Arch Dermatol Res (2002) 294 : 221–230
DOI 10.1007/s00403-002-0323-1

ORIGINAL PAPER

C. R. Harding · A. E. Moore · J. S. Rogers · H. Meldrum
A. E. Scott · F. P. McGlone

Dandruff: a condition characterized by decreased levels of intercellular lipids in scalp stratum corneum and impaired barrier function

Received: 13 November 2001 / Revised: 15 January 2002 / Accepted: 24 April 2002 / Published online: 7 June 2002
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Abstract Dandruff is a major problem, yet little is known about the underlying mechanism and subsequent biochemical changes occurring in the scalp skin that lead to its manifestation. The characteristic flaking and scaling of the scalp experienced by dandruff sufferers suggests, similar to the changes classically seen in xerosis, that the desquamation process is impaired. We initiated studies to quantify the biochemical nature of the stratum corneum in the scalp of healthy individuals and dandruff sufferers. Total amounts and relative ratios of stratum corneum lipids species were analysed in scalp stratum corneum samples collected during studies conducted in the UK and Thailand in order to examine ethnic differences. In both populations, dandruff was associated with a dramatic decrease in free lipid levels, with significant decreases in ceramides, fatty acids, and cholesterol. Detailed sub-analysis of the major ceramide species within the total ceramide fraction revealed a decrease in ceramide 1 and increased proportions of ceramide 6i and 6ii. In a separate study, we demonstrated that dandruff sufferers show both an elevated blood flow and an increased reported incidence of itch in response to histamine topically applied to the scalp compared with no-dandruff controls. Taken together these two studies indicate that the quality and resilience of the epidermal water barrier is impaired in the scalp of dandruff sufferers. We propose that the perturbed barrier leaves dandruff sufferers more prone to the adverse effects of microbial and fungal toxins, and environmental pollutants, thus perpetuating their impaired barrier.

C.R. Harding (✉) · A.E. Moore · J.S. Rogers · H. Meldrum
Unilever Research, Colworth House Laboratory,
Sharnbrook, Bedfordshire MK44 1LQ, UK
e-mail: Clive.Harding@unilever.com,
Tel.: +44-1234-222742, Fax: +44-1234-222552

A.E. Scott · F.P. McGlone
Unilever Research, Port Sunlight Laboratories, Wirral, UK

F.P. McGlone
Centre for Cognitive Neuroscience, University of Wales,
Bangor, UK

Keywords Lipids · Dandruff · Stratum corneum · Ceramide · Epidermal barrier · Scalp · *Malassezia*

Introduction

Dandruff is a common complaint and is suffered by as many as 50% of the population at some time during their life. The condition is generally characterized by the presence of flakes on the scalp and in the hair, and by itch. The symptoms can vary, and the severity can range from mild scaling, similar to dry skin, to severe scaling. Its prevalence and severity is greatest in young men, with children and older individuals suffering less frequently. It is generally accepted, although the data are equivocal, that the presentation of dandruff ranges from dry scalp to seborrhoeic dermatitis [1, 2].

Despite the fact that dandruff is a major global problem, and has been the subject of considerable research, there has been little progress in understanding the aetiology of the condition over the past decade. The central dandruff hypothesis remains that the lipophilic yeast, *Malassezia*, previously known as *Pityrosporum*, is the causal agent of dandruff [3]. The evidence supporting the significant involvement of *Malassezia* in the condition is primarily that the most effective antidandruff treatments are antifungals, and improvement and regression of the scalp condition correlates with removal of, and subsequent recolonization by, the yeast [4, 5, 6, 7]. In addition, the symptoms of dandruff, such as scaling, can be induced in animal models by inoculation with *Malassezia* [7].

However, *Malassezia* is a commensal organism that is found on healthy scalps as well as on dandruff scalps. No pathogenic mechanism, such as a hyphal transformation, has been associated with deterioration from a healthy to a dandruff scalp [1] and therefore a simple causal role for *Malassezia* in dandruff has yet to be unequivocally established. Taken together, these observations imply that other factors must play a significant role. It is possible that such factors make the host more susceptible to proinflammatory molecules released either directly by the yeast, or in-

directly by its interaction with other microbes within the scalp microenvironment. An altered immune response to *Malassezia* has also been proposed as a significant factor, although recent evidence suggests that seborrhoeic dermatitis may not be caused by an altered immune response to *Malassezia* yeasts [8].

It is probably misguided to try and identify a single aetiopathogenesis for dandruff, and it is likely that there are several complex mechanisms underlying the condition. The characteristic scaling appearance of the scalp in dandruff sufferers indicates that one or more of the critical maturation processes, including desquamation of the stratum corneum, are altered in a similar way to other skin disorders (reviewed in reference 9). A change in epidermal proliferation has been proposed by Ackerman and Kligman to explain some of the characteristic features of the dandruff condition [10], and it is known that parakeratosis and increased proliferation are features of the epidermis in dandruff scalps [10, 11]. Increased keratinocyte turnover in response to inflammation would result in incomplete keratinization, and an incorrect maturation of the stratum corneum, which is often associated with perturbation of the epidermal barrier. Barrier function is frequently impaired in skin disease, and anomalies in the major lipid species, namely ceramides, fatty acids and cholesterol [12], have been identified as a major characteristic in a number of scaling skin disorders ranging from winter xerosis to psoriasis [13, 14, 15, 16, 17, 18, 19, 20].

