The effect of water hardness on atopic eczema and skin barrier function

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The effect of water hardness on atopic eczema and skin barrier function

A Thesis submitted for the Degree of

Doctor of Philosophy

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ACKNOWLEDGEMENTS

First and foremost, I would like to convey my gratitude to my supervisors, Professor Carsten Flohr (St John's Institute of Dermatology) and Professor Janet Peacock (School of Population Health and Environmental Sciences) for their enduring support, guidance and supreme patience.

I would also like to thank Dr Michael Perkin (Population Health Research Institute, St George's, University of London) for his indispensable help with my understanding of the ins and outs of the EAT study and its data, Dr Anette Briley (Women's Health), Dr Danielle Greenblatt and Nikeeta Gurung at St John's for their amazing practical support during the SOFTER pilot trial.

The research would not have been possible without funding from the National Institute for Health Research (NIHR), the NIHR Biomedical Research Centre at Guy's & St Thomas' NHS Foundation Trust and King's College London, and Harvey Water Softeners Ltd, who provided financial support and technical expertise without which it would not have been possible to deliver the SOFTER pilot trial. I also very grateful for all the assistance provided by the NIHR Clinical Research Network (CRN), particularly the midwives and research staff of the CRN at Guy's & St Thomas' NHS Foundation Trust and Kingston Hospital NHS Foundation Trust.

A huge thanks goes to all the participants of the SOFTER pilot trial who generously gave their time at one of the most important periods in their lives to participate in the study.

Finally, I would like to thank my mother for her unwavering encouragement and support to help me pursue my dreams and my wife and daughters for their patience, encouragement and understanding.

Table of Abbreviations

A	Asthma
AMP	Antimicrobial Peptide
$CaCO_3$	Calcium Carbonate
CADESI	Canine Atopic Dermatitis Severity Index
CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
DNA	Deoxyribonucleic acid
EASI	Eczema Area and Severity Index
EAT	Enquiring About Tolerance
Eczema	Atopic eczema
FLG	Filaggrin
GRADE	Grading of Recommendations, Assessment, Development and Evaluation
HF	Hay fever
HR	Hazard ratio
IL	Interleukin
IMD	Index of Multiple Deprivation
ISAAC	International Study of Asthma and Allergy in Children
LL-37	Human cathelicidn LL-37
LOF	Loss of Function
OR	Odds Ratio
PAF	Population Attributable Fraction
POEM	Patient-Oriented Eczema Measure
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	Randomised Controlled Trial
SAP	Statistical Analysis Plan
SASSAD	Six Area Six Sign Atopic Dermatitis
SCORAD	Scoring Atopic Dermatitis
SD	Standard Deviation
SMD	Standardised Mean Difference
UK	United Kingdom
UPSW	Ultra-Pure Softened Water

ABSTRACT

Background: Atopic eczema, hereinafter referred to as eczema, is a common inflammatory skin condition that predominantly affects children. The cause of eczema is not known; however, is it likely that a combination of genetic and environmental factors leads to its development. In addition, several studies have identified an association between water hardness and eczema, suggesting that this might be an environmental factor of interest in the aetiology of eczema.

Objectives: The main objective of this thesis was to evaluate the role of water hardness in the development of eczema and to evaluate whether water hardness is an appropriate target for the prevention of eczema in early life.

Methods: A combination of methods was used to address these objectives: a systematic review and meta-analysis; an epidemiological analysis of an observational cohort, including evaluation of the interaction between water hardness and loss of function mutations in the skin barrier gene *filaggrin* (*FLG*); a pilot randomised controlled trial (RCT) to assess the feasibility of an eczema prevention trial using an ion-exchange water softener in the homes of neonates at high risk of eczema

Results:
Systematic review: The systematic review identified a positive association between living in a hard water area and eczema in children. There was a lack of longitudinal data on water hardness and eczema risk. Whilst there was evidence that domestic water softeners do not improve objective disease severity in established eczema, studies on the prevention of eczema using domestic water softeners were not identified.

Epidemiological analysis: A longitudinal analysis of data from infants in the Enquiring About Tolerance study found no overall association between exposure to harder (> 257 mg L-1 CaCO3) vs softer (≤ 257 mg L-1 CaCO3) water: adjusted hazard ratio (HR) 1·07, 95% confidence interval (CI) 0·92-1·24. However, there was an increased incidence of eczema in infants with *FLG* mutations exposed to hard water (adjusted HR 2·72, 95% CI 2·03-3·66), and statistically significant interactions between hard water plus *FLG* and

both risk of eczema (HR 1·80, 95% CI 1·17-2·78) and transepidermal water loss (0·0081 g m-2 h-1 per mg L-1 CaCO3, 95% CI 0·00028-0·016).

Pilot RCT: Of 149 eligible pregnant women, the target number of women, 80 were randomised (54% of those screened). Almost all, 92%, of families in the intervention arm found the study acceptable. By 6 months of age, 27 infants (35%) developed visible eczema, 31% vs 41% in the water softener and hard water groups, respectively. Similarly, a lower proportion of infants in the water softener arm had parent-reported, doctor-diagnosed eczema by 6 months compared to the hard water arm (15% vs 23%).

Conclusions: There may be a role of water hardness in initiating skin inflammation in early life. There is evidence of an interaction between water hardness and *FLG* skin barrier gene mutations in the development of infantile eczema. The results from the pilot RCT indicate that a definitive RCT of water softeners for the prevention of eczema in high-risk infants is feasible and acceptable.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 GENERAL INTRODUCTION

1.1 Background

Atopic eczema, synonymous with atopic dermatitis and hereinafter abbreviated as 'eczema', is a common inflammatory skin disease characterised by an itchy rash that often first develops in early infancy and affects around 20% of children.(1) It is associated with significant morbidity and affects health-related quality of life. Indeed, the health-related quality of life impairment of eczema, as measured by disability-adjusted life-years, is the highest of all skin diseases.(2) Eczema has long been recognised as a distinct dermatological disease, with the earliest accounts in the medical literature of an 'oozing and itchy condition in suckling infants' described in *De morbis cutaneis* in 1572.(3) The term 'eczema' comes from the Greek, *ekzein*, meaning 'to boil over' and, for much of the last millennium, the condition was considered to result from a humoral imbalance within the body, i.e., an *internal* imbalance that manifests in the skin. This idea of *intrinsic* eczema persists today (4), albeit understood to be mediated through immune system dysfunction rather than an imbalance in the 'humours'. However, given the skin's vast surface area and constant contact with the external environment, the idea of external allergens triggering eczema grew in popularity, so-called *extrinsic* eczema. Indeed, by the 20th century the term 'atopic dermatitis' was developed by combining the newly coined term 'atopy', which described a heritable hypersensitivity to allergens, together with 'dermatitis', meaning inflamed skin. The cause of eczema is still not fully understood, however the identification of the skin barrier gene *filaggrin* (*FLG*) has led to a growing understanding of how intrinsic and extrinsic factors may come together to influence a person's risk of developing eczema.(5) The filaggrin gene encodes profilaggrin, a large inactive phosphorylated polypeptide contained within keratohyalin granules in the granular layer of the epidermis. Profilaggrin is proteolytically cleaved by serine proteases, such as matriptase, to form functional filaggrin peptide units. Serine protease activity is controlled by protease inhibitors such as lymphoepithelial Kazal type–related inhibitor (LETKI) encoded by the serine protease inhibitor Kazal type 5 (*SPINK5*) gene. Filaggrin peptides within the stratum corneum are degraded into natural moisturising factor (NMF), a hygroscopic molecule that is an important contributor to the hydration and pH of the stratum corneum (Figure 1.1).(5) The role of *FLG* loss of function mutations, both as independent risk factors and as effect modifiers, has led to a greater understanding of

eczema as a multifactorial disease whereby several genetic and environmental aetiological factors have been identified.(6)

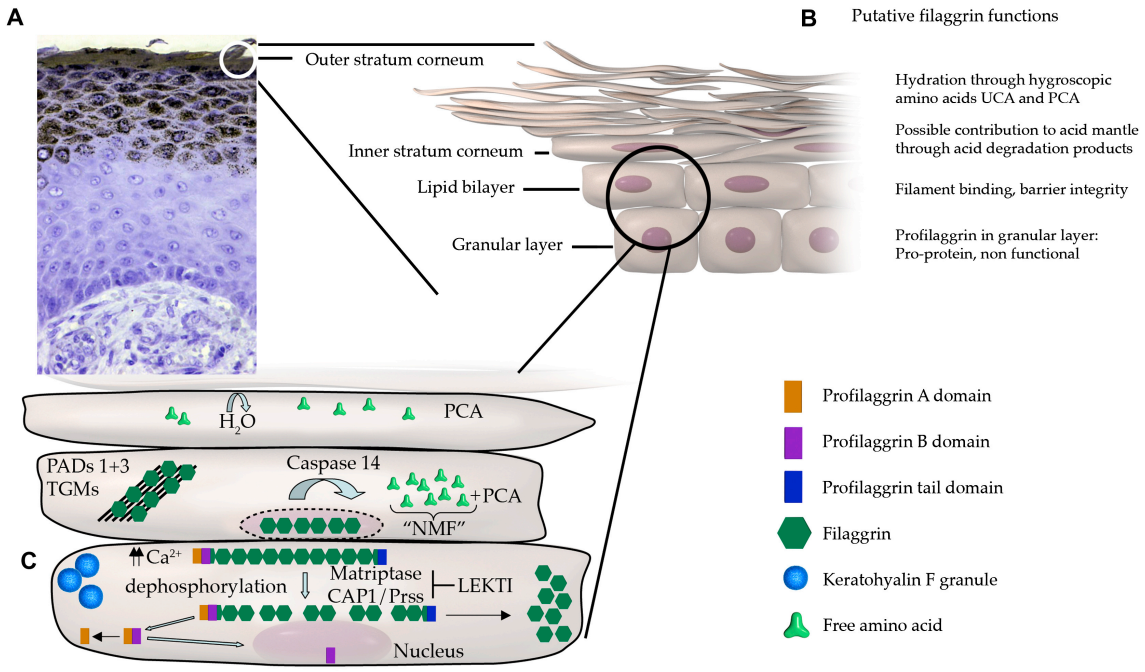

Figure 1.1 FLG expression and functions in the skin barrier

Profilaggrin is expressed in the granular layer (A) and then cleaved into free filaggrin peptide units by the action of proteases, regulated by protease inhibitors such as lymphoepithelial Kazal type–related inhibitor (LETKI). Filaggrin units are then cross-linked to keratin filaments by transglutaminases (TGMs) and subsequently deiminated by peptidylarginine deiminases (PADs) 1 and 3. Further posttranslational modification is undertaken by caspase 14 to produce the free amino acid hygroscopic degradation products urocanic acid (UCA) and pyrrolidone carboxylic acid (PCA; collectively known as natural moisturising factor (NMF)), which contribute to stratum corneum hydration and pH regulation. Reprinted from The Journal of Allergy and Clinical Immunology, Vol 122, Issue 4, O'Regan et al, Filaggrin in atopic dermatitis, Pages 689-683, Copyright (2008), with permission from Elsevier

1.2 Genetic risk factors for eczema

Subsequent studies have identified multiple risk loci, in addition to *FLG*, that encode genes related to other aspects of the skin barrier as well as genes linked to immunity.(7) For example, *SPINK5*, a gene involved in the formation of functional filaggrin peptides, as described above, is mutated in a rare monogenic disease, Netherton syndrome, that clinically resembles eczema and *SPINK5* mutations have also been associated with common atopic disease, including non-syndromic eczema.(8) A meta-analysis of genome-wide association studies examined over 15 million genetic variants in multiple ethnic groups and identified or confirmed 31 eczema risk loci, including those related to innate host defences, such as *CD207* (langerin) expressed in certain dendritic cells, and

adaptive immunity such as genes encoding cytokines interleukin (IL)-4 and IL-13 implicated in the type 2 inflammation underpinning eczema pathopysiology.(7, 9)

1.3 Environmental risk factors for eczema

Epidemiological studies have provided valuable insights into the likely contribution of environmental factors to the development of eczema. Several environmental factors have been identified as having an association with eczema. These include climate, diet, urbanisation, breastfeeding, early life antibiotic exposure, obesity, air pollution, and water hardness, amongst others. Some of these environmental factors are postulated to have a protective effect e.g., breastfeeding, however, others, such as water hardness, are considered risk factors for the disease.(6)

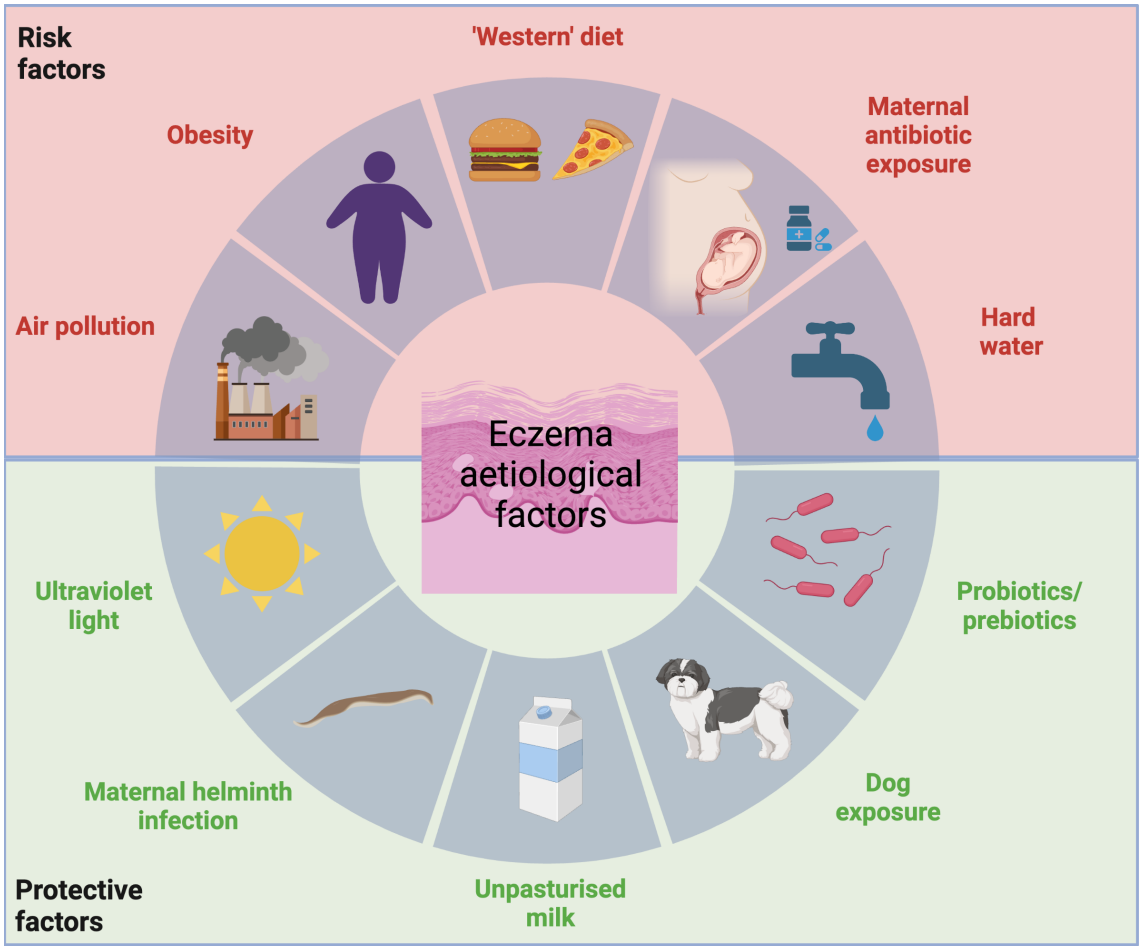

Figure 1.2 Key environmental factors implicated in the aetiology of eczema

Aetiological factors possibly conferring an increased risk of eczema are highlighted in red and those considered protective, i.e. associated with a reduced risk of eczema, are highlighted in green.
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The International Study of Asthma and Allergies in Childhood (ISAAC) was a global study that used standardised methodology to allow for comparisons to be made in the prevalence of eczema between countries and over time.(10) The ISAAC study identified that between Phase 1 and 3, eczema prevalence decreased in many developed countries with previously high prevalence rates. However, many formerly low prevalence developing countries experienced substantial increases, especially in the younger age group. Such changes were also observed over a relatively short period of time before and after German reunification in a repeated cross-sectional study that used a standardised examination protocol to identify cases of eczema. While the cumulative incidence of eczema was stable among preschool children in West Germany after the country's reunification, in East Germany there was an increase in the number of newly diagnosed eczema cases in pre-school children from 16.0% in 1991 to 23.4% in 1997.(11) Recent data from the Global Burden of Disease Study has shown that the global age-standardised prevalence of eczema was stable between 1990 and 2017, however, marked differences remain between countries and regions in terms of eczema prevalence and burden.(2) The short timeframe of the observed changes is unlikely to be explained by changes in genetic variants as these typically occur over many generations. Instead, these findings are more readily explained by changes in environmental factors or perhaps interactions between genes and the environment.

