**Bathing frequency is associated with skin barrier dysfunction and atopic dermatitis at three months of age**

**Running title:** Bathing frequency is associated with skin barrier dysfunction and atopic dermatitis at three months

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**CLINICAL IMPLICATIONS**

Higher bathing frequency was associated with a step-wise increase in skin barrier dysfunction and a greater prevalence of atopic dermatitis at three months of age. Interventional work is required to assess the impact of bathing on AD development alongside other skin care routines.

To the Editor,

It is widely believed that bathing practices have an impact on the development and progression of atopic dermatitis (AD). Skin barrier impairment, filaggrin (*FLG*) mutation carriage and AD are strongly interrelated.1 The former, as determined by transepidermal water loss (TEWL), has been shown to be linked to the development of AD2 and food allergy.3 AD management guidelines recommend that bathing should be minimised,4 however the evidence base for the impact of bathing on skin barrier function, AD and its development is limited.

We hypothesised that increased bathing frequency at three months of age was associated with raised TEWL and AD. We recruited a cohort of 1,303 three-month-old healthy babies from England and Wales to test a dietary intervention for the prevention of food allergy.5 Parents completed an online questionnaire at enrolment and twelve months, assessing frequency of bathing (‘hardly ever’, ‘once a week’, ‘2-4 times a week’, ‘5-6 times a week’, ‘daily’ or ‘more than daily’), frequency of moisturizing, the use of shampoo, soap, bubble baths, bath oils and baby wipes and whether they felt their baby had dry skin. We collected information on ethnicity, family history, siblings and domestic water hardness (CaC03 mg/L) for each household.. Participants were genotyped for *FLG* mutations (R501X, 2282del4, R2447X, S3247X, 3673delC, and 3702delG) by TaqMan allelic discrimination assay (Applied Biosystems, ABI 7900 HT, Foster City, CA)) and were examined for AD at three and twelve months in accordance with the UK diagnostic criteria–based photographic ISAAC protocol.6 Skin barrier function was measured through TEWL on uninvolved skin of participants’ left volar forearm at three and twelve months. TEWL was reported as medians with interquartile ranges and anddichotomized to generate an elevated TEWL variable using a threshold of ≥15g/m2\*h (75th percentile of TEWL distribution with no AD at three month visit),as previously reported.7 Categorical data were compared using chi-square analyses. Continuous TEWL was log-transformed prior to adjusted linear regression and presented as geometric mean ratios with 95% confidence intervals. Variables showing borderline (p<0.10) associations were included in adjusted models. Food sensitization to cow’s milk, egg, peanut, sesame, cod fish or wheat was measured by specific IgE (sIgE ≥0.35kIU) at three months.

At 3 months of age, few families bathed their infant “hardly ever” (n=23) or “more than daily” (n=3) and these categories were therefore combined with the adjoining category (once weekly and daily respectively). A binary bathing frequency variable comparing bathing once a week or less with more frequent bathing was also generated. Continuous number of baths were inferred from the categories of bathing frequency to assess the impact of each additional bath per week.

Bathing frequency was correlated between three and twelve months (nptrend p<0.001). Half of participants were bathed at the same frequency (51.9%; 567/1093) at both time points, while one third were bathed more frequently at twelve months (36.0%; 393/1093).

One quarter (24.4%; 317) of participants had AD on skin examination at three months. TEWL was skewed and the median value was 12.8g/m2\*h (interquartile range (IQR) 10.8-16.1g/m2\*h). TEWL was significantly higher among participants with AD (median 15.7g/m2\*h; IQR 12.3-22.0g/m2\*h) compared to those without (median 12.4g/m2\*h; IQR 10.4-15.0g/m2\*h, p<0.001).

Bathing frequency was associated with having an elevated TEWL with daily bathing being associated with a crude Odds Ratio (cOR) for raised TEWL (≥15g/m2\*h) of 4.62 (2.61-8.21, p<0.001) when compared to bathing up-to-weekly. There was a dose-response progression across bathing categories, whether participants had AD or not (trend p<0.001; figure 1).

Bath oils were used more often among children with elevated TEWL (24.1% versus 14.8%,p=0.001), as were emollients (84.0% versus 75.0%, p=0.002), while shampoo use was less common (26.1% versus 35.4%, p=0.004). After adjustment for these skin care variables in addition to participant AD, sex, non-European ethnicity, siblings, parental report of dry skin, water hardness, *FLG* mutation, parental history of AD, frequency of emollient application and use of wet wipes, elevated TEWL remained significantly associated with daily bathing (aOR 4.32 (2.27-8.22), p<0.001) when compared to up-to-weekly bathing.