In this study, we sought to determine whether the flaking and scaling which characterizes the dandruff lesion is associated with altered composition of the free and covalently bound lipids in scalp stratum corneum. The study was conducted in the UK (winter only) and in Thailand (both dry and humid seasons), in order to investigate possible ethnic differences in the aetiology of the condition and the influence of climate. In addition, we related our observations in lipid changes in dandruff scalp skin to an impairment of barrier performance in vivo by topical application of histamine. Histamine is the classic mediator of itch and is normally excluded by the skin if applied topically. However, should the skin barrier be compromised, histamine can penetrate, gain access to histamine H1 receptors, and thus provoke a sensation of itch and/or increased local blood flow [21]. In this study we demonstrated that dandruff scalp skin is characterized by a decrease in free intercellular lipids and an increased sensitivity to the topical application of histamine.

Methods

Subject recruitment and scalp assessment

Study I: Scalp stratum corneum lipid analysis

For both studies quantifying scalp barrier lipids (UK and Thailand), a mixed gender group of subjects were recruited with ages ranging from 20 to 40 years. Scalps were visually assessed by an experienced trichologist and classified as either normal and healthy (scalp score <32) or as having dandruff (scalp score >32) according to the assessment protocol outlined below. Subjects were examined under standardized lighting conditions and successive partings were made in four quadrants of the scalp using the handle of a tail comb. Within each quadrant, the area occupied by each of six severity grades was estimated to the nearest tenth of the area of the quadrant. The severity grades and their associated severity scores were defined as in Table 1. The estimate of the area for each grade was multiplied by the severity score, and these products were summed for each quadrant. The quadrant additions were summed to give a whole head score with a range of 0 to 200. All subjects were placed on a non-antidandruff shampoo for 3 weeks prior to scalp sampling. No hair washing was allowed within the 48 h prior to sampling. Scalps were reassessed on the day of sampling. In the Thai study, where possible, the same subjects were examined during both the dry season (average daily temperature 26.5ºC, average relative humidity 74.3±3.0%) and the humid season (average daily temperature 28.2ºC, average relative humidity 82.1±1.8%) studies. Average UK temperature during the study was 5.6ºC with an average relative humidity of 81.3%.

Table 1 Definition of severity grades and scores for adherent scalp flaking

Severity grade	Description	Severity score
O	Healthy scalp with no dryness or dandruff	0
A	Fine dryness on scalp surface	1
B	Small powdery flakes partially adhering to scalp	2
C	Moderately flaky scales loosely attached to scalp	3
D	Large pronounced crusty scaling adhering to scalp	4
E	Very large crusty scaling congealed into plates adhering to scalp	5

Study II: Response to topically applied histamine

Subjects classified as having dandruff were accepted onto the study and placed on a non-antidandruff shampoo for 2–3 weeks prior to the start of the study. They were asked not to wash hair for 48 h before returning for the start of the study. At the beginning of the study, subjects completed questionnaires on the level of scalp itch they experienced. Each subject's scalp was then visually reassessed. Subjects with a total weighted head score of <32 at this stage were allowed to remain in the study. These subjects could be regarded as having sub-dandruff with the movement of scores above and below the score criteria consistent with the hypothesis of dandruff being a cyclical condition. A total of 220 subjects (124 female and 96 male) took part in the study.

Analysis of stratum corneum lipids

Free lipids

Two partings (each approximately 0.5–0.6 cm in width by 6–7 cm in length) were made in the hair to reveal scalp sites of similar condition. Exposed scalp sites along the hair parting were swabbed briefly with isopropyl alcohol to remove surface sebum, and then sequentially tape stripped eight times (Tape 1601; Hadleigh Enterprises, Wickford, UK). Once collected, tape-strips were stored at –20ºC.

Corneocytes were removed from the tape by mild sonication in methanol. The methanolic suspension of corneocytes was then dried under nitrogen and extracted in chloroform/methanol (2:1) for 2 h. One-third of the clarified sample was removed for cholesterol sulphate analysis. The remainder of the sample was dried under nitrogen, reconstituted in chloroform, and the lipids separated

into major classes on aminopropyl-bonded silica gel columns (Bond Elut NH2, Analytichem Corporation, Rockville, Md.) exactly as previously described [18]. All lipid fractions were then fractionated by high-performance thin-layer chromatography (HPTLC) on 20×10-cm plates (Silica gel 60: Merck, Darmstadt, Germany). Ceramides were developed three times using chloroform/methanol/acetic acid (190:9:1). Fatty acids were developed with hexane/ethyl acetate (85:15, then 70:30). Cholesterol and fatty acids were separated with petroleum ether/diethyl ether/acetic acid (80:20:1). Plates were dried at 37ºC between each run and finally dried at 120ºC, prior to charring with 10% w/v copper sulphate/8% v/v orthophosphoric acid at 160ºC for 20 min. Lipids were quantified using a scanning densitometer (Shimadzu CS9000) at 420 nm against standard curves of cholesterol, palmitic acid, triolein (Sigma, Poole, UK) and ceramide 2 (Quest International, Ashford, UK).

Cholesterol sulphate was analysed from a dried lipid extract reconstituted in hexane and centrifuged at 20,000 *g*. The pellet was reconstituted in chloroform/methanol (2:1) and separated on HPTLC plates using chloroform/methanol/ammonia (40:10:1). Cholesterol 3-sulphate (Sigma) was used as a standard, and was quantified as above.

Stratum corneum protein quantitation

Lipid-depleted corneocytes were extracted in 1% w/v SDS, 10 m*M* Na_2HPO_4, pH 7.8, and 20 m*M* β-mercaptoethanol for 2 h at 60ºC. Samples were dried, reconstituted in deionized distilled water, and soluble protein was quantified using the BCA microtitre plate assay (Bio-Rad, Hemel Hempstead, UK). Absorbance was measured at 540 nm, and protein was compared to a standard curve of bovine γ-globulin. Lipid levels are expressed as nanograms lipid per microgram protein.