1.4 Gene-environment interactions in eczema

An understanding of gene-environment interactions has been sought to explain why individuals with different genotypes respond to the same environmental exposures in different ways. A recent systematic review of gene-environment interactions in eczema examined interactions with the *FLG* null genotype and a range of environmental exposures.(12) The focus was on *FLG* because this is the strongest and most widely replicated genetic risk factor for eczema, and because the role of *FLG* in skin barrier function provides *a priori* support for a hypothetical gene-environment effect in eczema.(12) The review identified some evidence for an interaction between *FLG* haploinsufficiency and environmental exposures including breastfeeding duration and early life cat exposure. However, the evidence for gene-environment interactions in eczema was limited and, in part, attributed to the lack of sufficiently powered studies designed specifically to answer such questions.(12)

1.5 Primary prevention strategies

Despite the plethora of environmental risk factors identified to date, no primary prevention strategy has been established.(13) However, several approaches have been proposed using either 'inside-outside' or 'outside-inside' approaches.(14)

'Inside-outside' approaches have focused on ante-natal maternal interventions to modify in-utero exposure or post-natal dietary changes in the infant. Ante-natal maternal interventions have included maternal dietary antigen avoidance, such as avoiding cow's milk, or the use of omega-3 or -6 fatty acid supplementation. Post-natal interventions have included promotion of exclusive breastfeeding for a defined period of time (15), hydrolysed protein formula for infants who are not exclusively breastfed (16), and pre/probiotics added to either breastmilk or infant formula.(17) An overview of systematic reviews of interventions to prevent eczema in infants and children concluded that there is no clear evidence that any of the interventions described above prevent eczema in the general population, however, there was some evidence that exclusive breastfeeding for at least six months and prebiotics might reduce eczema incidence in those at high-risk of allergic diseases.(18)

'Outside-inside' approaches have focused on enhancement of the barrier function of infant skin using either emollients, moisturisers, particular wash products or bathing practices. A recent Cochrane systematic review identified 17 studies that examined the effect of skincare interventions on risk of either eczema or food allergy.(19) Emollients used in early life infant skincare were the most common intervention evaluated in the included studies and were found to probably not be effective for preventing eczema and may indeed be associated with an increased risk of food allergy and skin infections. The studies included in the review evaluated any skin barrier intervention that could alter the skin barrier in the infant but did not identify any completed trials of interventions to reduce exposure to substances that might damage the skin barrier, such as elimination of hard water exposure.

1.6 Relationship between hard water and eczema

Hard water is the result of dissolved minerals from the percolation of water through rock in the environment. The key minerals that constitute hardness are calcium carbonate and magnesium carbonate. (20) A minor contribution to the total hardness of water is also made by other polyvalent ions, such as aluminium, barium, iron, manganese, strontium and zinc (20). England, especially in the south of the country, has hard to very hard (>250 mg/L calcium carbonate; $CaCO_3$) domestic water, related to the presence of limestone sedimentary rocks. This can lead to limescale build-up in domestic heating systems and the formation of soap scum (calcium stearate) on the skin, clothes and bedding. Anecdotally, patients report that their skin feels drier or their eczema gets worse if they move from a soft to a hard water area. Of course, there are many potential reasons for this and multiple confounders. In the late 1990s, a key study was published examining the prevalence of eczema in school-aged children living in hard and soft domestic water areas around Nottingham, UK.(21) It found that primary school-aged children living in hard water areas had an increased risk of eczema compared to children living in softer water areas. Following this study, two further cross-sectional studies among schoolchildren conducted in Japan and Spain confirmed this association.(22, 23) Subsequently, cross-sectional data from a UK-wide cohort has also confirmed this relationship in early life, which seems to be enhanced by increased chlorine concentrations, even after adjusting for likely confounders, and a possible interaction with *filaggrin* gene status.(24) Most recently, a large study from a Danish birth cohort has confirmed a 5% increase in prevalence of eczema for each 5 unit increase in domestic water hardness that was linear over the range of exposures evaluated (range, 6.60-35.90 German degrees of hardness [118-641 mg/L calcium carbonate]).(25)

1.7 Potential mechanisms for hard water causing eczema

Given the close contact between water used for bathing and the skin, the most plausible mechanisms by which hard water might be associated with eczema are through external contact with the skin. Whilst it is possible that ingestion of hard water, or a another component of water that is related to water hardness, actually induces skin inflammation, there are limited plausible mechanisms for this based on current knowledge (26) and furthermore the principal dietary source of both calcium and magnesium is food, rather than drinking water.(20)

In contrast, several potential mechanisms have been proposed for the way in which hard water may lead to eczema development through contact with the skin: increased deposition of detergents such as sodium lauryl sulphate (SLS) on the skin, altered calcium signalling in the epidermis, and a rise in skin surface pH with a resulting increase in protease activity, could all have a detrimental effect on skin barrier function (Figure 1.3).(24) Such hypotheses are supported by experimental work conducted with our collaborators at Sheffield University that examined the effect of water hardness on SLS-induced skin irritation in 83 people with or without eczema and with or without *FLG* null mutations. Increased deposition of SLS in skin washed with hard water vs. softened water was seen. Further, hardness was independently associated with greater skin redness following washing with an SLS-containing solution.(27) In a hairless mouse model, low extracellular concentrations of calcium ions in the upper epidermis led to exocytosis of lamellar bodies, required for skin barrier repair, independent of skin barrier disruption.(28) In an experimental pilot study of 11 dogs with pruritus there was evidence of an interaction between shampoo and hard water: a protective effect was seen on skin barrier function of shampoo with ultrapure soft water (<1 mg/L calcium carbonate) compared to shampoo and tap water (158 mg/L calcium carbonate).(29) In addition to these direct and indirect molecular effects on keratinocytes, such changes could also affect the delicate network of microorganisms living on the surface of the skin, known as the skin microbiome. Eczema is associated with a preponderance of *Staphylococcus aureus* and a reduction in microbial diversity.(30) It is not known whether these changes lead to the development of eczema or whether they merely reflect an alteration in skin microenvironment as result of eczematous changes.(31) In vitro studies have identified that there are changes in antimicrobial peptides (AMPs) resulting from changes in the pH and concentration of cations, such as calcium and magnesium, which can impact the activity of bacterial AMPs.(32) AMPs are immune defence molecules produced by both bacteria and human immune and epithelial cells. Recent work has identified a synergistic relationship between the human cathelin-related AMP LL-37 and AMPs produced by coagulase negative Staphylococcal species that selectively kill *Staphylococcus aureus*.(33) Human LL-37 activity against some bacterial species is decreased by the presence of calcium but not magnesium ions.(34)

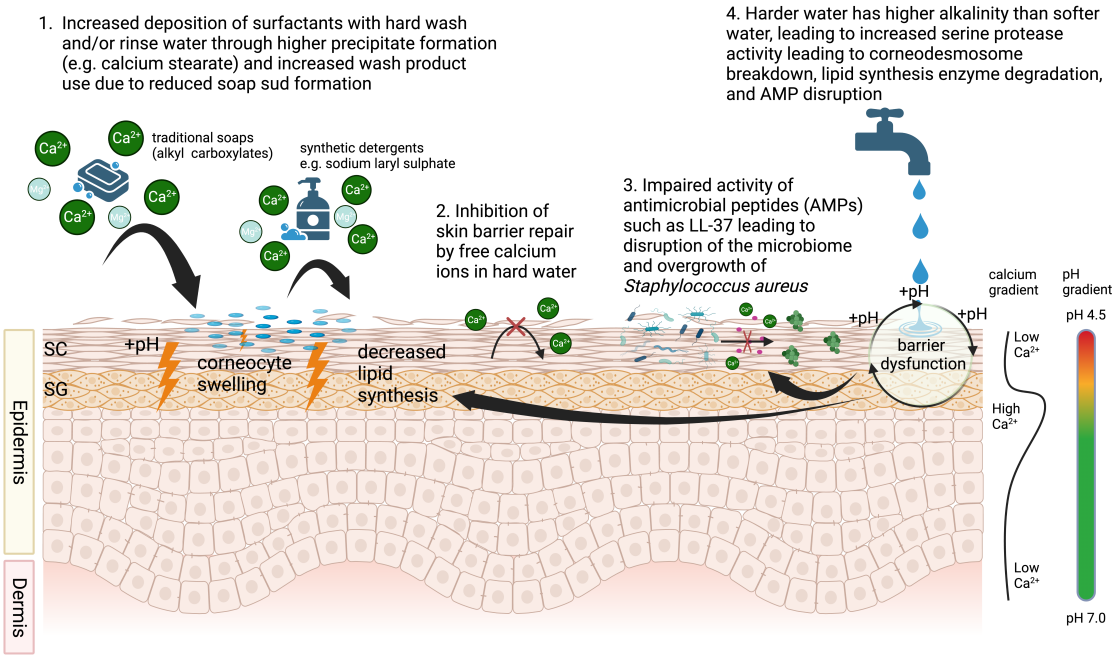

Figure 1.3 Overview of potential mechanisms through which hard water may lead to skin barrier disruption

There are several distinct but related mechanisms through which hard water may lead to skin barrier dysfunction. 1. Increased deposition of surfactants with hard water use may lead to higher deposition of precipitates, such as calcium stearate. Reduced soap-sud (foam) formation may lead to more wash product use, leading to further damage to the skin barrier. 2. Disruption of the calcium gradient in the stratum corneum (SC) may impair skin barrier repair, perpetuating the damage. 3. Impaired activity of antimicrobial peptides (AMPs) such as LL-37 may lead to disruption of the skin microbiome leading to overgrowth of pathogenic species such as Staphylococcus aureus. 4. More alkaline harder water may disrupt the natural pH gradient in the SC, affecting the activitiy of serine proteases leading to breakdown of corneodesmosomes, altered lipid synthesis and AMP disruption. Ca^{2+}, calcium; SG, stratum granulosum. Created with BioRender.com

1.8 Water softeners and eczema

Commercially available water softeners are sometimes used by people living in hard water areas, principally to counteract the detrimental effect of hard water on scaling of their domestic appliances and pipework. The multicentre Softened Water Eczema Trial (SWET), completed in 2011, examined the role of water softeners in treating children with established, moderate-to-severe eczema and found no overall benefit in terms of eczema severity reduction.(26) Early life is likely to be an important time in the development of eczema, particularly as most eczema develops before 2 years of age. Those early interactions between genes and the environment may be crucial in instigating

the cycle of inflammation and skin barrier dysfunction seen in eczema. Indeed, skin barrier dysfunction at just one week of age, as measured by transepidermal water loss, is a predictor of subsequent eczema risk.(35) Further, in a small pilot randomised controlled double-blind crossover trial of 12 patients aged 3-6 years with mild-moderate eczema compared ultra-pure soft water to tap water, after 6 weeks, no statistically significant differences in eczema area severity index (EASI) or transepidermal water loss (TEWL) were observed between the groups, although there was a statistically significant improvement in pruritus as measured by visual analogue score (-2.10, 95% CI -4.14, -0.063).(36) However, given the small sample size of this pilot study the lack of statistical significance may be explained by a lack of statistical power and so limited conclusions can be made. Furthermore, current recommendations for pilot trials do not support formal hypothesis testing in pilot studies for this reason.(37) To date, there are no published studies examining the role of water softeners in the prevention of eczema.

1.9 Objectives of the thesis

The literature suggests that there is evidence of an association between water hardness and eczema development and a plausible mechanistic rationale for this. However, there is a need to better characterise and quantify this relationship, particularly in the context of a genetic predisposition to weakened skin barrier function. There is also a need to identify whether modifying skin exposure to hard water in early life could be a viable strategy for primary prevention. The main objective of this thesis was therefore to evaluate the role of water hardness on the development of eczema in the setting of a large observational study and to evaluate whether water hardness is an appropriate target for the prevention of eczema in early life.

The main questions examined in this thesis were:

- What is the effect of domestic water hardness on eczema and skin barrier function?
- How is the effect of hard water on the skin modified by *filaggrin* mutation status?
- Is it feasible to conduct a trial of the installation of water softeners prior to birth for the prevention of eczema in infants?

1.10 Overview of the approach taken to address these questions

A combination of methods was used to address these questions:

1) a systematic review and meta-analysis
2) an epidemiological analysis of an observational cohort
3) the design and execution of a pilot randomised controlled trial

A comprehensive systematic literature review and meta-analysis was performed to identify and synthesise the known evidence around water hardness, skin barrier function, and eczema, as described in Chapter 2. The work contributing to Chapter 2 was published in Clinical and Experimental Allergy as an original manuscript (Jabbar-Lopez ZK, Ung CY, Alexander H, Gurung N, Chalmers J, Danby S, Cork MJ, Peacock JL, Flohr C. "The effect of water hardness on atopic eczema and skin barrier function: A systematic review and meta-analysis", *Clinical and Experimental Allergy*, 2021 Mar;51(3):430-451).(38)

This review was followed by an epidemiological analysis of water hardness and eczema risk in a large cohort of new-born babies, as described in Chapter 3. The work contributing to Chapter 3 was published as an original manuscript in the British Journal of Dermatology (Jabbar-Lopez ZK, Craven J, Logan K, Greenblatt D, Marrs T, Radulovic S, McLean WHI, Lack G, Strachan DP, Perkin MR, Peacock JL, Flohr C. Longitudinal analysis of the effect of water hardness on atopic eczema: evidence for gene-environment interaction. *Br J Dermatol*. 2019 Oct 10. doi: 10.1111/bjd.18597).(39)

The systematic review and epidemiological analysis were used to inform the design of a pilot randomised controlled trial (RCT) examining the feasibility of the installation of a domestic ion-exchange water softener around the time of birth to reduce the risk of skin barrier dysfunction and infants developing eczema, as described in Chapter 4. A pilot RCT was performed as full-scale prevention RCTs typically require large numbers of participants and significant resources to be invested, and there is an ethical imperative to ensure that the study is feasible prior to embarking on such an undertaking. The work contributing to Chapter 4 was published as an original manuscript in Clinical and Experimental Allergy (Jabbar-Lopez ZK, Ezzamouri B, Briley A, Greenblatt D, Gurung N, Chalmers JR, Thomas KS, Frost T, Kezic S, Common JEA, Danby S, Cork MJ, Peacock JL, Flohr C. Randomized controlled pilot trial with ion-exchange water softeners

to prevent eczema (SOFTER trial). Clin Exp Allergy. 2022 Mar;52(3):405-415. doi: 10.1111/cea.14071. Epub 2021 Dec 12. PMID: 34854157) (40) and BMJ Open (Jabbar-Lopez ZK, Gurung N, Greenblatt D, Briley A, Chalmers JR, Thomas KS, Frost T, Kezic S, Common JEA, Kong HH, Segre JA, Danby S, Cork MJ, Peacock JL, Flohr C. Protocol for an outcome assessor-blinded pilot randomised controlled trial of an ion-exchange water softener for the prevention of atopic eczema in neonates, with an embedded mechanistic study: the Softened Water for Eczema Prevention (SOFTER) trial. *BMJ Open*. 2019 Aug 20;9(8):e027168. doi: 10.1136/bmjopen-2018-027168).(41)

2 SYSTEMATIC LITERATURE REVIEW

2.1 Introduction

On initial searching of the literature there were no systematic reviews of water hardness and eczema identified. Therefore, in order to understand the existing body of evidence on water hardness and eczema, the first comprehensive literature review on the relationship between the effect of water hardness on a) the risk of developing eczema, b) existing eczema, and c) skin barrier function was conducted.

2.2 Methods

2.2.1 Literature search and study selection

A systematic literature search was performed to answer the question 'What is the effect of water hardness on skin barrier function and eczema?'. The reporting of this review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and the Meta-analyses of Observational Studies (MOOSE) checklist.(42, 43) The protocol was registered with PROSPERO (CRD42016051528). Systematic searches were conducted in MEDLINE, Embase, Cochrane CENTRAL, GREAT and Web of Science (including the Conference Proceedings Citation Index) from inception to 30th June 2020 using MESH terms such as 'Eczema' 'Atopic dermatitis' 'water' 'water softening' calcium' 'magnesium' and combinations of related free text keywords (full search strategy see Appendix 7.1). Searches were performed by a researcher trained in systematic reviews (ZKJ-L). No language restrictions were used. References of included studies were reviewed for additional papers of relevance.

2.2.2 Inclusion and exclusion criteria

Human and animal observational and experimental studies evaluating water hardness (calcium carbonate), water softening and/or filtration devices vs. naturally soft water, softened water, chlorine-free water, deionised water, filtered water were included. All study types were included, including conference abstracts. There were no language restrictions and no other exclusion criteria.

2.2.3 Outcome measures

The primary outcomes were risk of eczema and skin barrier function impairment, as measured by raised transepidermal water loss (TEWL). Transepidermal water loss measures the quantity of water lost from inside the body by diffusion across the stratum corneum and is the most widely used objective measurement for assessing the barrier function of the skin (44). Skin barrier dysfunction results in increased TEWL and eczema is associated with elevated TEWL (44). No standardised definition of eczema was specified. Secondary outcomes were also extracted, including clinician-assessed eczema severity, patient-reported eczema severity, eczema disease control, time to onset of eczema, wash product use including traditional alkyl carboxylate soaps and newer synthetic detergents (syndets), biological and cellular measures of skin barrier function, such as cutaneous cytokine release, and detergent deposition.

