggest that the presence of a stable TG pool may result from aberrant enzyme compartmentalization or deficiency of an enzyme activator. A similar mechanism has been postulated by Williams et al. [26, 28] for the accumulation of TG in fibroblasts derived from patients with neutral lipid storage disease. Since in these fibroblasts neither impairment in TG lipase activity in cell homogenates nor over synthesis of glycerolipids has been detected, Williams (reviewed in reference 26) has proposed that the storage of endogenous TG may arise from functional lipase deficiency within intact cells.

Previous studies have revealed [15, 19] that keratinocytes grown at the air-liquid interface synthesize substantial amounts of phospholipids, but upon prolongation of the culture the rate of phospholipid synthesis declines. The keratinocyte growth and differentiation pattern also changes during culture – during the first 2 weeks of air exposure the proliferation rate is high and the cellular maturation is rapid. This results in the formation of fully differentiated tissue. However, this tissue displays an altered expression of several differentiation-specific proteins, similar to that seen during wound healing [15]. After wound closure homeostasis is established resulting in a low level of proliferation and ordered differentiation. In contrast to the in vivo situation, in keratinocytes grown for a prolonged time at the air-liquid interface no epidermal homeostasis is established, but the proliferation rate decreases with time. This results in a decrease in the number of living cell layers and an increase in the number of stratum corneum layers, these layers being enriched in ceramides and partially depleted of phospholipids (reviewed in references 5, 24 and 25). Since, in proliferating cells, the presence of phospholipids is necessary for the formation of cell membranes, one can speculate that in keratinocytes grown at the air-liquid interface, phospholipid synthesis does not proceed optimally and this may be the cause of epidermal homeostasis not being reached upon prolongation of the culture time. This may be due to the presence of suboptimal concentrations of some (co)factors or a deficiency in the functional activity of enzymes involved in phospholipid synthesis, for example cytidyltransferase, resulting in synthesized diacylglycerol (a common precursor of TG and phospholipids) being utilized predominantly for TG synthesis. Further investigations will be required to establish what role the enzymes involved in phospholipid synthesis play in the regulation of keratinocyte proliferation and differentiation.

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