Assessment of response to topically applied histamine

Two contralateral sites were located on each subject's head. The hair was held back with grips and a scalp score on a scale of O to E (Table 1) was given for each site. The sites were cleaned using an isopropyl alcohol wipe, and laser Doppler blood perfusion and temperature measurement probes (Moor Instruments, Axminster, UK) attached to each location using a purpose-built chamber. Background blood flow measurements were taken over a period of 60 s before a 1% w/v histamine solution (Sigma H-7250) in 2% v/v methylcellulose was introduced into the chamber housing the laser Doppler probe. During the test, each subject was asked to estimate the intensity of any itch sensation experienced at the site using a visual analogue scale (VAS) anchored at "no itch" and "worst itch". A verbal descripta based upon the McGill Pain Questionnaire [22] of the VAS scale relating to the intensity of itch is shown in Table 2. The output of the VAS (voltage divider) was displayed and stored with the blood flow data. Subjective experience of itch was therefore assessed simultaneously with the objective measurement of blood flow. Analysis of the data showed that there was no difference in the flux responses whether the site was on the left or right side of the scalp.

Table 2 Ranking of itch response for visual analogue scale

Qualitative subject itch intensity	Percentage
None	0
Faint	10
Weak	20
Mild	30
Moderate	50
Strong	70
Intense	90
Extremely intense	100

Statistical analysis

Study I. All results are reported as means±SD, and were analysed using Student's *t*-test with the significance set at the 5% level.

Study II. The blood flow and VAS data are non-parametric; relationships between categorical assignments were therefore analysed using the chi-squared, Mann-Whitney and Wilcoxon Rank tests. The Mantel-Haenszel chi-squared test is a more powerful version of chi-squared for use when categories for each response are ordered such as with questionnaire data. VAS data tended to contain many zero responses and could not be considered normal. Such data were analysed by the Mann-Whitney test. The log-transformed flux data were normally distributed and were analysed by ANOVA.

Results

Scalp lipids are decreased in dandruff

Intercellular lipids were found to be decreased in dandruff sufferers compared to healthy individuals (Fig. 1A–C). This was true both in the barrier lipid studies conducted in the UK and in those conducted in Thailand. The generalized decrease in lipids was more pronounced in the Thai dry season (Fig. 1B) than in the humid season (Fig. 1C). Dandruff condition was worse in the Thai dry season than in the humid season, and the dandruff score in the dry season was twice the value in the humid season.

In UK subjects, a significant decrease ($P<0.05$) was seen in amounts of cholesterol sulphate, free fatty acids, cholesterol, triglycerides and total ceramides (Fig. 1A). Thai subjects demonstrated a significant decrease ($P<0.05$) in fatty acids, cholesterol, triglyceride, and total ceramide in the dry season (Fig. 1B) although only cholesterol and ceramides were significantly decreased in the humid season (Fig. 1C, $P<0.05$). Although there was a high level of individual variation in lipid levels, decreased levels were always evident in dandruff compared to healthy subjects, even though the decrease was not always significant. In general, UK and Thai subjects demonstrated similar levels of total lipids. The total lipid level in healthy UK subjects was 3498.4±2055 ng/μg protein, which decreased to 1414.4±1218.1 ng/μg protein in dandruff subjects. Healthy Thai subjects had total lipids of 6337±2618 ng/μg protein and 4008±1444 ng/μg protein in the dry and humid seasons, respectively. This amount decreased to 2429±1121 ng/μg protein and 2760±1552 ng/μg protein in dandruff subjects during the dry and humid seasons, respectively.

To examine specific changes in lipid ratios, the major classes of extracted lipids are expressed as percentages of total lipid (Fig. 2). The proportions of cholesterol sulphate and cholesterol were increased in dandruff subjects, but this was only significant ($P<0.05$) in UK subjects (cholesterol, Fig. 2A) and Thai subjects (cholesterol sulphate) during the dry season (Fig. 2B). The relative proportion of fatty acids and triglycerides was similar in healthy and dandruff subjects (Fig. 2A–C). The relative percentage of