2.2.4 Data abstraction and risk of bias assessment

After de-duplication, abstracts and titles were screened independently by two different researchers (ZKJ-L and CYU). Full-text articles of selected titles/abstracts were reviewed against a priori defined inclusion and exclusion criteria, again independently by two different researchers (ZKJ-L and CYU). Discrepancies were resolved by discussion with the senior author (CF). Data extraction and quality assessment were performed by one researcher and checked by another against the original article. Randomised controlled trial (RCT) risk of bias was assessed using the Cochrane Risk of Bias tool.(45) Study quality for other study types was assessed using domain-based approaches with tools appropriate to the specific designs of the included studies. The Newcastle-Ottawa Scale (46) was used for cohort studies without calculating a summary score. The Klimisch code was used to assess the quality of animal and in vitro studies using ToxRTool.(47) Data on outcomes were summarised and quality assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.(48) Non-randomised studies were considered low-quality evidence unless there was a compelling reason to grade up.(49)

2.2.5 Data synthesis and statistical analysis

Human and animal data were analysed separately. Where quantitative data from more than one study were identified for a particular exposure-outcome relationship, quantitative synthesis was to be performed using an inverse-variance weighted random effects model using RevMan v5.0 (Cochrane Collaboration) (50) and Open Meta-Analyst

(Brown University) (51). Otherwise, a narrative synthesis was to be used. Where a quantitative synthesis was performed, heterogeneity was explored by visual inspection of the forest plots and through the calculation of the I-squared (I^2) measure of heterogeneity. (52) Where non-trivial heterogeneity (I^2 >50%) was identified, this was explored through analysis of pre-defined subgroups: Infants (<1 year); primary school-aged children (5 to 11 years of age), secondary school-aged children (11-16 years), adults (>16 years of age); filaggrin loss of function (LOF) mutation status; clinician-reported versus patient-reported diagnosis eczema. Water hardness exposure was considered "hard" or "soft" according to the definitions reported in each study. Where water hardness was reported in >2 categories, the highest category was compared to the lowest. Eczema risk data were summarised as odds ratios (ORs) with 95% confidence intervals (CIs). Wherever possible, adjusted estimates were synthesised in preference. In the GRADE tables, anticipated absolute effects were calculated based on the assumed pooled risk in the comparison group and the relative effect of the intervention or exposure (and its 95% CI). Where different scales were reported for a particular outcome, comparisons were made using standardised mean differences with 95% CIs. When interpreting outcomes, a two-sided P-value of less than 0.05 was considered statistically significant. We planned to assess publication bias using funnel plots for quantitative syntheses of ≥10 studies.

2.3 Results

2.3.1.1 Studies included in the review

The literature search identified 5,981 studies, 5,931 of which were excluded either because their abstracts or titles were not relevant to the questions addressed in the review or because they contained duplicate records. Fifty studies were selected for full review; 16 were included: 2 RCTs (26, 36), eight observational studies(21-24, 39, 53-55), two experimental studies with a randomised component (56, 57), three experimental non-randomised studies (58-60), two animal studies (29, 60). Study characteristics are presented in Table 2.1. Figure 2.1 represents the PRISMA flow diagram for study selection. Excluded studies are listed in Appendix 7.2.

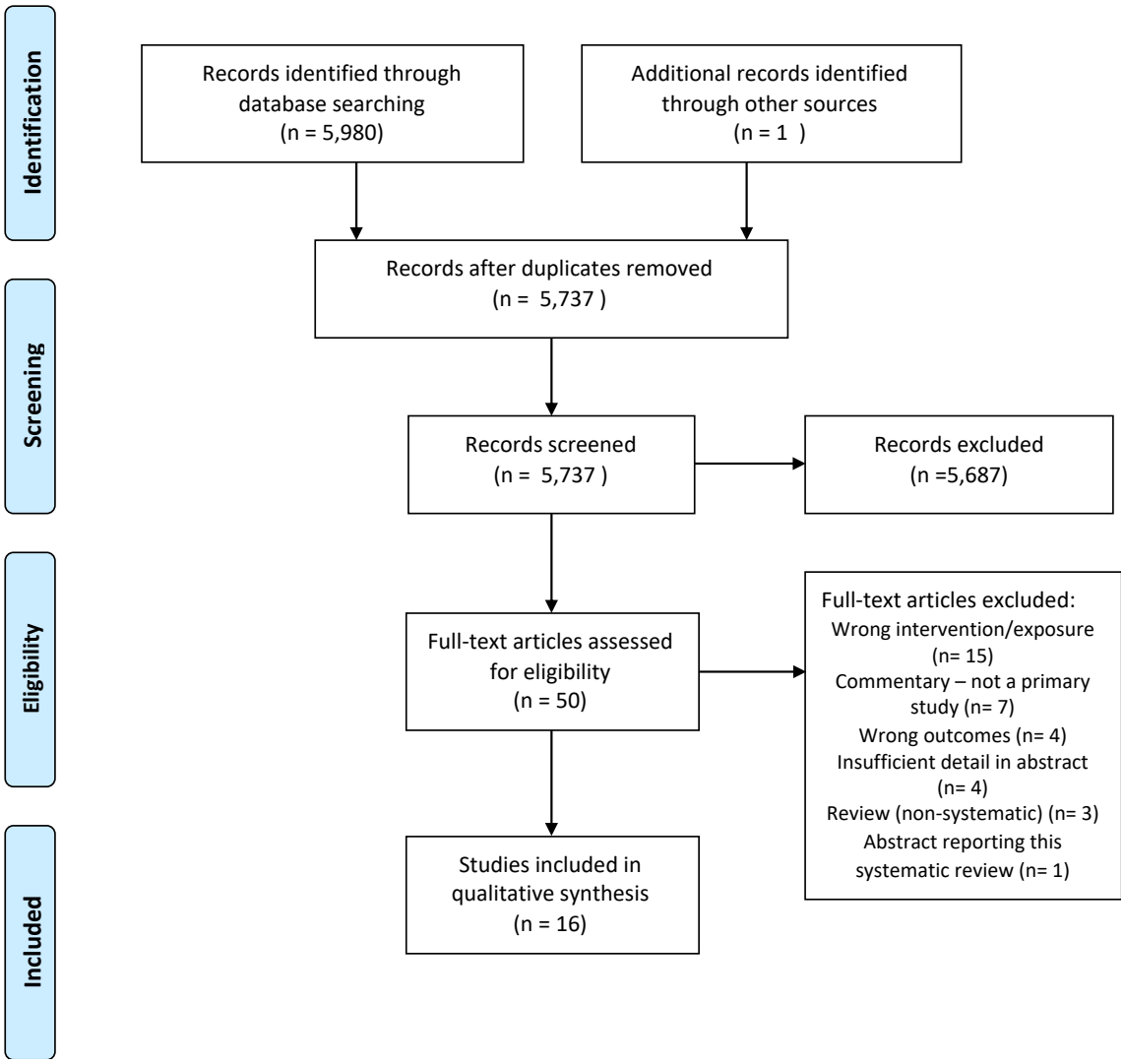

Figure 2.1 PRISMA Flow diagram of studies

PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Table 2.1 Characteristics of included studies

Author, Year Country Report type	Design	N	Population	Exposure(s) Comparator(s)	Outcome(s)	Covariates adjusted for	Effect (adjusted, where reported)
Hard water exposure and risk of eczema							
McNally et al, 1998 (21) UK Full paper	Observational, Cross-sectional	4,141	Primary school children (4-11 years)	$CaCO_3$ categories (mg/L): I) 118-135, II) 151-157 III) 172-214 IV) 231-341	Questionnaire-based parent-reported lifetime occurrence and 1-year-period prevalence of atopic eczema, defined by International Study of Asthma and Allergies in Childhood (ISAAC) study-based questionnaire	-Age -Sex -Socio-economic status -Health care status	1-year period prevalence of eczema: adjusted OR = 1.54 (95%CI 1.19-1.99) Lifetime prevalence of eczema (highest vs lowest hardness category): OR 1.28 (95%CI 1.04-1.58)
		3,499	Secondary school children (11-16 years)				1-year period prevalence of eczema: adjusted OR = 1.03 (95%CI 1.79-1.33) Lifetime prevalence of eczema(highest vs lowest hardness category): OR 0.99 (95%CI 0.80-1.23)
Miyake et al, 2004 (23) Japan Full paper	Observational, Cross-sectional	458,284	Primary and secondary school children (6-12 years)	$CaCO_3$ categories (mg/L) I) 35.2-48 II) 48-53.9 III) 54-75.9 IV) 76-100 Yearly average of water hardness levels supplied to municipalities	Questionnaire-based parent-reported physician-diagnosed atopic eczema since birth	-Smoking in the household -Pollution due to traffic around residential area -Medical conditions -Socioeconomic status -Health care status	OR 1.12 (95% CI 1.06-1.18) for eczema in highest vs lowest category of water hardness *P* value for linear trend <0.0001

Arnedo-Pena et al, 2007 (22) Spain Full paper	Observational, Cross-sectional	3,024	Primary school children (6-7 years)	CaCO3 level (mg/L) Zone1: <200 Zone 2: 200-250 Zone3: >300 Water hardness based on average levels measured between 1993-2002 across the Castellon region	Questionnaire-based parent-reported lifetime occurrence and 1-year-period prevalence of atopic eczema, defined by ISAAC questionnaire	-Age -Sex -Family history of atopic eczema -Social class -Number of siblings -History of otitis	Age 6-7 1-year period prevalence of eczema (highest vs lowest hardness category): OR 2.29 (95%CI 1.19-4.42) Lifetime prevalence of eczema (highest vs. lowest hardness category): OR 1.58 (95%CI 1.04-2.39)
		3,112	Secondary school children (13-14)				Age 13-14 1-year period prevalence of eczema (highest vs lowest hardness category): OR 0.41 (95% CI, 0.12, 1.33) Lifetime prevalence of eczema (highest vs lowest hardness category): OR 0.89 (95% CI 0.53, 1.49)
Chaumont et al, 2012 (53) Belgium Full paper	Observational Cross-sectional	358	Primary school children (mean age 5.7 years)	3 categories of $CaCO_3$ (mg/L) Soft <150 Moderately hard 150-350, Very hard >350 Municipality-based average water hardness levels between 2003-2007	Questionnaire-based parent-reported physician-diagnosed atopic eczema at any point	-Pool attendance during infancy -Parental AD and/or allergy -Breastfeeding -Parent's education level -Presence of older siblings -Passive smoking -Maternal smoking during pregnancy	Prevalence of eczema (highest vs. lowest hardness category): OR 1.97 (95% 0.97-3.96) *P* trend = 0.08 In atopic* individuals only (highest vs. lowest hardness category): OR 3.36 (95% CI 1.02-11.1) *defined as positive Rhinostick test to common allergens or reports receiving allergy medication
Font-Ribera et al, 2015 (55) Spain Full paper	Observational, Birth cohort	1,638	Infants (14 months) Children (4 years)	$CaCO_3$ levels in tertiles: <173 173-209 >209 Water hardness level based on address of residence	Questionnaire-reported 'ever eczema' (based on ISAAC) Current eczema at 4 years if responded as having used eczema	-Cohort (geographical location) -Sex -Maternal allergy -Maternal education	'Eczema ever' at 14 months (highest vs. lowest hardness tertiles): OR 0.79 (95%CI 0.45, 1.39) "Eczema ever" at 4 years (highest vs. lowest hardness tertiles): OR 0.94 (95% CI 0.57,1.53) "Current eczema" at 4 years (highest vs. lowest hardness tertiles): OR 1.25 (95% CI 0.71, 2.20)

					medication in last 12 months		
Perkin et al, 2016 (24) UK Full paper	Observational, Cross-sectional	1,303	3 month-old infants	Categories based on water hardness & chlorine: 1) Low $CaCO_3$/ low total chlorine 2) High $CaCO_3$/ low total chlorine 3) Low $CaCO_3$/ high chlorine 4) High $CaCO_3$/ high chlorine	Atopic eczema using UK diagnostic criteria-based photographic protocol adapted for infants	-Sex -Ethnicity -Home location -Maternal age -Socioeconomic status (maternal age at leaving full-time education) -Water softener installation -Family history of AD or other atopy -Frequency of bathing and the use of emollients and bathing products	H $CaCO_3$/L Chlorine OR 1.87, 95%CI 1.25-2.80 H $CaCO_3$/H Chlorine OR 1.61, 95%CI 1.09-2.38
Engebretsen et al, 2017 (54) Denmark Full paper	Observational, Birth cohort	52,950	Children aged up to 18 months	Water hardness in German degrees	Parent-reported physician-diagnosed atopic eczema	- Sex - Maternal history of eczema - Maternal socioeconomic status - Maternal education - Urban location	Relative prevalence of atopic eczema during the first 18 months of life: 1.05 (95% CI: 1.03, 1.07) for every 5-degree increase in hardness
Jabbar-Lopez et al, 2019 (39) UK Full paper	Observational, Cohort	958	Infants aged 3-36 months enrolled in the Enquiring About Tolerance study	Harder water (>255 mg/L $CaCO_3$) versus softer water (<255 mg/L $CaCO_3$)	Composite of atopic eczema using UK diagnostic criteria-based photographic protocol adapted for infants and	Adjustment for: - Ethnicity - Home location (urban vs. rural) - presence of a water softener	Adjusted HR 1.07 (95% CI 0.92, 1.24) Stratification by *FLG* mutation status showed a significant interaction with water hardness: adjusted HR 2.72 (95% CI: 2.03, 3.66).

			without atopic eczema by 3 months of age		parent-reported, doctor-diagnosed atopic eczema between 3-36 months of age		
Hard water exposure and skin barrier function							
Warren et al, 1996 (57) *Part A* USA Full paper	Experimental Order of forearm testing sites was randomly determined	36	Healthy female volunteers Aged 18 to 65 years	Forearm controlled application test (FCAT) Controlled wash volar forearm with 3 test cleanser bars: 1) Sodium cocoate 2) Triethanolamine-coconut (TEA) 3) Sodium cocoyl isethionate (syndet) twice daily for two 5-day periods along with 4 wash/rinse water combinations: 1) 0 gr/0 gr 2) 0 gr/11 gr 3) 11 gr/0 gr 4) 11 gr/11gr (1 grain = 6.86 mg/L $CaCO_3$)	Visual assessment of erythema by expert grader pre-wash and after final wash (>3 h), dryness, skin capacitance	None	Mean dryness grade: 1.75 (SEM 0.07) with 11 gr rinse versus 1.14 (SEM 0.07) with 0 gr water, P<0.001 Mean erythema grade: 1.2 (SEM 0.07) with 11 gr rinse versus 0.80 (SEM 0.07) with 0 gr rinse Mean capacitance (pF): 15.38 (SEM 0.25) with 11 gr versus 17.28 (SEM 0.25) with 0 gr rinse No statistically significant difference in dryness, erythema or capacitance with 11 gr versus 0 gr wash water hardness Statistically significantly higher dryness (P<0.01), erythema (P<0.05) and lower capacitance (P<0.01) with hard (11 gr) water rinse and syndet. Statistically significantly higher dryness, erythema and lower capacitance with sodium soap and TEA soap.
Warren et al, 1996 (57) *Part B* USA Full paper	Experimental Subjects were randomly assigned to a water hardness group	24	Healthy volunteers aged 18 to 65 years	Flex wash Test bar rubbed with a sponge 3 x per day, each followed by 10s rinse for 5 days	Visual assessment of erythema by expert grader pre-wash and after 3rd wash each day, dryness, capacitance, soap deposition	None	In 11 gr water: Erythema grade 2.19 with TEA soap versus 0.77 with syndet (P<0.01). In 0 gr water: Erythema grade 2.08 with TEA soap versus 0.67 with syndet (P<0.01).
Warren et al, 1996 (57) *Part C*	Experimental	30, divided into 3 groups	Healthy volunteers	Forearm rinse with 70% isopropyl alcohol followed by a wash	Soap deposition using Fourier transform	None	When hard water rinse was preceded by a 0 gr rinse there was significantly less soap deposition