In an unadjusted regression model, log transformed TEWL was significantly associated with increasing bathing frequency. The geometric mean ratio of TEWL per unit increase in bathing frequency was 1.03 (95% Confidence Interval 1.02-1.04, p<0.001), with a baseline (bathing less than weekly) TEWL of 11.93g/m2\*h. . After adjustment, the relationship remained statistically significant with a geometric mean ratio of TEWL per unit increase in bathing frequency of 1.03 (1.02-1.03, p<0.001) with a baseline (bathing less than weekly) TEWL of 10.63g/m2\*h. A sensitivity analysis excluding infants with AD at enrolment or parental report of ever having had dry skin yielded the same results (geometric mean ratio of 1.02 (1.01-1.03), p<0.001 with a baseline (bathing less than weekly) TEWL of 10.89g/m\*h).

Bathing frequency showed a non-linear relationship with AD at three months. Babies bathed up-to-weekly demonstrated a 14.6% AD prevalence, while the prevalence associated with more frequent bathing categories varied between 23.2 and 26.9% (table 1). In an adjusted logistic regression analysis bathing more often than weekly, compared with bathing weekly or less, was significantly associated with AD at three months (aOR 1.98 (1.06-3.71), p=0.03), but not associated with food sensitization (≥0.35kIU) at the same time point (aOR 0.52 (0.18-1.51), p=0.23).

A sub-group analysis was undertaken among participants who were bathed at the same frequency at both three and twelve months of age to avoid reverse causality. Investigating the relationship in this subgroup between bathing frequency and TEWL at the 12 month visit, there was a statistically significant relationship with the geometric mean ratio of TEWL per unit increase in bathing frequency of 1.03 (1.01-1.04, p<0.001) with a baseline (bathing weekly or less) geometric mean TEWL of 12.51g/m2\*h. AD on examination and bathing frequency were not associated at 12 months (aOR 0.70 (0.24-2.03), p=0.51).

In summary, bathing frequency was significantly associated with a dose-response increase in skin barrier dysfunction at both three and 12 months of age, but with AD only at three months. There was insufficient statistical power to explore associations with food sensitization or allergy at any of the time points. These data suggest that increased frequency of bathing may negatively influence the integrity of the immature skin barrier and is associated with AD by three months of age. In this context, future studies should investigate the impact of the length of time babies are bathed for and whether moisturizer application directly after bathing can ameliorate the negative effect on skin barrier function, ideally tested in an intervention study.

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**Tables and figures:**

**Table 1.** Adjusted associations between bathing frequency, transepidermal water loss (TEWL), AD and serum food sensitization at three months of age.

**Figure 1.** Box plot showing median transepidermal water loss (TEWL) at three months in relation to bathing frequency at three months a) in all participants and b) among those without signs of AD on skin examination.

**Table 1.** Adjusted associations between bathing frequency, transepidermal water loss (TEWL), AD and serum food sensitization at three months.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Weekly bathing frequency** | | | | | | | | **Binary bathing** | |
|  |  | **Up to once weekly** | | **2-4 times** | | **5-6 times** | | **At least daily** | | **More than weekly** | |
|  |  | N (%) | aOR (95% CI) | N (%) | aOR  (95% CI) | N (%) | aOR  (95% CI) | N (%) | aOR  (95% CI) | N (%) | aOR  (95% CI) |
| **Skin barrier dysfunction** | TEWL; ≥15g/m2\*h; 31.6% (299/946) | 14.6% (16/110) | 1.00 | 26.4% (104/394) | 1.85  (0.99-3.47) \* | 30.4% (35/115) | 2.34  (1.23-4.84) \* | 44.0% (144/327) | 4.32  (2.27-8.22) \* | 33.9% (283/836) | 2.53  (1.38-4.62) \* |
| **Atopic dermatitis (AD)** | AD;  24.0% (227/946) | 14.6%  (16/110) | 1.00 | 26.9%  (106/394) | 2.21  (1.16-4.22) | 25.2%  (29/115) | 1.87  (0.86-4.06) | 23.2%  (76/327) | 1.63  (0.82-3.24) | 25.2% (211/836) | 1.98  (1.06-3.71) |
| **Any serum food sensitization** | Any food sIgE ≥0.35kIU; 6.1% (53/867) | 6.0% (6/100) | 1.00 | 6.9% (25/360) | 0.58  (0.19-1.74) \* | 4.8% (5/104) | 0.35  (0.09-1.41) \* | 5.6% (17/303) | 0.53  (0.17-1.70) \* | 6.1% (47/767) | 0.52  (0.18-1.51) \* |

All Odds Ratios adjusted for sex, non-European ethnicity, having siblings, parental report of participant having dry skin, *FLG*, water hardness, *FLG* mutation inheritance, parental history of AD, frequency of emollient application, and use of bath oil, shampoo, soap and wetwipes.

\* denotes adjustment for participant examined AD at 3 months in addition to the above

**Figure 1.** Box plot showing median transepidermal water loss (TEWL) at three months in relation to bathing frequency at three months a) in all participants and b) among those without signs of AD on skin examination.

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