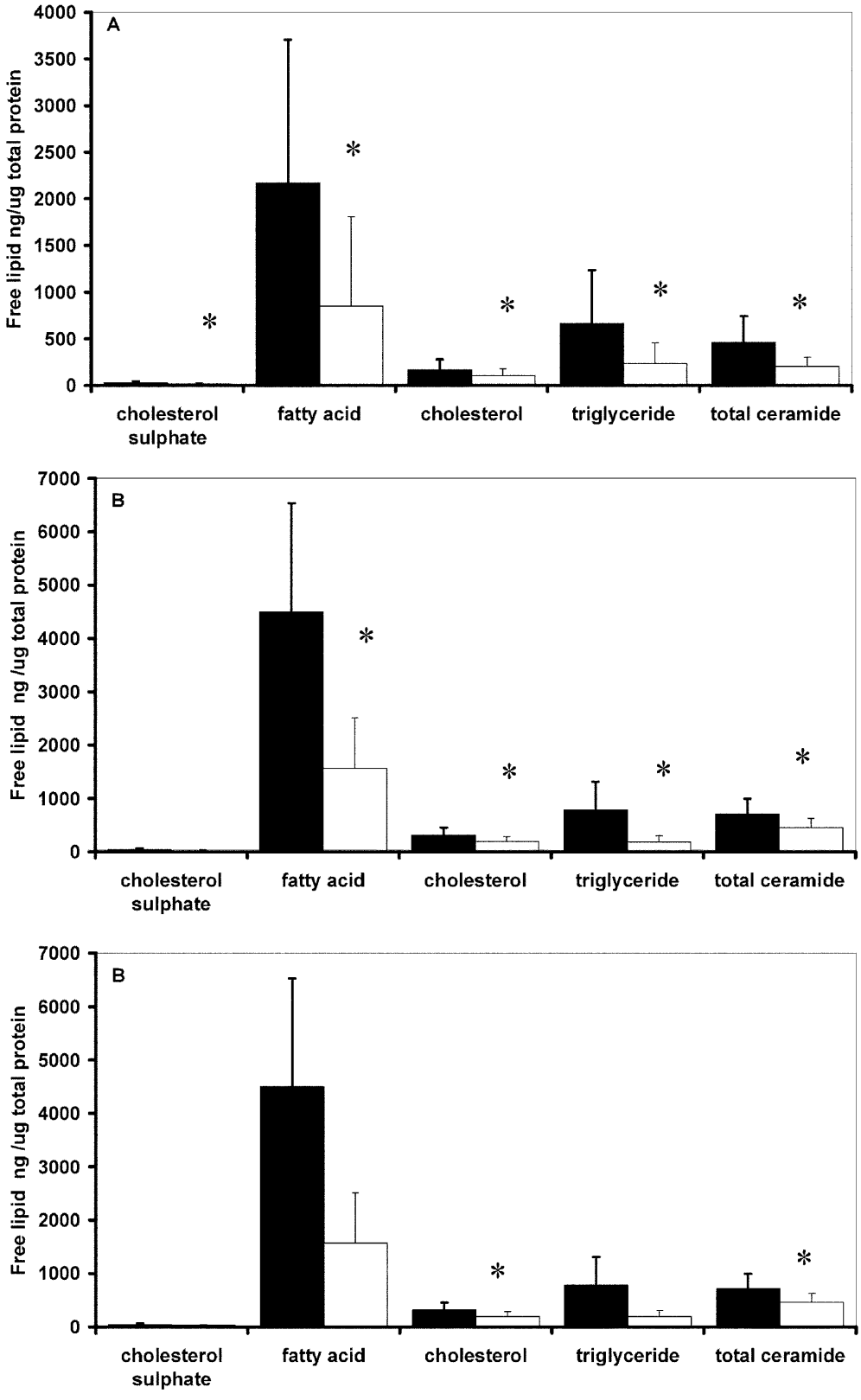

Fig. 1A–C Dandruff sufferers have lower levels of free lipids. Amounts of cholesterol sulphate, total ceramides, free fatty acids, cholesterol and triglycerides were assayed in tape strips from subjects. The lipids were quantified using a scanning densitometer and were compared with known standards. **A** UK subjects: *filled bars* healthy (n=17), *open bars* dandruff (n=24); **B** Thai subjects (dry season): *filled bars* healthy (n=11), *open bars* dandruff (n=20); **C** Thai subjects (humid season): *filled bars* healthy (n=11), *open bars* dandruff (n=20). *P<0.05

total ceramides showed a tendency to increase in dandruff subjects in the UK, and in both Thai dry and humid seasons, although this increase was significant only in Thai subjects during the dry season (Fig. 2B).

Precise changes in the heterogeneous ceramide population were investigated further by analysing individual ceramide classes, and again expressing each species as a percentage of total ceramides (Fig. 3A–C). Consistent changes