USA Full paper	Subjects were randomly assigned to different wash waters			with sodium cocoate soap bar and 0 gr or 8 gr (tap) water Group 1a: wash 0 gr, rinse 15 s 8 gr Group 1b: wash 0 gr, rinse 15 s 0 gr followed by 15 s 8 gr Group 2a: wash 8 gr, rinse 15 s8 gr Group 2b: wash 8 gr, rinse 15 s 0 gr followed by 15 s 8 gr Group 3a: wash 0 gr, rinse 30 s 8 gr Group 3b: wash 0 gr, rinse 15 s 0 gr followed by 15 s 8 gr	infrared spectroscopy (FTIR)		Distilled wash water wash followed by hard (8 gr) rinse mean soap deposition: 0.12 (±0.04) versus soft (0 gr) rinse mean soap deposition 0.07 (±0.02) (P<0.01). Hard (8 gr) wash water followed by hard (8 gr) mean soap deposition 0.09 (±0.04) versus hard then soft (0 gr) water rinse mean soap deposition 0.05 (±0.02) (P<0.05) Distilled wash water wash followed by hard (8 gr) water longer rinse mean soap deposition: 0.10 (±0.04) versus hard (8 gr) then soft (0 gr) rinse mean soap deposition 0.05 (±0.03) (P<0.05).
Tanaka et al, 2015 (60) (human) Japan Full paper	Experimental, non-randomised	10 Group 1: UPSW Group 2: Tap water	Eight female patients with mild eczema, median age 37 years Healthy volunteers	Daily showering with UPSW for 4 weeks	Dryness Scaling Pruritus Skin hydration TEWL Lauric acid residue on tape stripping determined by gas chromatography		Pruritus “almost completely resolved in this cohort of 8 females with mild AD and dry skin.” Showering with UPSW also significantly (P<0.001) increased the water content of the stratum corneum from a median of 21 to 30 arbitrary units at Day 29. This was associated with a significant (P<0.05) reduction in TEWL at 29 days compared to both before and after 15 days of UPSW treatment When the skin was rinsed with UPSW, the lauric acid was almost completely gone by 90 s, while even after 180 s of washing with tap water over 80% of the fatty acid remained in the stratum corneum
Danby et al, 2018 (56) UK Full paper	Experimental Allocation of the test water to the test areas was randomized	Group 1: n=26; Group 2: n=8; Group 3: n=24; Group 4: n=22	Adult volunteers Group 1: healthy skin Group 2: *FLG* LOF mut without current/past eczema Group 3: Eczema and normal *FLG*	Volar forearm skin washing with SLS 10% solution and one of: Hard domestic tap water Softened water using ion-exchange water softener Deionised water	Visual grading of erythema Objective redness (Mexameter) TEWL (AquaFlux AF 200) Skin surface pH (PH905)	None	Objective erythema: statistically significantly lower skin redness with deionised versus hard water. TEWL: mean 10.19 (SEM 0.74) g/m2/h with hard water, versus 7.43 (SEM 0.74) g/m2/h with deionised water SLS deposition 2.8 +/-0.6-fold greater with hard water versus deionised water. Chlorine level in water did not affect SLS deposition AD patients carrying the FLG gene mutation were affected by SLS deposits to a significantly greater extent compared to individuals with no FLG mutation and healthy skin

			Group 4: Eczema and *FLG* LOF mut	With or without chlorine (1.5 ppm) Patch testing	Detergent deposition using FTIR Soluble IL-1a (ELISA) *FLG* genotyping		
Engebretsen et al, 2018 (58) Denmark Full paper	Experimental, non-randomised	40	Healthy volunteers aged 18-49 years without *FLG* mutations	Exposure to different water types using Finn chambers: Hard water Soft water Chlorinated water 0.5% sodium lauryl sulfate	Skin measurements taken from volar forearms 24 hours and 48 hours after application. Transepidermal water loss (TEWL) Tape stripping for NMF, cytokines	None	Mean (SD) TEWL at 24 hours: No difference between soft 13.0 (±6.2) g/m2/h and hard 13.1 (±6.1) g/m2/h compared with occlusion alone 11.8 (±3.3) g/m2/h. Mean (SD) TEWL at 48 hours: Significant difference between soft 9.1 (±3.0) g/m2/h and hard 9.2 (±2.8) g/m2/h individually compared with occlusion alone 8.2 (±2.2) g/m2/h. Mean (SD) NMF at 24 hours: soft 0·51 (± 0·19) mmol/g and hard water (0·61 (± 0·32) mmol/g compared with occlusion alone 0·71 (± 0·18) mmol/g. No difference in NMF was found between hard and soft water. Mean (SD) NMF at 48 hours: soft 0.64 (± 0·22) mmol/g and hard water 0·61 (± 0·19) mmol/g compared with occlusion alone 0·82 (± 0·17) mmol/g. No difference in NMF was found between hard and soft water. There was an increase in mean (SD) stratum corneum cytokine levels: IL-4 -1.69 (±0.25) log(pg/µg protein), IFN- γ -1.12 (±0.22) log(pg/µg protein), and IL-10 -1.90 (±0.24) log(pg/µg protein) after 24 hours with exposure to hard water compared to the control. There was no significant increase in IL-1α, IL-β. No significant increase in IFN-γ, IL-4, IL-1α, IL-β, IL-10 was found for soft water compared to the control. No direct comparison was reported between hard and soft water.
Matsuda et al, 2018 (59) Japan Full paper	Experimental, non-randomised	26 recruited, 15 participants with data Group 1: n=7 Group 2: N=8	Elderly care home residents aged 67-97 years with no history of skin diseases.	Twice-weekly bathing Group 1: Twice-weekly bathing with UPSW (<0.1 mg/L $CaCO_3$) Group 2: Twice-weekly bathing with tap water (79.9 mg/L $CaCO_3$)	Skin measurements taken from volar forearm 1.5 hours after bathing: Stratum corneum water content (Skicon-200EX)	None	Statistically significantly higher skin hydration at 12 weeks in adults bathed with UPSW compared to those bathed with tap water (P<0.01). No statistically significant difference in TEWL or skin dryness between groups at 12 weeks.

					Transepidermal water loss (AquaFlux AF200) Dermatologist-assessed skin dryness		
Water softeners for the treatment of eczema							
Thomas et al, 2011 (26) UK Full paper	RCT (Parallel groups)	336 Group A: 159 Group B: 164	Children with atopic eczema aged 6 months to 16 years	Group A: ion-exchange water softener + normal eczema care for 12 weeks Group B: Usual eczema care for 12 weeks, then 4 weeks of softened water	Mean change in Six Area Six Sign Atopic Dermatitis (SASSAD) score Mean change in Patient-Oriented Eczema (POEM) score Well-controlled weeks (WCW) Totally-controlled weeks (TCW)	N/A	Mean (SD) SASSAD improvement: Group A -5.0 (8.8) Group B -5.7 (9.8) Mean change (A-B) in SASSAD between 2 groups at 12 wk = 0.66 (95% CI -1.37, 2.69) Mean (SD) POEM improvement: Group A -5.7 (7.2) Group B -3.6 (6.7) Mean change (A-B) in POEM between groups at 12 wk = -2.03 (95% CI -3.55, -0.51) Mean (SD) WCW Group A 8.3 (3.8) weeks Group B 7.3 (4.1) weeks Mean difference (A-B) in WCW 0.99 (95% CI 0.04, 1.95) weeks Mean (SD) TCW Group A 2.9 (3.5) weeks Group B 1.7 (2.8) weeks Mean difference (A-B) in TCW 1.19 (95% CI 0.43, 1.95) weeks
Togawa et al, 2014 (36) Japan Full paper	RCT (Crossover)	12 Group 1: 5 Group 2: 6	3-6-year-olds with mild-moderate eczema	Group 1: UPSW by cation-exchange resin 6-week shower treatment	Eczema Area and Severity Index (EASI)	N/A	Non-significant difference in mean change in EASI between groups: -2.61 (95% CI -7.03, 1.81) in UPSW group versus tap water group Mean difference in pruritus VAS in the UPSW group was -2.1 points (95% CI -4.14 to -0.063)

				Group 2: Standard tap water (mean 91.67 mg/L $CaCO_3$)	Pruritus visual analogue scale (VAS) Transepidermal water loss (TEWL)		Abdominal region: Mean difference in TEWL in the UPSW group: -5.77 g/m^2h (95% CI -13.9 to 2.41) Upper back region: Mean difference in TEWL in the UPSW group: 2.26 g/m^2h (95% CI -16.9 to 21.4)
Animal studies							
Ohmori et al, 2010 (29) Japan Full paper	Experimental, randomised	11	Dogs with atopic eczema	Tap water: hardness 158 mg/L $CaCO_3$ UPSW: hardness <1 mg $CaCO_3$ mg/L Group1: weekly shampoo wash with UPSW for 4 weeks, then washout period 12 weeks then tap water 4 weeks Group 2: weekly shampoo wash with tap water for 4 weeks then washout period 12 weeks, then UPSW 4 weeks	Pruritus visual analogue scale (PVAS) 0-10 Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) Transepidermal Water Loss (TEWL)	N/A	PVAS -UPSW mean 4.7±0.6 -Tap water mean 5.3±0.6 CADESI scores -UPSW, mean 76.5±20.7 range (14-195) -Tap water, mean 88.1±18.9 range (24-199) TEWL -UPSW mean 12.5±2.3 g/m^2/h; range (3.2-27.2 g/m2/h) -Tap water mean 17.8±4.6 g/m^2/h (range 5.2-54.5 g/m2/h)
Tanaka et al, 2015 (60) (animal) Japan Full paper	Experimental, non-randomised	15-16 mice in each group	NC/Tnd mice with moderate atopic dermatitis	Clipped dorsal skin washed with soap and rinsed with hard tap water (151.9 mg/L CacO3) or UPSW (<1 mg/L) daily for 3 weeks	Dermatitis severity score Scratching frequency Total scratching duration TEWL	N/A	Statistically significantly lower clinical severity scores in mice treated with UPSW versus tap water No statistically significant difference in TEWL between groups. Statistically significant reduction in TEWL pre- to post-treatment in UPSW group (P<0.05).

$CaCO_3$, calcium carbonate; CADESI, canine atopic dermatitis extent and severity index; EASI, eczema area and severity index; FLG, filaggrin; HR, hazard ratio; IFN, interferon; IL, interleukin; LOF, loss of function; OR, odds ratio; POEM, patient-oriented eczema measure; PVAS, pruritus visual analogue scale; RCT, randomized controlled trial; SASSAD, six area six sign atopic dermatitis severity score; SD, standard deviation; TCW, totally-controlled week; TEWL, transepidermal water loss; UPSW, ultrapure softened water; VAS, visual analogue scale; WCW, well-controlled week

2.3.1.2 Studies in humans

2.3.1.2.1 Hard water exposure and risk of eczema

The pooled estimate based on 385,901 participants in five cross-sectional and two birth cohort studies (21-24, 53-55) showed statistically significant increased odds of eczema in infants and children exposed to harder (range: 76 to >350 mg/L $CaCO_3$) versus softer (range: 35.2 to 256 mg/L $CaCO_3$) water (OR 1.28, 95% CI 1.09, 1.50) (Figure 2.2). No studies were identified that examined the risk of eczema with hard water exposure in adults. Certainty in this estimate is very low due to high risk of bias and heterogeneity (I^2 63%; GRADE profile; Table 2.2; Appendix 7.3). Time to eczema development was not reported.

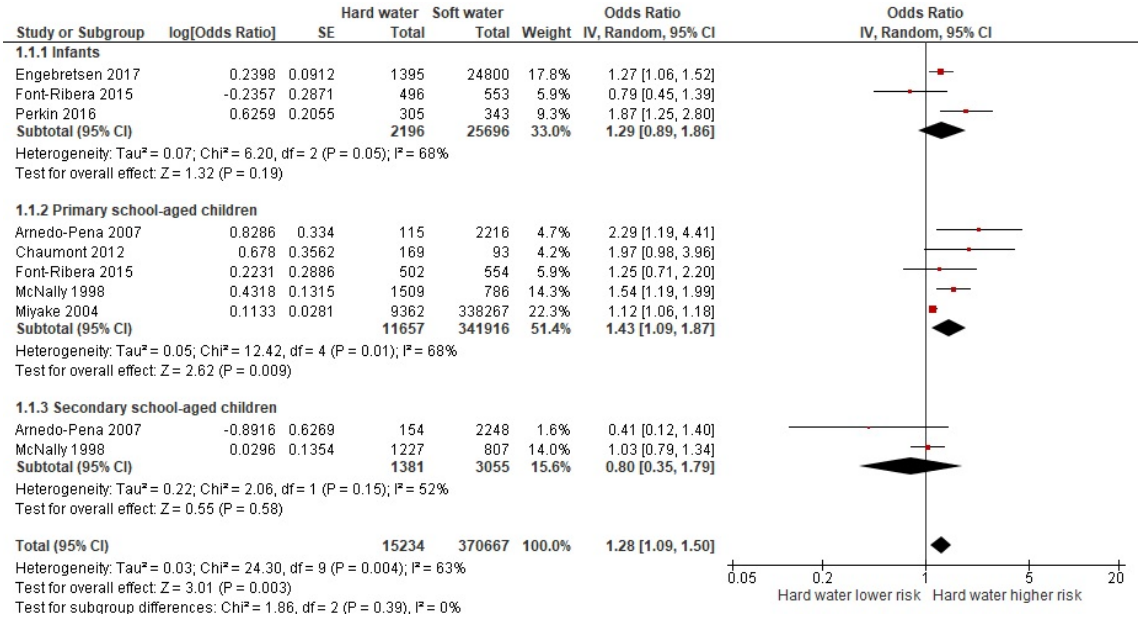

Figure 2.2 Forest plot of observational studies of water hardness and eczema risk
CI, confidence interval; IV, inverse variance

Table 2.2 GRADE Summary of findings table – hard water exposure and risk of eczema

What is the effect of hard water exposure on the risk of eczema?

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with soft water	Risk with hard water				
Risk of eczema in infants and children assessed with: Parent-reported doctor-diagnosed eczema or visible dermatitis Follow up: birth to 16 years	211 per 1,000	**255 per 1,000** (226 to 286)	**OR 1.28** (1.09 to 1.50)	385901 (7 observational studies)(54, 61-66)	⊕◯◯◯ VERY LOW [a,b]	Hard water may increase the risk of eczema in children but we are very uncertain.
Risk of eczema in infants assessed with: Parent-reported, doctor-diagnosed eczema or visible dermatitis Follow up: birth to 18 months	126 per 1,000	**157 per 1,000** (114 to 212)	**OR 1.29** (0.89 to 1.86)	27892 (3 observational studies)(54, 63, 66)	⊕◯◯◯ VERY LOW [a,b]	We are very uncertain about the effect of hard water on the risk of eczema in infants.
Risk of eczema in primary school-aged children assessed with: Parent-reported physician-diagnosed Follow up: lifetime prevalence up to 7 years	227 per 1,000	**296 per 1,000** (243 to 355)	**OR 1.43** (1.09 to 1.87)	353573 (5 observational studies)(61-65)	⊕◯◯◯ VERY LOW [a,b]	Hard water may increase the risk of eczema in primary school-aged children but we are very uncertain.
Risk of eczema in secondary school-aged children assessed with: Child-reported doctor-diagnosed atopic eczema Follow up: lifetime prevalence up to 16 years	70 per 1,000	**63 per 1,000** (46 to 86)	**OR 0.90** (0.65 to 1.25)	4436 (2 observational studies)(61, 64)	⊕◯◯◯ VERY LOW [a]	We are very uncertain about the effect of hard water on risk of eczema in secondary school-aged children.
Risk of eczema in adults - not measured	-	-	-	-	-	No studies identified reporting the risk of eczema in adults

CI, confidence interval; GRADE, Grading of Recommendations, Assessment, Development and Evaluation; OR, odds ratio

[a] Rated down for high risk of bias due to study design.

[b] Rated down for inconsistency due to high heterogeneity in effect estimates (I^2 = 63%).

* The risk in the intervention group (and its 95% confidence interval) is based on the assumed pooled risk in the comparison group and the relative effect of the intervention (and its 95% CI).
GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

2.3.1.2.2 Hard water exposure and risk of eczema in infants

Two studies (24, 54) reported a higher risk of eczema in infants living in a hard water area and one (55) reported no statistically significant difference in eczema risk between harder and softer water areas (Figure 2.2). These studies adjusted for potential confounders, including geographical location (Table 2.1). The pooled estimate based on 27,892 infants was almost identical to that for all children but was not significant (OR 1.29, 95% CI 0.89, 1.86). Certainty in this estimate is very low due to high risk of bias and heterogeneity (I^2 68%; Figure 2.2). This heterogeneity may be explained by the different study designs and populations studied – younger infants assessed at a single timepoint (3 months) in the Perkin cross-sectional study, older infants assessed at two timepoints (6 or 18 months) in the Engebretsen study and at 14 months in the Font-Ribera birth cohort studies.(24, 54, 55) The use of different diagnostic criteria, i.e. questionnaire-derived diagnosis vs skin examination by a physician following validated diagnostic criteria, as well as covariates adjusted for, might also have contributed to the heterogeneity (Table 2.1). Importantly, there were also differences in the precision of water hardness estimation – postcode-based (around 100 m) in the Perkin study compared with wider, municipality-based estimates in the Engebretsen and Font-Ribera studies. Interestingly, the Font-Ribera study reported no significant variation in water hardness with repeat measurements over one year, suggesting that season is less likely to be a significant source of heterogeneity. The longitudinal analysis of data from the Perkin presented in Chapter 3 found no overall statistically significant association between exposure to harder versus softer water and parent-reported eczema (adjusted HR 1.07, 95% CI 0.92, 1.24).(39)

2.3.1.2.3 Hard water exposure and risk of eczema in non-infant children

Five studies (21-23, 53, 55) reported on the risk of eczema in children exposed to harder versus softer water. The pooled estimate based on 358,009 participants showed a statistically significant increased risk of eczema in children exposed to hard water (OR 1.27; 95% 1.03, 1.57). Two studies (21, 22) reported different risks in primary and secondary school-aged children and so these differences were explored further using a post hoc analysis of subgroups based on these categories.