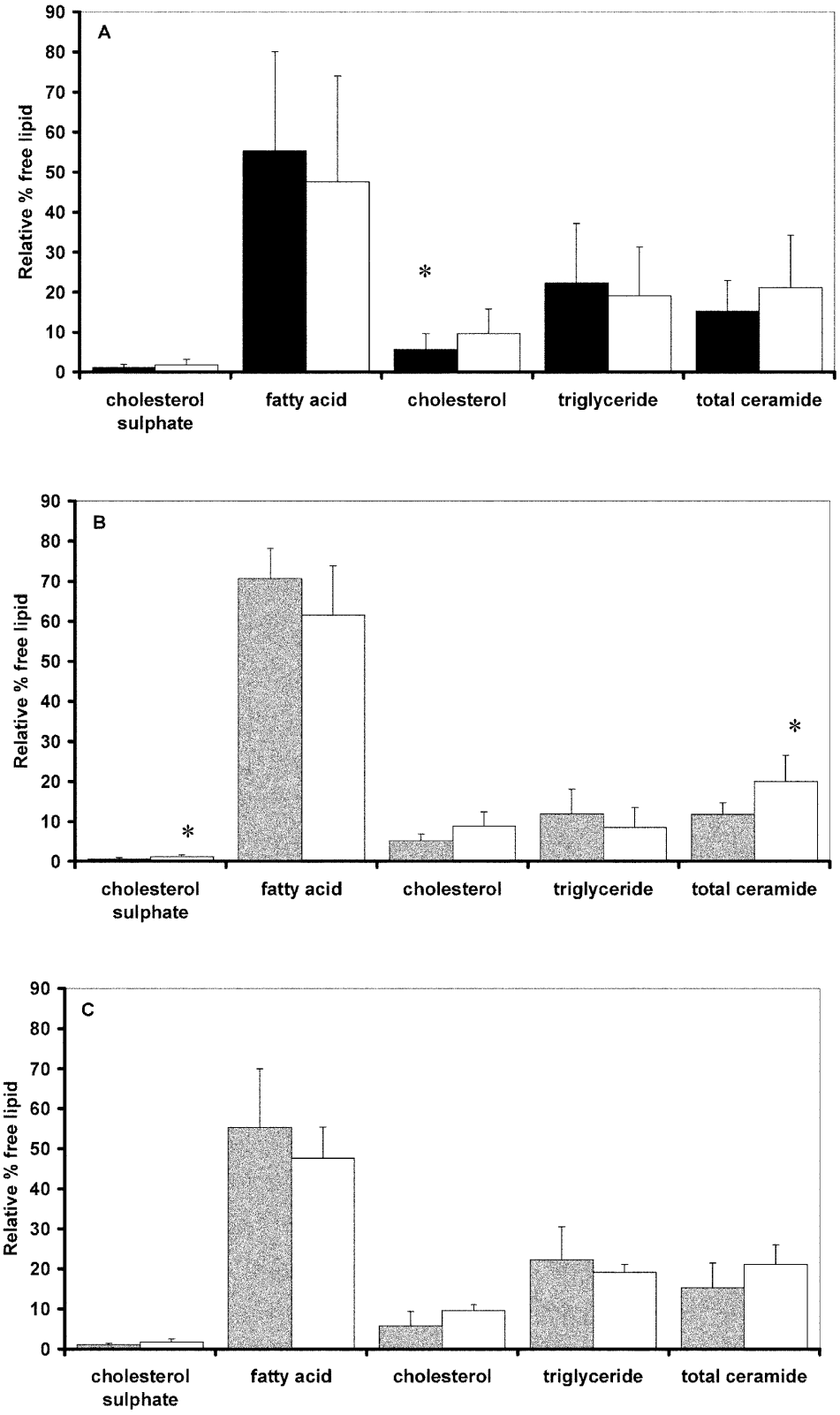

Fig. 2 Free lipid species (cholesterol sulphate, total ceramides, free fatty acids, cholesterol, triglycerides) as a percentage of total free lipids. **A** UK subjects: *filled bars* healthy (n=17), *open bars* dandruff (n=24); **B** Thai subjects (dry season): *filled bars* healthy (n=11), *open bars* dandruff (n=20); **C** Thai subjects (humid season): *filled bars* healthy (n=11), *open bars* dandruff (n=20). *P<0.05

in the ceramide profiles in dandruff versus healthy populations were not observed. A significant increase in ceramides 6i and 6ii was evident in UK and Thai subjects (during the dry season) and decreased levels of ceramides 1 and 3 were seen in dandruff subjects, but this decrease was significant (P<0.05) only in UK subjects. No change in the ceramide subclasses was evident between healthy and dandruff Thai subjects during the humid season. There