2.3.1.2.4 Hard water exposure and risk of eczema in primary school children

Three cross-sectional studies (21-23) reported a higher risk of eczema and two studies (53, 55) reported no statistically significant difference in eczema risk in primary school children living in harder versus softer water areas (Figure 2.2). Studies adjusted for potential confounding covariates, such as socio-economic status (Table 2.1). The pooled estimate based on 353,573 participants showed a statistically significant increased risk of eczema in primary school-aged children exposed to hard water (OR 1.43; 95% CI 1.09, 1.87). Certainty in this estimate is very low due to high risk of bias and heterogeneity (I^2 68%; Table 2.2). This heterogeneity may be explained by the differences highlighted in the previous section around study design and assessment of the outcome. The heterogeneity may also partly be explained by global differences in what is considered 'hard' water: up to 100 mg/ml $CaCO_3$ in the highest quartile in the Miyake et al. (23) study in Japan, compared to up to 341 mg/ml $CaCO_3$ in the highest quartile in the McNally et al. (21) study in the UK. Removing the large Miyake (23) study in a post hoc leave-one-out sensitivity analysis increased the pooled effect estimate (OR 1.59, 95% CI 1.29, 1.97) (Figure 2.3).

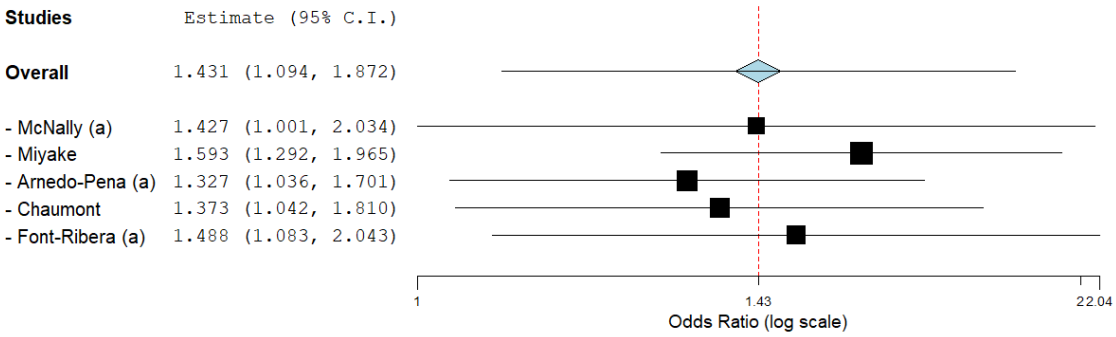

Figure 2.3 Leave-one-out meta-analysis of risk of eczema in primary school-aged children exposed to harder versus softer water

Odds ratios show the effect on the overall pooled effect estimate from removing the named study. Removing the Miyake study increases the pooled effect estimate.
CI, confidence interval

2.3.1.2.5 Hard water exposure and risk of eczema in secondary school children

Two cross-sectional studies (21, 22) in the UK and Spain reported no statistically significant difference in eczema risk in secondary school children living in harder versus softer water areas. The pooled estimate based on 4,436 participants also showed no statistically significant association between hard water exposure and ISAAC

questionnaire-based eczema risk among secondary school-aged children (OR 0.80, 95% CI 0.35, 1.79) (Figure 2.2). However, certainty in this estimate is very low due to high risk of bias, moderate heterogeneity (I^2 52%) and imprecision (Table 2.2).

2.3.1.2.6 Effect of FLG mutation status on risk of eczema in infants exposed to hard water

Perkin et al. investigated the interaction between hard water and the presence of a loss-of-function mutation in the *filaggrin* (*FLG*) gene in 1,303 healthy, breastfed 3-month-olds but this was not statistically significant (OR 2.10 95% CI 0.74, 5.99).(24) A longitudinal analysis of infants aged 3-36 months in the same study showed that, after adjustment for confounders, there was a significantly higher risk of parent-reported, doctor-diagnosed eczema in infants with *FLG* mutations exposed to hard water, compared to those with wild-type *FLG* living in softer water areas (adjusted HR 2.72 [95%CI 2.03, 3.66]).(39)

2.3.1.2.7 Hard water compared to deionised water and skin barrier dysfunction in adults

TEWL was measured by Danby et al. (56) in 80 adults with or without eczema, with and without *FLG* loss-of-function mutations. After 72 hours, mean TEWL at the volar forearm skin sites washed with hard water was 2.76 g/m^2/h significantly higher than in those washed with deionised water (Table 2.3). Danby et al. reported no statistically significant effects of hard water on skin surface pH or IL-1α. Warren et al.(57) washed the forearms of 36 healthy adult female volunteers with various types of soap and hard (188 mg/L) or deionised water. There was no difference in skin hydration between washing with hard and deionised water (Table 2.3). However, skin rinsed in hard water had a 1.9 pF lower (95% CI -2.59, -1.21) mean level of skin hydration at five days. Both studies reported statistically significant increased erythema 72 hours to 5 days after washing with hard water. Overall, due to the small sample size and non-randomised experimental design, there is a very low certainty of the evidence for these estimates (Table 2.3).

Table 2.3 GRADE Summary of findings table – hard water exposure and skin barrier function

Hard water compared to Deionised water for Skin barrier dysfunction

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with Deionised water	Risk with Hard water				
Transepidermal water loss at volar forearm (TEWL) follow up: mean 72 hours	The mean transepidermal water loss at volar forearm was **10.19** g/m^2/h	The mean transepidermal water loss at volar forearm in the intervention group was 2.76 g/m^2/h higher (0.71 higher to 4.81 higher)	-	80 (1 observational study)(56)	⨁◯◯◯ VERY LOW [a]	Hard water may increase short-term transepidermal water loss at volar forearm slightly but we are very uncertain.
Transepidermal water loss at volar forearm (TEWL) follow up: 12 weeks	Matsuda et al: “There was no significant difference in TEWL between the two groups”		-	15 (1 observational study)(59)	⨁◯◯◯ VERY LOW [a]	We are very uncertain about the medium-term effect of hard water on transepidermal water loss.
Erythema (Erythema) assessed with: Mexameter/visual erythema scale 0-6 follow up: range 72 hours to 5 days	Danby, et al: "There was a significant effect of water type on skin redness (repeated measures analysis of variance, p<0.0001)." A larger change in skin redness was seen in the skin sites exposed to hard water compared to those exposed to deionised water. Warren, et al: no statistically significant difference between hard and deionised water used for washing, but statistically significant (p<0.001) higher erythema with skin washed with hard water (1.20) compared to deionised water (0.80).			116 (2 observational studies)(56, 67)	⨁◯◯◯ VERY LOW [a]	Hard water may increase short-term skin erythema slightly but we are very uncertain.

Skin cytokine release assessed with: IL-1a follow up: mean 72 hours	"The use of hard water without chlorine did not lead to elevated IL-1a levels compared with the deionised water control."			80 (1 observational study)(56)	⨁◯◯◯ VERY LOW [a]	We are very uncertain about the effect of hard water on skin (stratum corneum) cytokine release.
Skin surface pH assessed with: pH probe follow up: mean 72 hours	No apparent statistically significant difference in skin surface pH between sites exposed to hard water compared to those exposed to deionised water.			80 (1 observational study)(56)	⨁◯◯◯ VERY LOW [a]	Hard water appears to result in little to no difference in skin surface pH but we are very uncertain.
Skin hydration follow up: mean 5 days	The mean skin hydration was **17.28** pF	The mean skin hydration in the intervention group was 1.9 pF lower (95% CI -2.59, -1.21).	-	36 (1 observational study)(56, 67)	⨁◯◯◯ VERY LOW [a]	Hard water may reduce short-term skin hydration slightly but we are very uncertain.
Skin hydration follow up: 12 weeks	"Water content was increased on 8 and 12 weeks in the older adults with UPSW bathing as compared with those with tap water that contained 79.9 mg CaCO3/L." P<0.01 at 12 weeks between groups.		-	15 (1 observational study)(59)	⨁◯◯◯ VERY LOW [a]	Hard water may increase medium-term skin hydration slightly but we are very uncertain.

CI, confidence interval; IL, interleukin; MD, mean difference; TEWL, transepidermal water loss; UPSW, ultra-pure softened water

[a] Rated down for imprecision due to small study size.

* The risk in the intervention group (and its 95% confidence interval) is based on the assumed pooled risk in the comparison group and the relative effect of the intervention (and its 95% CI).

GRADE Working Group grades of evidence

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect

Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Engebretsen et al. studied the short-term effect of hard water on TEWL, NMF and cytokines in 40 healthy adults without FLG mutations using Finn chambers on the volar forearm.(58) At 24 hours there was no difference between mean (SD) TEWL in volar forearm skin exposed to soft 13.0 (±6.2) g/m2/h and hard 13.1 (±6.1) g/m2/h compared with occlusion alone 11.8 (±3.3) g/m2/h. At 48 hours there was a statistically significant difference between soft 9.1 (±3.0) g/m2/h and hard 9.2 (±2.8) g/m2/h individually compared with occlusion alone 8.2 (±2.2) g/m2/h. At 24 hours, a significant decrease in NMF on tape strips was observed for soft (0·51 (0·19) mmol/g) and hard water (0·61 (0·32) mmol/g) compared with occlusion alone (0·71 (0·18) mmol/g), but no significant difference in mean NMF was found between hard and soft water. At 24 hours, there was a significant increase in mean interleukin-4 (IL-4), interferon-gamma (IFN-y) and interleukin-10 (IL-10) on tape strips after exposure to hard water compared to the control. No significant increase was found for soft water compared to control. The study did not report a direct comparison of the effect between hard and soft water. Matsuda et al. studied the effect of ultra-pure softened water (UPSW) on skin hydration in 15 elderly care home residents without a history of skin diseases.(59) Statistically significantly ($P<0.01$) higher mean skin hydration was reported at 12 weeks in adults bathed with UPSW compared to those bathed with tap water (Table 2.3). However, there was no statistically significant difference in mean TEWL or skin dryness between the groups at 12 weeks.

2.3.1.2.8 Water softeners and usual care compared to usual care alone in treating eczema

Two RCTs (26, 36) compared water softeners plus usual skincare to usual skincare alone in the treatment of eczema in children. Thomas et al. found no objective improvement in disease severity from the addition of an ion-exchange water softener to usual care in 323 children aged 6 months to 16 years with moderate eczema after 12 weeks (mean change in SASSAD 0.66 [95% CI -1.37, 2.69]).(26) A smaller study by Togawa et al. subsequently reported a non-statistically significant reduction in mean EASI of -2.61 points (95% CI -7.03, 1.81) at 6 weeks in 12 children aged 3-6 years with mild eczema.(36) The pooled estimate of objective eczema severity across both RCTs showed no statistically significant additional benefit of ion-exchange water softeners in the treatment of eczema (standardised mean difference (SMD) 0.06, 95% CI -0.16, 0.27)

(Figure 2.4). Certainty in this estimate is moderate (Table 2.4). Thomas et al. reported an improvement in subjective eczema severity (POEM) with the use of an ion-exchange water softener in addition to usual care (mean reduction -2.03 points, 95% CI -3.55, -0.51, GRADE: moderate certainty).(26) Slight improvement in disease control as measured by well-controlled weeks (WCW) was reported with the use of an ion-exchange water softener in addition to usual care (mean increase in WCW 0.99 week, 95% 0.04, 1.95, GRADE: moderate certainty). Totally-controlled weeks (TCW) also increased (mean 1.19 weeks, 95% CI 0.43, 1.95, GRADE: moderate certainty) (Table 2.4) (Appendix 7.3).

In Togawa et al. the changes in mean TEWL were not statistically significant at abdominal (-5.77 g/m2/h; 95% CI -13.9, 2.41) or upper back sites (2.26 g/m2/h; 95% CI -16.9, 21.4) over 12 weeks.(36) The same study reported a statistically significant reduction in mean pruritus visual analogue scale (-2.1 points, 95% CI -4.14, -0.063, GRADE: low certainty) (Table 2.4).

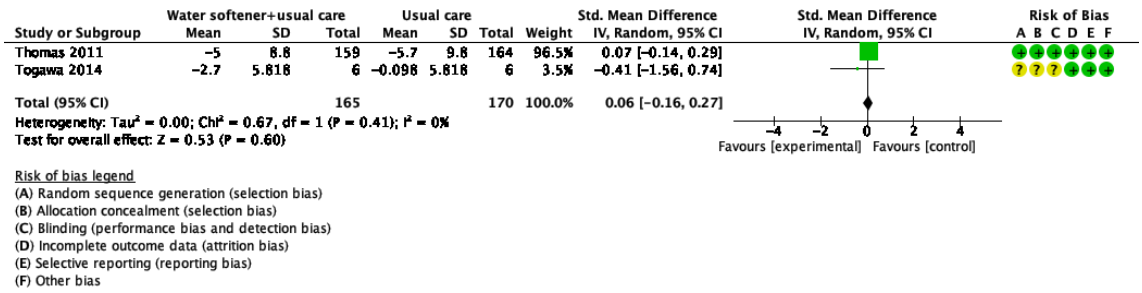

Figure 2.4 Forest plot of randomised controlled trials comparing water softeners to usual care on eczema severity

CI, confidence interval; IV, inverse variance; SD, standard deviation

Table 2.4 GRADE Summary of findings table – water softeners for the treatment of eczema

Water softeners for the treatment of eczema

Outcomes	Anticipated absolute effects* (95% CI)		№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with usual care	**Risk with water softeners**			
Eczema severity assessed with: SASSAD, EASI follow up: mean 12 weeks	-	SMD 0.06 SD higher (0.16 lower to 0.27 higher)	335 (2 RCTs)(68, 69)	⨁⨁⨁◯ MODERATE [a]	Water softeners in addition to usual care probably do not reduce eczema severity.
Skin barrier function (TEWL) assessed with: Transepidermal water loss (TEWL) (Abdominal region), g/m^2h follow up: mean 12 weeks	The mean change in TEWL was **-0.037** g/m^2h	The mean change in TEWL in the intervention group was 5.77 g/m^2h lower (13.9 lower to 2.41 higher)	11 (1 RCT)(69)	⨁◯◯◯ VERY LOW [a, b, c]	Water softeners in addition to usual care may result in little to no difference in skin barrier function in children with eczema.
Skin barrier function (TEWL) assessed with: Transepidermal water loss (TEWL) (Upper back region), g/m^2h) follow up: mean 12 weeks	The mean skin barrier function was **-4.62** g/m^2h	The mean skin barrier function in the intervention group was 2.26 g/m^2h higher (16.9 lower to 21.4 higher)	11 (1 RCT)(69)	⨁◯◯◯ VERY LOW [a, b, c]	Water softeners in addition to usual care may result in little to no difference in skin barrier function in children with eczema.

Patient-reported eczema severity (Pruritus VAS) assessed with: Pruritus Visual Analogue Scale Scale from: 0 to 9 follow up: mean 12 weeks	The mean change in patient-reported eczema severity was **0.56** points	The mean change in patient-reported eczema severity in the intervention group was 2.1 points lower (4.14 lower to 0.063 lower)	11 (1 RCT)(69)	⊕◯◯◯ VERY LOW [a, b, c]	Water softeners may reduce patient-reported pruritus slightly.
Patient-reported eczema severity assessed with: Patient-oriented eczema measure (POEM) Scale from: 0 to 28 follow up: mean 12 weeks	The mean patient-reported eczema severity was **-3.6** points	The mean patient-reported eczema severity in the intervention group was 2.03 points lower (3.55 lower to 0.51 lower)	323 (1 RCT)(68)	⊕⊕⊕◯ MODERATE [c]	Water softeners probably reduce patient-reported eczema severity slightly.
Eczema disease control assessed with: Well-controlled weeks (WCW) Scale from: 0 to 12 follow up: mean 12 weeks	The mean eczema disease control was **7.3** WCW	The mean eczema disease control in the intervention group was 0.99 WCW higher (0.04 higher to 1.95 higher)	267 (1 RCT)(68)	⊕⊕⊕◯ MODERATE [c]	Water softeners probably improve eczema disease control slightly.

Eczema disease control assessed with: Totally-controlled weeks (TCW) Scale from: 0 to 12 follow up: mean 12 weeks	The mean eczema disease control was **1.7** TCW	The mean eczema disease control in the intervention group was 1.19 TCW higher (0.43 higher to 1.95 higher)	265 (1 RCT)(68)	⨁⨁⨁◯ MODERATE [c]	Water softeners probably improve eczema disease control.

EASI, eczema area and severity index; GRADE, Grading of Recommendations, Assessment, Development and Evaluation; POEM, patient-oriented eczema measure; RCT; randomized controlled trial; SASSAD, six area six sign atopic dermatitis severity score; SMD, standardised mean difference; TCW, totally-controlled week; TEWL, transepidermal water loss; VAS, visual analogue scale; WCW, well-controlled week

[a] Rated down for imprecision due to wide confidence intervals.