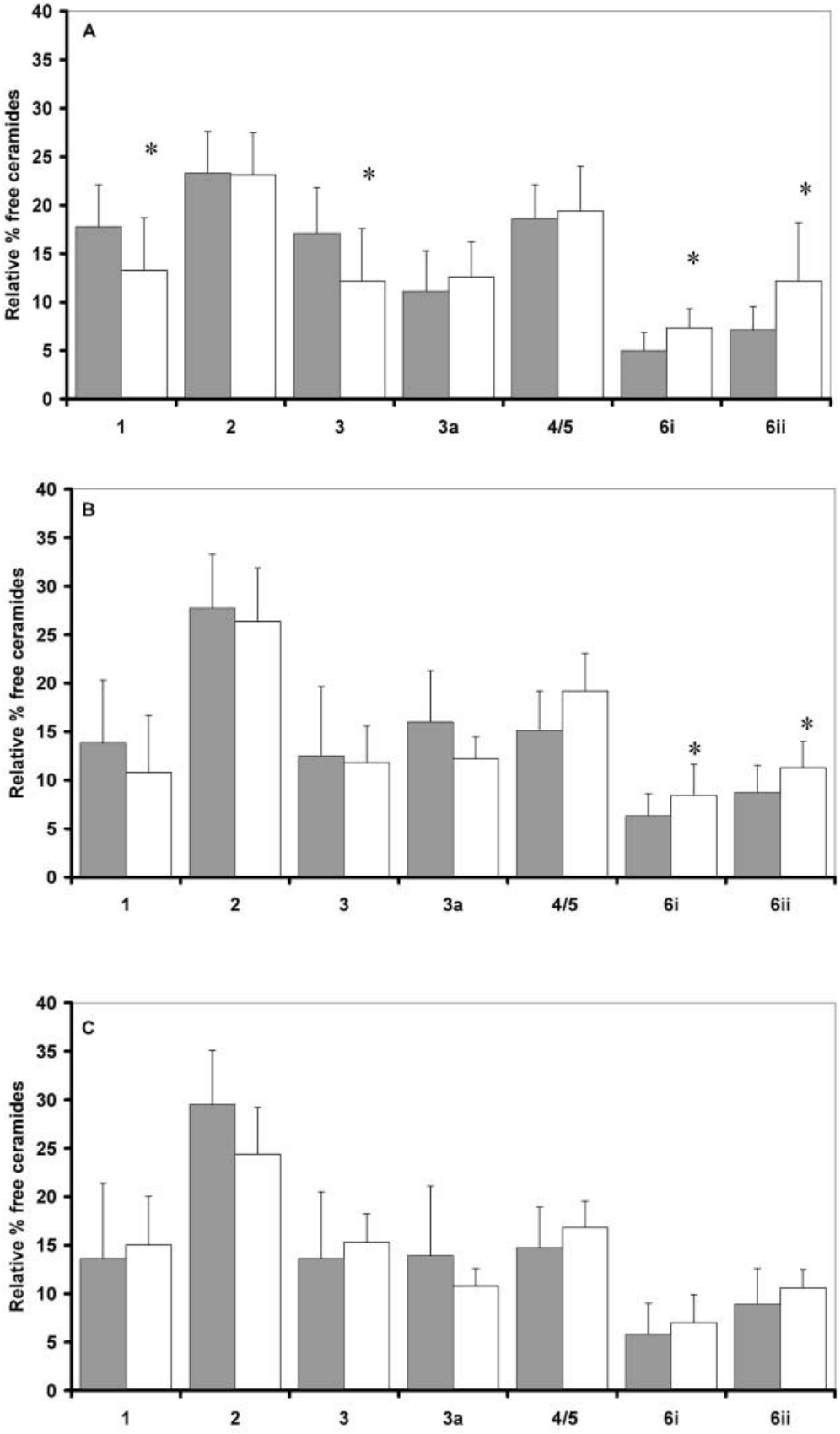

Fig. 3 Ceramide classes as a percentage of total ceramide levels. **A** UK subjects: *filled bars* healthy (*n*=17), *open bars* dandruff (*n*=24); **B** Thai subjects (dry season): *filled bars* healthy (*n*=13), *open bars* dandruff (*n*=24); **C** Thai subjects (humid season): *filled bars* healthy (*n*=5), *open bars* dandruff (*n*=8)

were also no significant differences between healthy and dandruff subjects in the amounts of covalently bound lipids or in the amounts and ratios of sebum lipids (triglycerides, fatty acids, squalene and cholesterol) (data not shown).

Response to topically applied histamine

Histamine is a classical mediator of itch, but when topically applied to most body sites it does not elicit a significant itch response unless the barrier is perturbed or the skin surface is deliberately damaged. Itch is often associated with the dandruff condition and in preliminary investigations (data not shown) we studied the varied itch response in subjects when histamine was iontophoresed into the scalp. In certain individuals an itch response was elicited before the current was switched on. In this study, we examined the response to histamine in the absence of iontophoresis in more detail. We applied histamine topically to the scalp skin, and investigated whether subjects had an objective and subjective response.

An objective response to histamine was defined as an increase in blood flow as measured by laser Doppler. Of 84 subjects with a healthy scalp (scalp score O and A), 26% showed an objective response, and of 90 more severely affected subjects (scalp score C), 64% showed an objective response (Fig. 4), The remaining 46 subjects with a score of B formed an intermediate category between scalp scores O/A and C and 41% of these subjects showed an objective response to histamine. A significant relationship between the blood flow response and site score was found ($P<0.001$, $\chi^2 = 30.5$).

A subjective response to histamine was defined as any indication of itch on the VAS scale within 5 min of the application of histamine to the scalp. In a similar pattern to

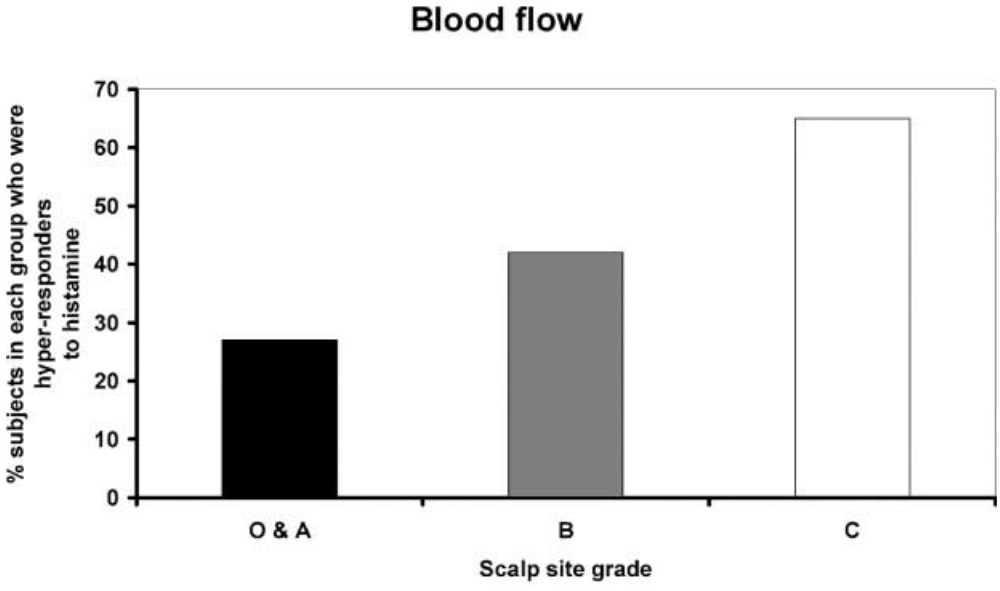

Fig. 4 Objective responses (increased blood flow) to topically applied histamine. Percentages of subjects who showed an increased scalp blood flow in response to the topical application of histamine grouped according to their scalp condition (severity grades O–C, see Table 1)