[b] Rated down for imprecision due to small study size.

[c] Rated down due to unclear risk of bias.

GRADE Working Group grades of evidence

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect

Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

2.3.1.3 Studies in animals

2.3.1.3.1 Animal studies of softened water and skin barrier dysfunction

Ohmori et al. conducted a randomised crossover study of 11 dogs with eczema.(29) Dogs received a weekly shampoo for 4 weeks using either hard (158 mg/L $CaCO_3$) or UPSW (<1 mg/L $CaCO_3$) followed by a washout period of at least 12 weeks before switching to the other water type. The authors reported the changes in skin barrier dysfunction in each treatment period separately but did not directly compare the changes overall using a within-subject statistical analysis. The authors reported significantly improved canine atopic dermatitis severity (CADESI) score and TEWL after shampoo treatment with soft water and no significant change after tap water (Table 2.1), but with no direct, within-subject comparison these results cannot be used to infer a significant benefit of soft water and certainty in the reported findings is very low (Table 2.5).

Tanaka et al. examined the effects of treatment with UPSW, tap water versus no treatment on the skin of 30 NC/Tnd mice with phenotypic eczema (Table 2.1).(60) The graphical results showed the change in clinical severity score over time but are difficult to interpret since there is no overall analysis of the trend over time in the three groups. Klimisch category was 2 (reliable with restriction) Appendix 7.3.

Table 2.5 GRADE Summary of findings table – water softeners for the treatment of eczema in animals

Water softeners compared to hard water for animals

Outcomes	Impact	№ of participants (studies)	Certainty of the evidence (GRADE)
Animal Eczema Severity assessed with: Canine Atopic Dermatitis Extent and Severity Index (CADESI) in dogs, Clinical skin severity score in mice follow up: range 22 days to 0	In dogs, CADESI score was measured pre- and post-treatment within each group (UPSW, tap water). Comparisons were not reported between UPSW and tap water. In the UPSW group there was a decrease from a mean score of 96.8 (+/- 21.0 SEM) to a mean score of 76.5 (+/- 20.7), P<0.05. In the tap water group the CADESI score changed from a mean of 87.7 (+/- 19.9) to 88.1 (+/- 18.9). In mice, Blinded assessment of clinical skin severity score of AD-type lesions in mice. Severity score reduced in the group of mice treated with UPSW compared to tap water, P<0.05, at 21 days after treatment.	39 (2 observational studies)(70, 71)	⨁◯◯◯ VERY LOW [a,b]
Transepidermal water loss (TEWL) assessed with: Tewameter follow up: range 22 days to 0	In dogs, TEWL was measured pre- and post-treatment within each group (UPSW, tap water). Comparisons were not reported between UPSW and tap water. In the UPSW group there was a decrease from mean 12.5 g/m2/h (+/- 2.3 SEM) to 6.7 g/m2/h (+/- 0.8), P=0.0096. In the Tap water group, TEWL changed from a mean 16.85 g/m2/h (+/- 4.2) pre-treatment to a mean 17.8 g/m2/h (+/- 4.6) post-treatment. In mice, TEWL was measured at the dorsal area pre- and post-treatment within each group (UPSW, tap water, untreated). Comparisons were not reported between groups. In the UPSW group, there was a statistically significant reduction in TEWL at Day 22, P<0.05. No statistically significant difference was seen at Day 22 in the untreated mice or in those treated with tap water.	39 (2 observational studies)(70, 71)	⨁◯◯◯ VERY LOW [a]

Pruritus (PVAS) assessed with: Owner pruritus visual analogue score (PVAS) in dogs, Scratching frequency/30 minutes in mice follow up: range 22 days to 0	In dogs, PVAS was measured pre- and post-treatment within each group (UPSW, tap water). Comparisons were not reported between UPSW and tap water. In the UPSW group, there was a decrease in PVAS from a mean score of 6.2 (+/- 0.7 SEM) to 4.7 (+/- 0.6). In the tap water group, PVAS changed from a mean score of 5.5 (+/- 0.6) to 5.3 (+/- 0.6). In mice, scratching behaviour was recorded by an automated system. Comparisons were made pre- and post-treatment within each group (UPSW, tap water, untreated). Scratching frequency per 30 minute interval increased in the untreated and tap water groups at Day 22, P<0.05. There was no statistically significant difference in scratching frequency in the UPSW group.	39 (2 observational studies)(70, 71)	⊕◯◯◯ VERY LOW [a,b]

CADESI, canine atopic dermatitis extent and severity index; CI, confidence interval; GRADE, Grading of Recommendations, Assessment, Development and Evaluation; PVAS, pruritus visual analogue score; TEWL, transepidermal water loss; UPSW, ultrapure softened water

[a] Rated down for imprecision due to small study size.

[b] Rated down for indirectness due to differences in outcome assessment.

* The risk in the intervention group (and its 95% confidence interval) is based on the assumed pooled risk in the comparison group and the relative effect of the intervention (and its 95% CI).

2.3.1.4 Publication bias

Publication bias was not formally assessed due to the limited number of studies included in the quantitative syntheses (<10).

2.4 Discussion

We performed a systematic literature review and meta-analysis of the relationship between water hardness and the primary outcomes of eczema and skin barrier function. Overall, in non-infant children exposed to harder versus softer water, there were slightly increased odds of eczema, although there is very low certainty in the evidence. In infants and secondary school-aged children, there was no clear evidence of overall increased risk of eczema with hard water exposure. However, infants with *FLG* mutations exposed to hard water may be at a higher risk of eczema. In adults, hard water may result in skin barrier dysfunction, as measured by slight increases in short-term TEWL and skin erythema, with a more marked effect seen in adults with eczema who have FLG mutations. Hard water appears to result in little to no difference in skin IL-1α release or skin surface pH but may reduce short-term skin hydration slightly. Secondary outcomes of clinician- and patient-reported eczema severity and control were also reviewed. There is evidence that water softeners provide no objective improvement in eczema severity, based on clinician assessed signs, when used in addition to usual care, with low certainty in the evidence. However, there is moderate-quality evidence that water softeners reduce patient-reported eczema severity slightly and improve eczema disease control slightly in addition to usual care.

2.4.1.1 Strengths and limitations

A comprehensive systematic literature search was conducted, including the 'grey' literature. Risk of bias was assessed for included studies and the certainty of the evidence was rated for each outcome using the GRADE approach. There were key methodological limitations in the studies of eczema risk that preclude firm conclusions being drawn regarding the role of hard water exposure on eczema risk in children. There was a lack of studies in very young children and in adults. Definitions of hard water and eczema varied across studies, potentially leading to misclassification. Most studies used questionnaire-based child- or parent-reported eczema to determine eczema status, some of which were

based on the validated ISAAC questionnaire. Exposure to hard water is a product of water hardness and bathing frequency. Observational studies only look at the association between living in a hard water area and developing eczema, rather than the causal effect of hard water exposure *per se* on the risk of eczema development. Some studies included adjustment for confounders, however, the studies were mainly of a cross-sectional design and therefore do not allow causal interpretations to be made. Furthermore, the definition and nature of the confounders accounted for in the different studies varied, and so as with any observational study, there is a risk of bias due to residual confounding.

A limitation of this review is that single, rather than double, data extraction was used. There was a high degree of heterogeneity between studies that persisted after subgroup analyses, suggesting that the results must be interpreted with caution. An obvious difference between studies is in the levels of hard water between study areas. It could be that the baseline is too high, and/or that the comparator is too low. Indeed, removing the Miyake study where the comparison was between 70 mg/L CaCO3 and <48 mg/L CaCO3 increases the overall pooled OR to 1.53 (95% CI 1.29, 1.97) (Figure 2.3). The observed heterogeneity between studies may also be driven by differences in study design, for example, cross-sectional versus cohort design.

2.4.1.2 Interpretation of main findings

The development of eczema is multifactorial and likely due to genetic determinants of skin barrier function and immunity along with environmental exposures that initiate and perpetuate the cycle of inflammation and skin barrier damage seen in chronic eczema. The potential effect of environmental exposures, such as hard water, on skin barrier function has been hypothesised to be greater in early life because the skin barrier is immature. Our finding of an increased risk of eczema in younger rather than older children fits with this hypothesis. Data from the Warren et al and Danby et al studies suggest that detergents in wash products may interact with hard water resulting in deposition of detergents on the skin leading to a detrimental effect on skin barrier function.(56, 57) Whilst the focus in the literature has been on calcium carbonate as the main determinant of water hardness, magnesium also influences hardness. Evidence from in vitro studies suggests that magnesium has a beneficial effect on skin barrier function. Furthermore, this effect may be enhanced by the addition of calcium, so long as the ratio

of calcium to magnesium is <1.32 Dead Sea water has a very high calcium level (17,600 mg/L) yet it is reported as having beneficial properties for the skin. This may in part be because the magnesium ion concentration is higher still at 45,900 mg/L, i.e. a ratio of 0.38.33 As ion-exchange water softeners remove both calcium and magnesium, along with other divalent cations such as iron, zinc, and manganese, this may partly explain the lack of efficacy of water softeners in the treatment of eczema. Softened water is different from naturally soft water in both its mineral content and pH, in that softened water retains the alkaline pH of the original hard water, whereas naturally soft water has a lower (more acidic) pH due to lower alkalinity. The short-term data from the study by Danby et al, however, suggest that in normal skin hard water does not make a significant difference to the pH of the skin surface, which may be due to the innate buffering potential of the skin.8 Wash products have a major effect on skin surface pH and are also the source of irritant surfactants that bind to calcium. In recent years, the use of mild syndet liquid soaps has increased whereas the use of traditional alkyl carboxylate bars of soap has declined, probably leading to lower skin pH levels. This change in practice, in addition to varying early skincare advice around the world, make wash product use an important potential confounder to be considered when measuring the effect of water hardness on skin surface pH.

Clearly, people living in softer water areas also develop eczema, suggesting that hard water is not a necessary cause. However, hard water may be a component cause. For example, following an initial barrier insult, either chemical or physical, there may be impaired barrier repair due to exposure to high levels of calcium. How much of a contribution to this initial barrier insult is made directly by hard water or indirectly through increased detergent deposition remains unclear. Further complicating the picture, some cleansers may reduce free calcium ion concentrations in water.34 However, the effect of such calcium-surfactant complexes on the skin also remains unclear.

Experimental studies in controlled environments in humans and animals provide some evidence for short-term irritant effects of hard water on the skin. It is unclear how this translates into real-world exposure to hard water where there is repeated exposure at varying frequencies and duration and in combination with multiple wash products that, again, may be used in varied ways.

2.4.1.3 Implications for clinical practice and future research

Water hardness needs to be considered in the context of other risk factors for the development of eczema, both genetic and environmental, particularly in younger children. Based on the evidence, it is unclear how the evidence may be used practically for clinicians working in hard water areas. While there is good evidence that water softeners do not help in the treatment of eczema, there is an open question as to whether water softeners may reduce the risk of eczema in children. This review has highlighted a need for RCT evidence and has helped to shape the design of a randomised controlled trial of water softeners for the prevention of eczema.

2.4.1.4 Conclusions

These findings have highlighted a lack of high-quality evidence on the effect of water hardness exposure on eczema risk in adults and very young children. There is evidence for an increased risk of eczema in primary-school-aged children exposed to harder water (range: 76 to >350 mg/L CaCO3). Water softeners provide no additional objective benefit in the treatment of eczema, however, they may provide subjective improvements in eczema severity. There may be a role of water hardness in the initiation of skin inflammation in early life, but there is a need for further longitudinal and interventional studies to test this.

3 EPIDEMIOLOGICAL STUDY ON WATER HARDNESS AND ECZEMA

3.1 Introduction

The systematic review highlighted the need for high-quality longitudinal studies of water hardness exposure and eczema risk in infants and young children. The review also provided crucial information on what confounders should be considered in such an analysis. Perkin et al. has previously shown in a cross-sectional analysis from the Enquiring About Tolerance (EAT) study among three-month old infants from England and Wales that combined exposure to hard water and high chlorine was associated with a 61% increase in the odds of eczema on skin examination (adjusted OR 1.61 85% CI 1.09, 2.38) (24). These results also suggested an interaction between hard water and *FLG* LOF mutations, not only for eczema but also skin barrier dysfunction measured by transepidermal water loss (TEWL), although formal interaction testing was not statistically significant. The present study extends the analysis beyond three months of age to longitudinally test the hypothesis that early life exposure to hard water is associated with eczema and skin barrier dysfunction in infants with and without *FLG* LOF mutations.

3.2 Methods

A secondary analysis of data on infants aged 3-36 months enrolled in the EAT study was performed. The EAT study was a randomized controlled trial comparing early versus standard introduction of allergenic foods in 1,303 generally well, breastfed infants born at term (≥37 weeks) (72). Infants were recruited at three months of age from the general population across England and Wales. The aim of the EAT study was to determine whether early introduction of common dietary allergens would prevent food allergies. The sample size was determined by the intervention component in the EAT study.

3.2.1 Primary outcome

Two definitions of eczema were used for the primary analyses: parent-reported, doctor-diagnosed eczema and visible eczema on skin examination. Visible eczema was

determined at the three-, 12- and 36-month visits using a UK diagnostic criteria–based photographic protocol adapted for infants (73). Parents were asked about new onset eczema, including whether this diagnosis was confirmed by a doctor, through online questionnaires at monthly intervals from 3-12 months, then three-monthly thereafter. The outcome of parent-reported eczema was used for the survival analysis. Time of onset of eczema prior to three months was imputed using a combination of parent-reported, doctor-diagnosed eczema, combined with time of first topical steroid use. Eczema severity was determined by the Scoring Atopic Dermatitis (SCORAD) index (74).

3.2.2 Secondary outcomes

A secondary outcome was TEWL as a measure of skin barrier function. TEWL was measured at three and 12 months with the Biox Aquaflux AF200 closed condenser chamber device (Biox Systems Ltd, London, UK) on unaffected skin of the volar aspect of the forearm (75). Parents were advised not to use any skin care products on the infant's arms for the preceding 24 hours. Measurements were performed in our environmentally controlled clinical research facility (ambient temperature, 20°C ± 2°C; relative room humidity, 32% to 50%) after at least 20 minutes of acclimatization. In all children the mean of three separate TEWL measurements was calculated.

3.2.3 Water hardness exposure and covariates

Data on domestic water $CaCO_3$ concentrations in milligrams per litre (mg/L) were obtained from local water supply companies for each participant's household based on postcode at the time of study recruitment. Data were collected on covariates, including sex, ethnicity, home location, maternal age, socioeconomic status (maternal age at leaving full-time education), ownership of a water softener, family history of eczema and other allergic diseases, frequency of bathing, and use of topical moisturizers and bathing products, through parental questionnaires. Data on indices of multiple deprivation (IMD), a measure of socio-economic position, were obtained from official statistics based on postcode of residence.

3.2.4 Filaggrin (FLG) genotyping

Venous blood samples were screened for the six commonest *FLG* mutations by using TaqMan allelic discrimination assays (mutations R501X, 2282del4, R2447X, S3247X; ABI 7900 HT; Applied Biosystems, Foster City, Calif) or by sizing of fluorescent PCR products on an Applied Biosystems 3130 DNA sequencer (mutations 3673delC and 3702delG). These six mutations detect 99% of *FLG* mutation carriers in the UK population.

3.2.5 Statistical analysis

As in the previous cross-sectional analysis of the effect of water hardness at three months of age in the EAT study, water hardness exposure was dichotomized based on the median value of $CaCO_3$ across the whole EAT cohort (24). Our *a priori* hypothesis was that the risk of eczema with hard water would be increased in those with *FLG* LOF mutations. We therefore planned to test for an interaction between water hardness and *FLG*, even in the absence of a statistically significant main effect of water hardness. *FLG* status was modelled using a two-level dominant genetic model whereby infants were assigned as having a *FLG* LOF mutation if they were heterozygous or homozygous for the null allele in at least one of the two single nucleotide polymorphisms. Children were retained in the analysis from three months until the first of: development of eczema, drop-out, or 36 months of age. We did not create a combined water hardness-chlorine variable, as was done in our cross-sectional analysis at three months, since our own subsequent mechanistic work in patients with and without eczema and *FLG* LOF mutations showed no additional increase in skin barrier disruption secondary to chlorine exposure (27). SCORAD was categorized as mild (1-15) or moderate/severe (>15). The relationships between covariates and eczema were explored using Kaplan-Meier (K-M) plots and univariate Cox regression. Multivariable adjustment was made for likely confounders: home location (rural/urban), ethnicity (White/non-White), IMD (deciles), and water softener present (yes/no). The effect of adding in study randomization group as a covariate was examined. Analyses were conducted using Stata, version 15 (StataCorp, College Station, TX). An exact partial-likelihood method was used to handle tied failures. Interactions among selected variables in the main effects model were examined based on

plotting the ratios of hazard ratios using *fintplot*.(76) Likelihood ratio tests were used to compare model fit with and without addition of the selected interaction terms. The proportional hazards assumption for the overall model was tested using *stphtest*. A cut-off of ≥15 g/m^2/h was used to define 'high' TEWL, based on the upper quartile of value of TEWL in EAT participants at enrolment without visible eczema, and consistent with our previous publications.(24, 77) The relationship between water hardness and TEWL was analysed using a generalized estimating equation with an equal-correlation model and conventionally derived variance estimator for standard error. All estimates are reported with 95% confidence intervals (CI) in brackets. Population Attributable Fraction (PAF) was calculated as PAF (%) = p(HR-1)/(p(HR-1)+1) x 100%, where p is the prevalence of the risk factor and HR is the hazard ratio of the disease risk in the exposed over the non-exposed.

3.3 Results

Of 1,303 infants enrolled in the EAT study, 91.3% (n=1,189) attended the final clinic visit and 94.0% (n=1,225) of participants' families completed the 36-month questionnaire. Water hardness data based on $CaCO_3$ levels were available for all participants. Water hardness values ranged from 3 mg/L to 490 mg/L, with a median of 257 mg/L (Figure 3.1).

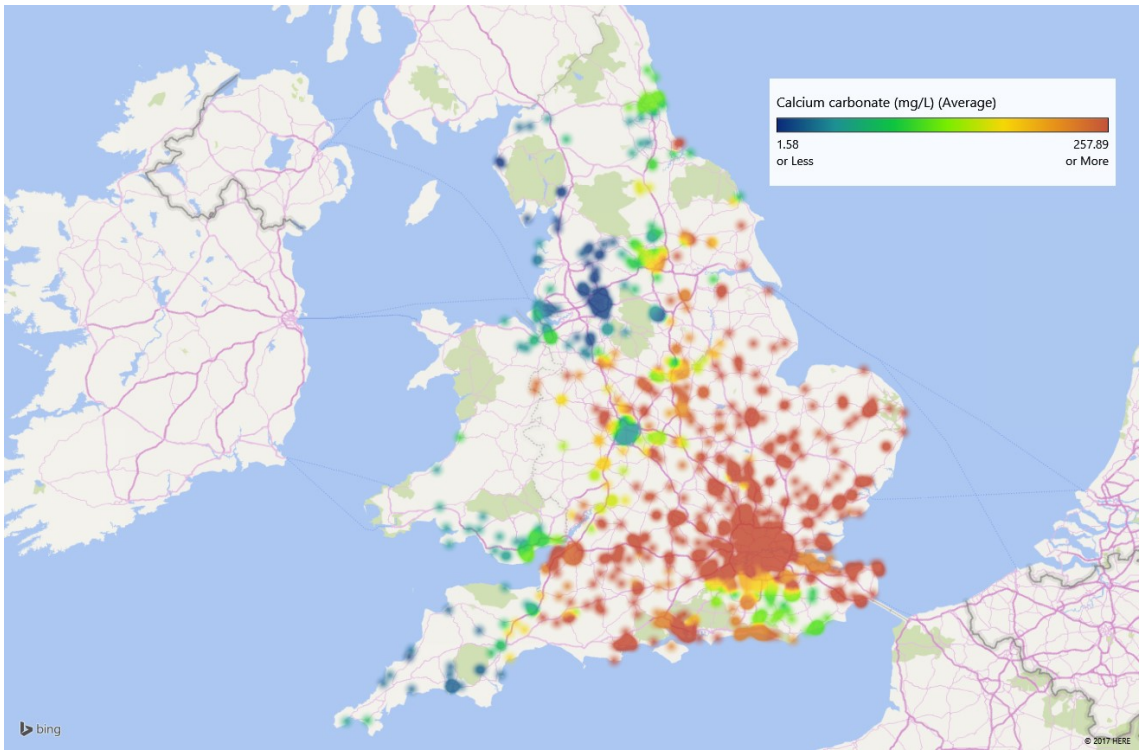

Figure 3.1 Heat map of England and Wales showing average calcium carbonate levels (mg/L) based on participants' postcodes at enrolment

The distribution of water hardness values was negatively skewed. *FLG* genotype was available for 1,206 participants (92.6%). Exposure to hard water was independent of *FLG* mutation status (X□1 $d.f.$ □2□ P = .84). Infants living in hard water areas were, at enrolment, more likely to be of non-white ethnicity, more likely to use a moisturizer and have a water softener installed and were less likely to use bubble bath or have pets (Table 3.1). 1,204 participants were included in this analysis and their demographic characteristics were broadly similar to the full EAT cohort (Table 3.2).

Table 3.1 Population demographics by exposure to ≤ or > 256 mg/L calcium carbonate concentrations at enrolment into the Enquiring About Tolerance study

	$CaCO_3$ below median (≤256 mg/L) (N =639) n (%)	**$CaCO_3$ above median (>256 mg/L) (N = 664) n (%)**	***P* value***
Demography			
Sex			
Male	316 (49.5)	337 (50.8)	.64
Age at enrolment, mean (SD)	3.40 (0.23)	3.38 (0.23)	.16
Ethnicity			
White	562 (87.9)	542 (81.6)	**.002**
Home location			
Urban	479 (75.1)	527 (79.5)	.06
Maternal education (age at completion [y])			
≤16	35 (5.5)	39 (5.9)	.93
17 -18	86 (13.5)	86 (13.0)	
≥19	517 (81.0)	539 (81.2)	
Index of multiple deprivation (deciles)			
1 – most deprived	34 (5.3)	17 (2.6)	.20
2	47 (7.4)	44 (6.6)	
3	56 (8.8)	63 (9.5)	
4	56 (8.8)	63 (9.5)	
5	64 (10.0)	78 (11.8)	
6	68 (10.6)	87 (13.1)	
7	64 (10.0)	75 (11.3)	
8	84 (13.1)	79 (11.9)	
9	89 (13.9)	75 (11.3)	
10 – least deprived	77 (12.1)	82 (12.4)	
EAT study randomization group			
Assigned to intervention	318 (49.8)	334 (50.3)	.85
Family atopy status			
Maternal			
Atopy (Eczema, A, or HF)	404 (63.3)	410 (61.8)	.58
Eczema	228 (35.7)	221 (33.3)	.36
Paternal			
Atopy (Eczema, A, or HF)	349 (54.7)	342 (51.6)	.26
Eczema	124 (19.4)	136 (20.5)	.63
Parental			
Atopy (Eczema, A, or HF)	524 (82.1)	542 (81.7)	.86
Skincare & bathing			
Water softener present at home	12 (1.9)	54 (8.1)	**<.001**
Bathing ≥5 times per wk	259 (43.0)	245 (39.4)	.21
Moisturizer use ≥5 times per wk	180 (29.9)	234 (37.6)	**.004**
Bubble bath used	215 (35.7)	173 (27.8)	**.003**
Bath emollient used	104 (17.2)	128 (20.6)	.14
Shampoo used	202 (33.5)	190 (30.5)	.27
Soap used	50 (8.3)	60 (9.6)	.41
FLG loss of function mutation	68 (11.7)	75 (12)	.84
Antibiotic exposure	122 (19.1)	123 (18.5)	.79
Pet ownership	316 (49.5)	238 (35.9)	**<.001**
Any household members smoking	87 (13.6)	81 (12.2)	.45
Vaginal delivery	479 (74.7)	496 (74.7)	.91

A, asthma; $CaCO_3$, calcium carbonate; EAT, enquiring about tolerance; FLG, filaggrin; HF, hay fever; wk, week

**P*-values based on Chi-squared test for proportion differences and independent samples t-test for means

Table 3.2 Comparison of baseline characteristics between full and analytic datasets

	Full EAT study dataset N=1,303 % (n)	Analytical dataset* N=1,204 % (n)
Demography		
Sex		
Male	50.1 (653)	49.8 (600)
Ethnicity		
White	84.7 (1,104)	84.6 (1,019)
Home location		
Urban	77.3 (1,006)	77.6 (934)
Maternal education (age at completion [y])		
≤16	5.7 (74)	5.7 (68)
17 -18	13.2 (172)	13.5 (162)
≥19	81.1 (1,056)	80.9 (974)
Index of multiple deprivation (deciles)		
1 – most deprived	3.9 (51)	4.2 (50)
2	7.0 (91)	6.7 (80)
3	9.1 (119)	9.0 (108)
4	9.1 (119)	8.8 (106)
5	10.9 (142)	10.8 (130)
6	11.9 (155)	12.0 (144)
7	10.7 (139)	10.9 (131)
8	12.5 (163)	12.9 (155)
9	12.6 (164)	12.9 (155)
10 – least deprived	12.2 (159)	12.0 (144)
Water hardness		
CaCO3 (mg/L), median (IQR)	257 (123.8)	258 (98.4)
EAT study randomization group		
Assigned to intervention	50.0 (652)	50.4 (607)
Family atopy status		
Maternal		
Atopy (Eczema, A, or HF)	62.6 (814)	62.8 (756)
Eczema	34.5 (449)	34.1 (410)
Paternal		
Atopy (Eczema, A, or HF)	53.1 (691)	52.9 (637)
Eczema	20.0 (260)	20.2 (243)
Parental		
Atopy (Eczema, A, or HF)	81.9 (1,066)	82.0 (987)
Skincare		
Water softener present at home	5.1 (66)	5.0 (60)
Bathing ≥5 times per wk	41.1 (504)	40.6 (459)
Moisturizer use ≥5 times per wk	33.8 (414)	34.6 (391)
Bubble bath used	31.7 (388)	31.0 (351)
Bath emollient used	18.9 (232)	18.9 (214)
Shampoo used	32.0 (392)	32.1 (363)
Soap used	9.0 (110)	8.5 (96)
FLG loss of function mutation	11.9 (143)	11.9 (143)
Antibiotic exposure	18.8 (245)	19.0 (229)
Pet ownership	42.6 (554)	43.7 (467)
Any household members smoking	12.9 (168)	13.0 (156)
Vaginal delivery	25.2 (328)	25.0 (300)

A, asthma; $CaCO_3$, calcium carbonate; EAT, enquiring about tolerance; FLG, filaggrin; HF, hay fever; IQR, interquartile range; wk - week

3.3.1 Visible eczema with domestic hard water exposure

At three months, 183 infants (27.6%) exposed to harder water had visible eczema, compared to 134 (21.0%) exposed to softer water (*P*=.005) (Table 3.3). By 36 months, this difference had attenuated (*P*=.69). Cumulatively, a high proportion of infants overall (47.9% in the softer water group, 52.1% in the harder water group) had visible eczema on examination during at least one assessment visit at three, six, or 12 months, although there was also no statistically significant difference in the frequencies between the two groups (*P*=.47). Moderate-severe eczema (SCORAD >15) was more common in harder water areas compared to softer water areas (6.6% versus 4.4%, *P* = .02) at three months, however, this relationship was not present at 12 or 36 months (Table 3.3).

Table 3.3 Point and cumulative prevalence of visible eczema at 3, 12 and 36 months, stratified by water hardness exposure

Visit age, Outcome	$CaCO_3$ <median (<257 mg/L) (N =639) n (%)	$CaCO_3$ >median (≥257 mg/L) (N = 664) n (%)	$CaCO_3$ Q1 (N = 327) n (%)	$CaCO_3$ Q2 (N = 329) n (%)	$CaCO_3$ Q3 (N = 325) n (%)	$CaCO_3$ Q4 (N = 322) n (%)
3 m						
Eczema	134 (21.0)	183 (27.6) *P* = .005	71 (21.7)	71 (21.6)	95 (29.3)	80 (24.8) *P* = .07
Eczema Severity						
Mild	106 (16.6)	140 (21.1)	54 (16.5)	60 (18.2)	71 (21.8)	61 (18.9)
Mod-severe	28 (4.4)	44 (6.6) *P* = .02	17 (5.2)	11 (3.3)	25 (7.7)	19 (5.9) *P* = .12
Raised TEWL	196 (30.7)	224 (33.8) *P* = .23	98 (30.0)	104 (31.7)	114 (35.1)	104 (32.5) *P* = .57
12 m						
Eczema	144 (25.2)	156 (26.9) *P* = .52	79 (26.7)	71 (24.6)	73 (25.6)	77 (27.4) *P* = .88
Eczema Severity						
Mild	115 (20.1)	122 (21.0)	63 (21.3)	58 (20.1)	59 (20.7)	57 (20.3)
Mod-severe	28 (4.9)	31 (5.3) *P* = .87	16 (5.4)	12 (4.2)	12 (4.2)	19 (6.8) *P* = .83
Cumulative eczema	222 (46.9)	251 (53.1) *P* = .24	117 (35.8)	115 (35.0)	124 (38.3)	117 (36.3) *P* = .84
Raised TEWL	203 (37.2)	221 (39.7) *P* = .39	107 (37.7)	101 (36.7)	115 (42.0)	101 (37.4) P = .58
36 m						
Eczema	124 (21.2)	132 (22.1) *P* = .69	67 (22.1)	62 (20.9)	68 (23.6)	59 (20.1) *P* = .75

Eczema Severity						
Mild	74 (12.6)	94 (15.6)	39 (12.8)	39 (13.1)	50 (17.2)	40 (13.5)
Mod-severe	45 (7.7)	36 (6.0)	24 (7.9)	21 (7.1)	15 (5.2)	21 (7.1)
		P = .20				P = .83
Cumulative eczema	265 (47.9)	288 (52.1)	136 (41.6)	139 (42.2)	149 (46.0)	129 (40.1)
		P = .47				P = .47

CaCO3, calcium carbonate; TEWL, transepidermal water loss
Data are presented as n (%). P-values for heterogeneity were calculated using Chi-squared statistics.

3.3.2 Parent-reported eczema risk with domestic hard water exposure

Overall, 761 infants (58.4%) developed parent-reported eczema by 36 months of age. There was no significant difference in the risk of parent-reported eczema between infants exposed to harder versus softer water ($P_{Log\text{-}rank}$ = .20) (Figure 3.2). There was no association between $CaCO_3$ exposure (per mg/L) and risk of parent-reported eczema (HR 1.00 95% CI 1.00, 1.00; *P* = .62), and this was unchanged after adjustment for confounders (HR 1.00 95% CI 1.00, 1.00; *P* = .33). When water hardness was dichotomized on the median value of $CaCO_3$ in the cohort, there was a small, non-statistically significant increased risk of parent-reported eczema with exposure to above versus below median water hardness levels (HR 1.10 95% CI 0.95, 1.28; *P* = .20) even after adjustment for confounders (HR 1.07 95% CI 0.92, 1.24; *P* = .39). This risk equates to a PAF of 3.4% overall with water hardness exposure. There was evidence of violation of the proportionality assumption, based on water hardness and ethnicity. Stratification by ethnicity improved proportionality and the effect estimates were unchanged (*P* = .39).

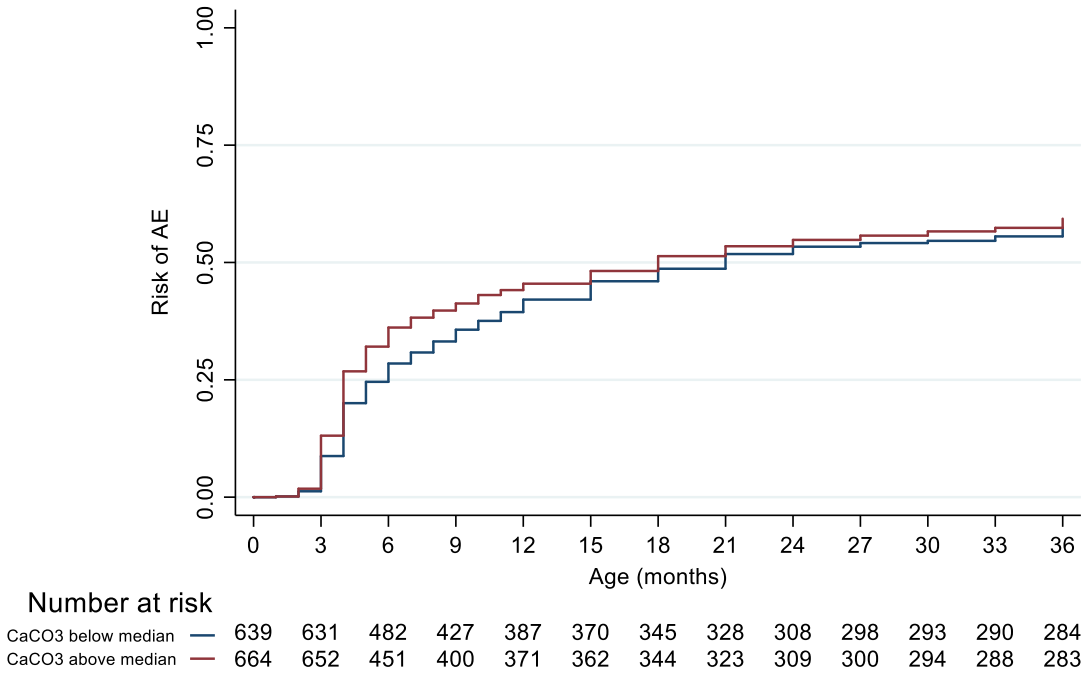

Figure 3.2 Kaplan-Meier plot of parent-reported eczema risk with exposure to harder versus softer water

Harder (>256 mg/L $CaCO_3$) versus softer water (≤256 mg/L $CaCO_3$); AE, atopic eczema; $CaCO_3$, calcium carbonate; $P_{logrank}$ = .20

3.3.3 Effect of *FLG* LOF mutation status on eczema risk

A total of 141/1,206 (11.9%) carried a *FLG* LOF mutation and, of those, 102 (72.3%) developed visible eczema on skin examination at one or more timepoints up until 36 months, compared to 413/988 (41.8%) of those with wild-type (WT) *FLG*. The risk of parent-reported eczema was also significantly increased in those with a *FLG* LOF mutation: HR 2.04 95% CI 1.64, 2.53 (P <.001) (Figure 3.3).