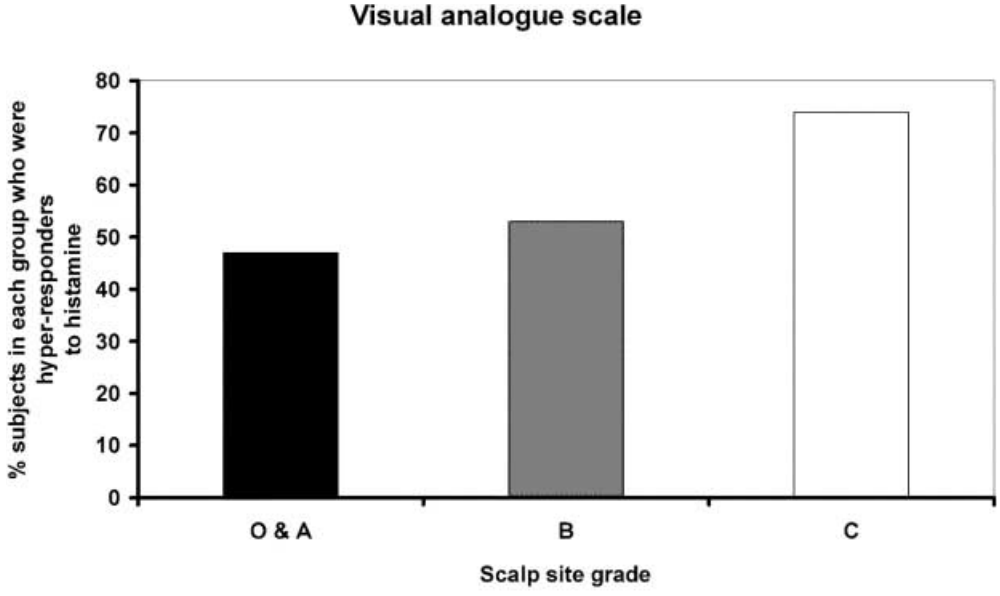

Fig. 5 Subjective responses (reported itch) to topically applied histamine. Percentages of subjects who reported an increased incidence of itch in response to topically applied histamine grouped according to their scalp condition (severity grades O–C, see Table 1)

the blood flow data, of 84 subjects with a healthy scalp (scalp score O and A), 46% reported an itch response, and of 88 more severely affected subjects (scalp score C), 74% reported an itch response (Fig. 5), There was a significant correlation between the subjective and objective responses to histamine. Correlation coefficients were 0.41 ($P<0.0001$), 0.42 ($P<0.03$) and 0.27 ($P<0.01$) for groups O/A, B and C, respectively.

Discussion

In this study, we demonstrated that a dramatic depletion of the major intercellular lipids in scalp stratum corneum is a characteristic feature of individuals suffering from dandruff. In particular, decreased levels of free fatty acid, cholesterol, and ceramides, which are all critical components of the epidermal water barrier [12], was evident in both UK and Thai dandruff sufferers. In general, the overall levels of scalp lipids were similar in the UK and Thai subjects, although in the latter, higher levels of free lipids were evident in the dry season. In addition, we observed that dandruff sufferers demonstrated an increased sensitivity to histamine application in terms of both objective and subjective responses. These two findings clearly demonstrate that the scalp skin barrier is perturbed in dandruff.

Decreased stratum corneum lipids are associated with a number of pathological and cosmetic skin disorders where the epidermal barrier is perturbed, and it is known that correct expression of lipids is necessary for a functional skin barrier [12, 13, 14, 15, 16]. Our observations suggest that the decreased levels of intercellular lipids associated with dandruff may underlie the poor scalp condition. These changes in lipid content may in turn perturb the water-holding capacity of the tissue, and reduce corneodesmosomal degradation leading to the characteristic scaling visible on the scalp surface in dandruff lesions (see review reference 9).

In contrast to winter xerosis and soap-induced dryness, which demonstrate increased ratios of fatty acids [19, 20], the relative proportions of the intercellular lipids did not show dramatic changes in dandruff. However, direct comparisons between lipids in dandruff and dry skin are difficult due to the presence of scalp sebum, and elevated levels of triglycerides were detected in samples despite attempts to remove surface sebaceous lipids. This suggests that sebum was absorbed within the surface layers of the scalp stratum corneum which has been reported to occur at sites elsewhere on the body [23] and is most marked on the scalp. In turn, we cannot assume that the free fatty acid was solely of stratum corneum origin as it may have resulted from microbially induced hydrolysis of sebaceous-derived triglycerides. Long-chain ceramides, which are the most critical components of the epidermis, and are not found in sebaceous secretions, were decreased in individuals suffering from dandruff. It is unknown whether this decrease reflects decreased synthesis, elevated leaching from the scalp surface or depletion resulting from microbial metabolism.

In parallel with typical dry skin, changes in the ratio of ceramides were also detected in dandruff. Ceramide 1 was decreased whereas ceramides 6i and 6ii were increased. A reduction in ceramide 1 has been associated with a variety of skin disorders including winter xerosis, atopic dermatitis and aged skin (reviewed in reference 24). Ceramide 1-linoleate plays a particularly important role in barrier structure and function and its presence is believed to be critical for maintaining the fluidity of the barrier [25]. There is a poorly understood seasonal decline in ceramide 1 linoleate levels in the winter which may predispose skin to dryness under these conditions [26]. It is tempting to speculate that ceramide 1 levels may fluctuate in scalp skin, which may explain why dandruff is more prevalent in winter in northern Europe.

Changes in lipids were also more pronounced during the Thai dry season than during the humid season, and this correlated with increased severity of dandruff. This seasonal difference suggests that decreased lipid levels are associated with dandruff severity, but of course, whether the relationship is causal or consequential is unknown. The influence of environmental factors on the seasonal predisposition to dandruff in Thailand remains to be elucidated. Although the daily temperature and relative humidity (averaged over 24 h) is not dramatically different between the dry and humid seasons, temperatures during the daytime in the humid season reach 37–40ºC. At 35ºC the water vapour pressure is some twofold higher than at 25ºC (6 versus 3 kPa). Under these very uncomfortable conditions, the incidence of scalp itch is reported to be much higher and the habit of scratching in response to this itch may in itself exacerbate a poor scalp condition. Although speculative, these considerable changes in atmospheric conditions may also influence the speciation of the bacteria and fungi colonizing the scalp surface potentially leading to increased irritation (see below).

The mechanism leading to decreased lipids in scalp skin in dandruff is currently unknown but there are a number of intrinsic and extrinsic factors that may contribute. First, *Malassezia*, or other scalp microbes, could actively metabolize and deplete the barrier lipids. *Malassezia* has an absolute requirement for fatty acids for growth [27], and is known to express lipase activity, which has been detected in supernatants of cultured *Malassezia* [28]. *Pseudomonas aeruginosa* has been found to possess ceramidase activity, which cleaves ceramide between the fatty acid and sphingosine moieties [29]. It is currently unknown whether any of the scalp-colonizing microbes, and in particular *Malassezia*, possess ceramidase activity.

Second, the decreased lipid levels may reflect an impairment of the epidermal differentiation process associated with an underlying inflammation. Epidermal hyperproliferation and inflammation are classically associated with psoriasis, which is a prevalent scalp condition also associated with reduced synthesis of stratum corneum lipids and impaired barrier function. Dandruff shares several other features with psoriasis: both are characterized by nuclear and neutrophil debris within the stratum corneum and the presence of similar chemotactic factors [30]. In

dandruff, the inflammatory stimulus may be the penetration of *Malassezia*-derived toxins, lipophilic metabolites such as peroxidated lipid [31], or possibly proinflammatory cytokines [32]. Although detailed electron microscopic studies were not undertaken in this study, it is also likely that there would be defective secretion of lamellar bodies associated with the epidermal hyperproliferation as reported for psoriasis [33] and atopic dermatitis [34].

Third, the reduced levels of stratum corneum lipids may reflect an intrinsic predisposition. Individuals susceptible to dandruff may have inherently low stratum corneum lipid levels and a permeability barrier which is either more readily damaged, or less able to repair itself following damage. In this scenario, decreased lipid levels may represent a cause rather than a consequence of dandruff. In some respects, the resting scalp condition of dandruff sufferers may be similar to that of the skin in aged individuals. Ceramide levels are known to decrease with age but under resting conditions barrier functionality, as measured by TEWL, is comparable to that of young skin. However, when the skin is challenged or 'stressed' by external factors, the ability to repair the permeability barrier in aged skin is inferior compared to young skin [35], and an acute insult can lead to a chronic skin problem. If the intrinsic barrier quality were an important factor in predetermining whether an individual is predisposed to dandruff, then this would help to explain the paradox of "everybody has *Malassezia* but not everybody has dandruff". In addition, other factors known to influence barrier functionality and repair, such as hormonal status (during the menstrual cycle), release of histamine itself [36], and even psychological stress [37, 38], could also initiate or exacerbate a dandruff episode in susceptible individuals.

The observations on histamine response are novel and of particular interest. Histamine is a classical mediator of itch although the mechanism underlying this skin sensation is poorly understood. The involvement of unmyelinated, histamine-sensitive nerve fibres has been established for experimental pruritus [39]. However, although itch cannot be induced by topically applied histamine on certain body sites, e.g. the forearm, unless the skin surface is perturbed, we have observed that topical application to apparently healthy scalp leads to reported itch in certain individuals. Paradoxically, studies to investigate the sensitivity of healthy scalp skin to histamine following intradermal microdialysis in vivo have indicated that scalp skin is actually less sensitive than forearm skin to histamine-induced experimental itch (submitted for publication). The observations in the present study cannot therefore simply be explained by a higher sensitivity of very superficial epidermal nerve fibres on the scalp as suggested elsewhere [40], but indicate instead that the histamine response is complex.

In the current study 46% of the healthy group reported a response to histamine. This high percentage may reflect the fact that the barrier is intrinsically weaker even in healthy scalps compared with other body sites, or the influence of follicular shunt pathways. However, this group also included subjects with fine dryness (severity grade A) and some individuals had previously cycled in and out of dandruff episodes before presenting a healthy scalp at the study onset. These factors may have contributed to the relatively high percentage of responders. Nevertheless, individuals with poor scalp condition responded more frequently to the application of topical histamine (74%). The increased frequency of both subjective and objective responses to topical histamine is consistent with underlying perturbation of the stratum corneum barrier function indicated by the reduced lipid levels in dandruff sufferers. More recent studies, in which corneosurfametry has been used to investigate barrier integrity, have also indicated a weakened barrier in dandruff sufferers (data not shown), which supports our hypothesis further.

Additional studies to understand the heightened response to histamine in dandruff and the incidence of a response in healthy scalps are clearly needed to examine whether there are differences in the underlying epithelium, and/or simple barrier perturbation. There is evidence of a marked increase in nerve fibre density and production of substance P, vasoactive intestinal peptide and calcitonin gene-related peptide following skin damage induced by tape stripping [41, 42, 43, 44], and similar changes may occur in dandruff sufferers. In normal skin, nerve growth factor (NGF), produced by basal keratinocytes, acts via a high-affinity receptor to increase keratinocyte sensitivity especially in inflammation, and NGF levels are increased in psoriatic skin [45]. Levels of NGF in dandruff lesions are unknown.

In conclusion, these studies have demonstrated that scalp skin in dandruff sufferers contains significantly reduced levels of intercellular lipids and shows an increased response to histamine. Skin barrier function is critical to life, since it acts as a primary defence mechanism against environmental stresses. We propose that an impaired barrier makes dandruff sufferers more prone to the adverse irritant effects of microbial activity on scalp skin, and to other environmental factors such as styling products, surfactants and pollutants. It is likely that both perturbed stratum corneum maturation and *Malassezia* yeasts contribute to the incidence and severity of the condition, although the question as to whether impaired epithelial differentiation is a predisposing cause of dandruff or a consequence of *Malassezia* interaction with the scalp remains to be answered.

Acknowledgements We would like to thank Susan Welch for organizing all the studies and conducting the scalp mapping analysis and David Hitchcock for statistical analyses. We would also like to thank Prof. Peter Friedmann, Prof. Terence Kealey, Prof. Jan Faergemann and Dr. Gillian Westgate for many fruitful discussions.

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