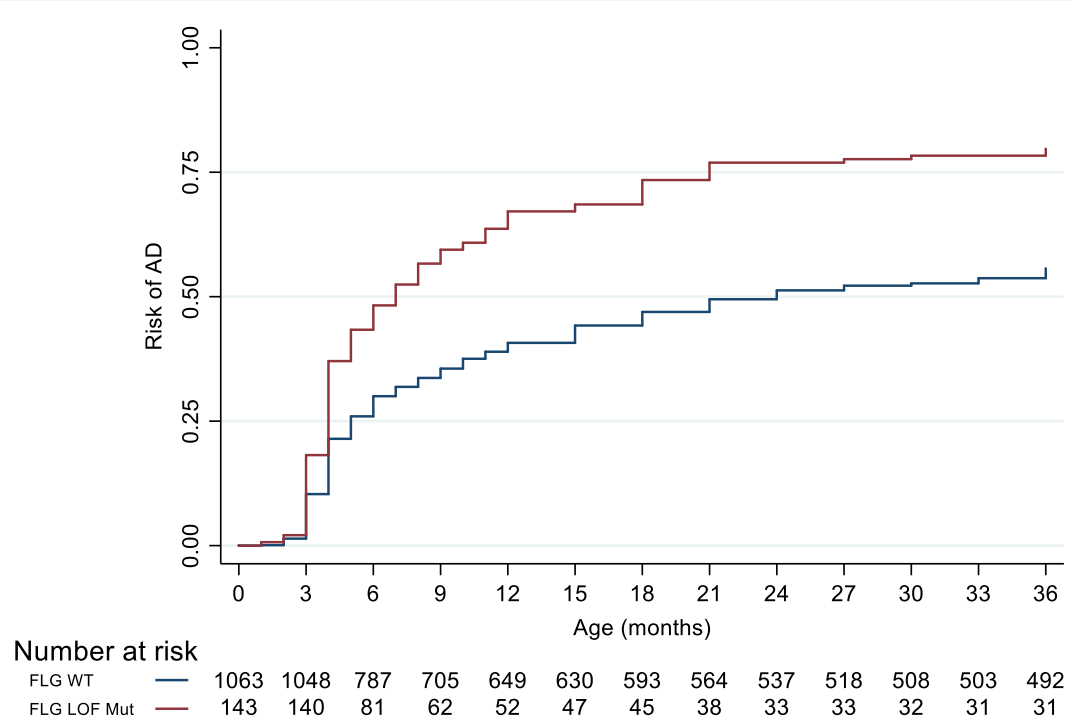

Figure 3.3 Gene-only effect of *filaggrin* loss-of-function mutations on risk of parent-reported eczema

Abbreviations: AD, eczema; *FLG*, *filaggrin*; HR, hazard ratio; LOF, loss-of-function

3.3.4 Effect of *FLG* LOF mutation status on risk of eczema with domestic hard water exposure

Stratified by *FLG* LOF mutation status, there was an increase in the cumulative prevalence of visible eczema at visits up to 36 months with higher quartiles of calcium carbonate water hardness exposure (Figure 3.4). There was also a higher risk of parent-reported eczema in those with *FLG* LOF mutations exposed to higher versus lower calcium carbonate levels at the various time points up to 36 months (Figure 3.5) ($P_{Log\text{-}rank} < .001$).

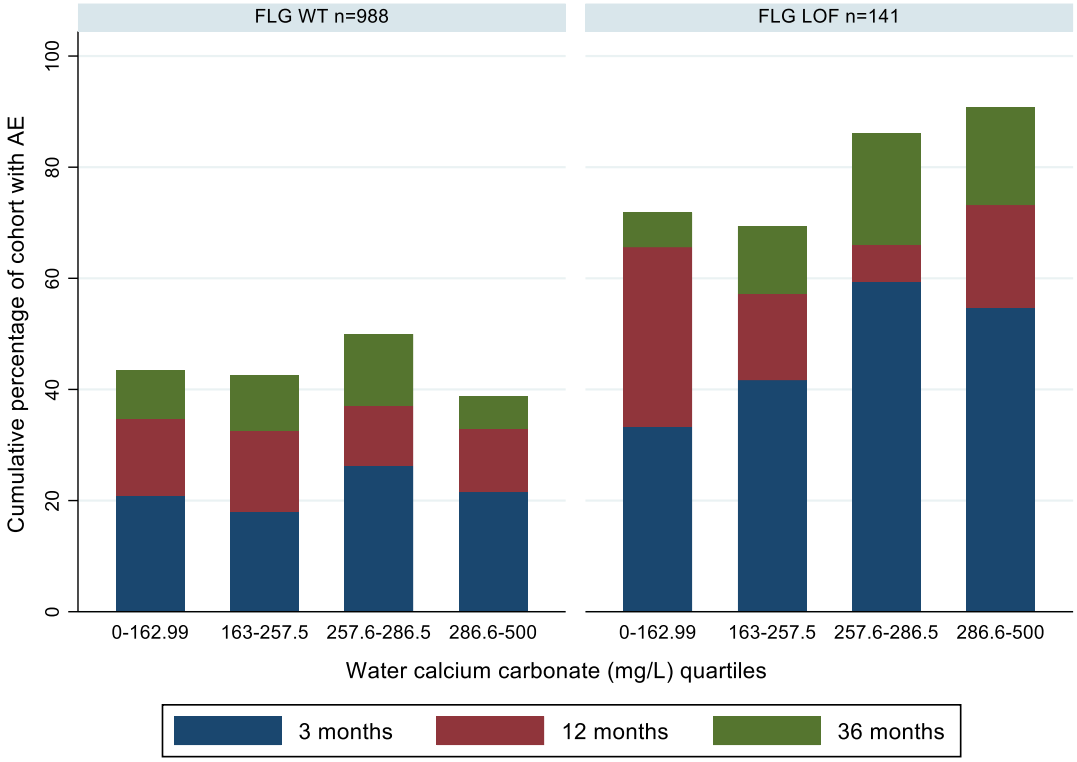

Figure 3.4 Cumulative prevalence of visible eczema at 3, 12 and 36 months stratified by water hardness exposure in infants with and without *filaggrin* loss-of-function mutations

AE, eczema; FLG, filaggrin, LOF, loss of function; WT, wild-type

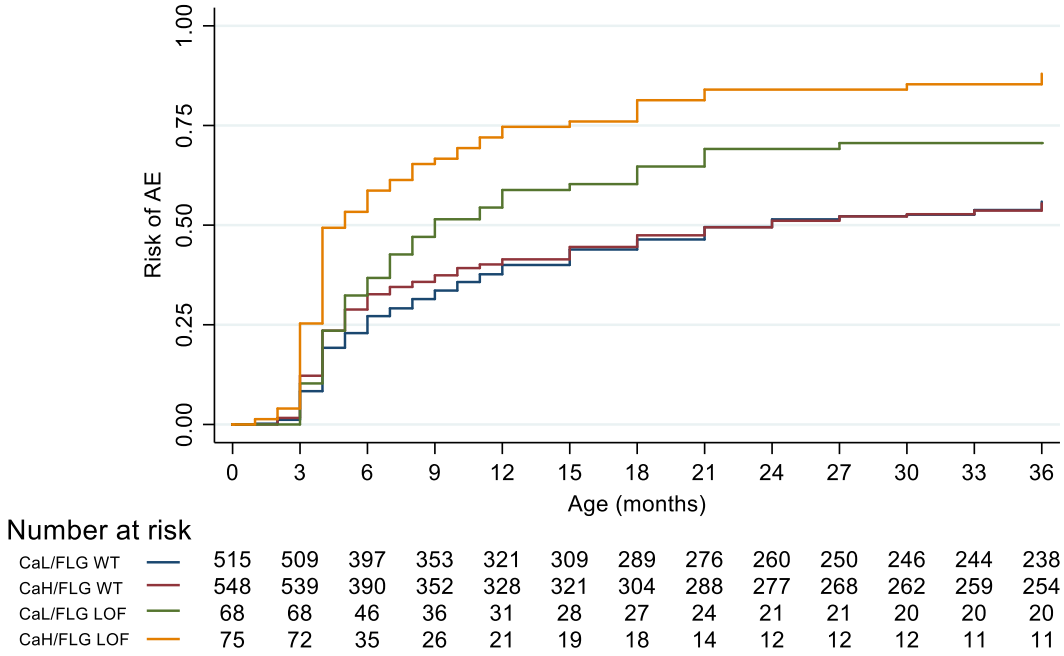

Figure 3.5 Kaplan-Meier plot of parent-reported eczema risk stratified by *filaggrin* status

AE, eczema; CaH, high calcium carbonate; CaL, low calcium carbonate; FLG, filaggrin; LOF, loss of function; WT, wild type

In the adjusted multivariable model there was a higher risk of eczema in those with *FLG* LOF mutations exposed to harder versus softer water (HR 2.72 95% CI 2.03, 3.66; Table 3.4), with a statistically significant multiplicative interaction term ($P_{Interaction}$= .008), which improved the fit of the model. This risk equates to a PAF of 23.2% in those with *FLG* and hard water co-exposure. Besides the interaction between water hardness and *FLG* LOF mutations, there were no significant interactions between water hardness and other key variables (Figure 3.6).

Table 3.4 Crude and adjusted hazard ratios for parent-reported eczema stratified by water hardness exposure (high/low) and *filaggrin* mutation status (yes/no)

*Adjusted for likely confounders (ethnicity (White/other), home location (urban/rural), index of multiple deprivation, water softener use)
Abbreviations: $CaCO_3$, calcium carbonate; CI, confidence interval; *FLG*, *filaggrin*; HR, hazard ratio; Ref, reference group

	Crude HR (95% CI) N=1,206	Adjusted* HR (95% CI) N=1,203	Interaction term with FLG N=1,203
Low CaCO3/FLG normal	1 (Ref)	1 (Ref)	
High CaCO3/FLG normal	1.03 (0.87, 1.22)	0.99 (0.84, 1.18)	P = 0.008
Low CaCO3/FLG mutation	1.54 (1.11, 2.12)	1.52 (1.10, 2.11)	
High CaCO3/FLG mutation	2.79 (2.09, 3.74)	2.72 (2.03, 3.66)	

*Adjusted for likely confounders (ethnicity (White/other), home location (urban/rural), index of multiple deprivation, water softener use)
Abbreviations: $CaCO_3$ – calcium carbonate; CI – confidence interval; *FLG* – *filaggrin*; HR – hazard ratio; Ref – reference group

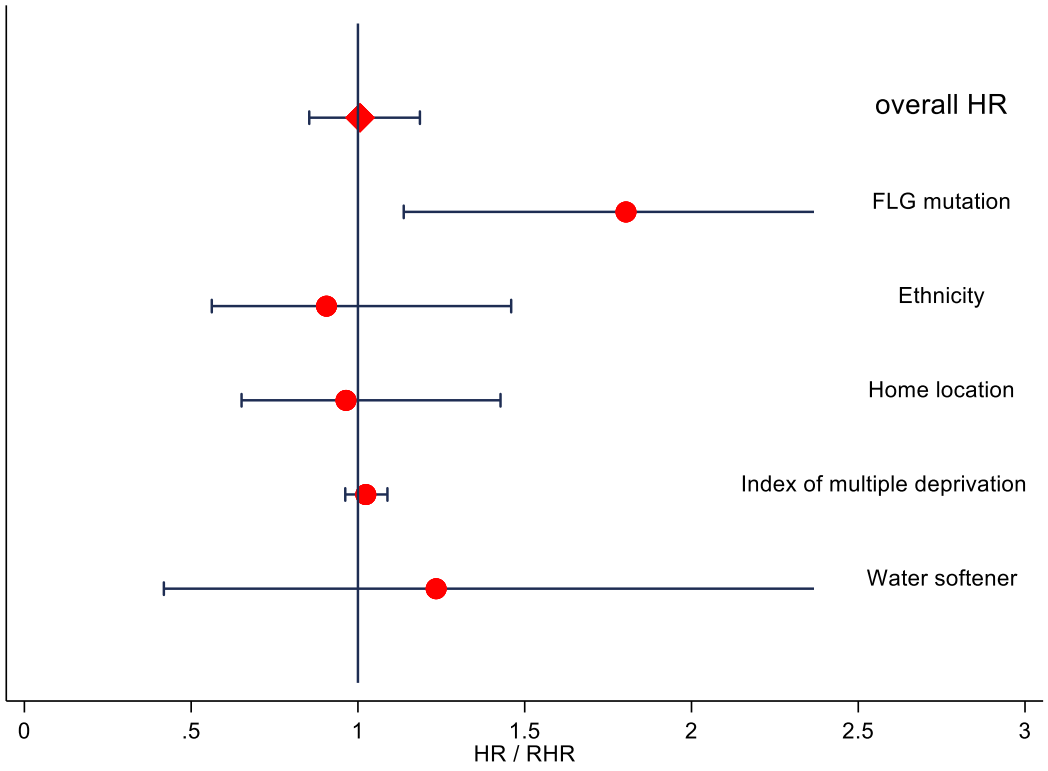

Figure 3.6 Forest plot summarising interactions of key variables with water hardness in relation to parent-reported eczema risk.

Hazard ratios are calculated using the Cox proportional hazards model and the ratio of hazard ratios of an interaction for the variables.
HR, hazard ratio; *FLG*, *filaggrin;* RHR, ratio of hazard ratios,

3.3.5 Transepidermal water loss and domestic hard water exposure

TEWL was measured in 1,300 infants (99.8%) at 3 months, and 1,103 infants (84.7%) at 12 months. Median TEWL levels increased overall by 0.94 g/m^2/h (IQR 5.29) between 3 and 12 months. Of those with TEWL measured, increased TEWL (≥15 g/m^2/h) was observed in 32.3% at three months and 38.4% at 12 months. Table 3.5 shows the effect of hard water on TEWL stratified by *FLG* LOF mutation status. In children without *FLG* LOF mutations, there was a higher proportion of infants with visible eczema and high TEWL in those exposed to harder versus softer water at 3 months (11.9% versus 9.1%, *P* =.138), however, this difference was greater in those with *FLG* LOF mutations (46.7% versus 23.5%, *P*= .004). The differences did not persist at 12 months (10.7% versus 11.1% and 29.0% versus 28.8%, respectively, *P* > 0.05 for both). Interestingly, in children with *FLG* LOF mutations there was a higher proportion (42.6% versus 22.7%, *P*=.011) of infants with neither visible eczema nor increased TEWL at 3 months (i.e. 'normal' skin) in those exposed to softer versus harder water. The difference was attenuated at 12 months (32.2% versus 24.6%, *P*=.340).

Table 3.5 Influence of water hardness (high/low) on transepidermal water loss and eczema prevalence by *filaggrin* mutation status

			No *FLG* mut			*FLG* mut		
Category	**Visible eczema**	**Increased TEWL**	**$CaCO_3$ High % (n)**	**$CaCO_3$ Low % (n)**	***P*-value**	**$CaCO_3$ High % (n)**	**$CaCO_3$ Low % (n)**	***P*-value**
	3 months							
	Yes	No	12.5 (68)	9.9 (51)	0.181	10.7 (8)	13.2 (9)	0.645
	Yes	Yes	11.9 (65)	9.1 (47)	0.138	46.7 (35)	23.5 (16)	**0.004**
	No	Yes	17.1 (93)	18.9 (97)	0.446	20.0 (15)	20.6 (14)	0.929
	No	No	58.5 (319)	62.1 (319)	0.232	22.7 (17)	42.6 (29)	**0.011**
Total, n			545	514		75	68	
	12 months							
	Yes	No	12.2 (56)	12.2 (54)	1.00	18.8 (13)	11.9 (7)	0.284
	Yes	Yes	10.7 (49)	11.1 (49)	0.847	29.0 (20)	28.8 (17)	0.980
	No	Yes	26.2 (120)	22.9 (101)	0.251	27.5 (19)	27.1 (16)	0.960
	No	No	50.9 (233)	53.7 (237)	0.401	24.6 (17)	32.2 (19)	0.340
Total, n			458	441		69	59	

Increased TEWL defined as ≥15 g/m^2/h. P-values based on two-sample tests of proportions.
$CaCO_3$, calcium carbonate; *FLG* mut, *filaggrin* mutation; TEWL, transepidermal water loss

There was no statistically significant relationship between water hardness and TEWL as a continuous outcome between 3 and 12 months: 0.00061 g/m^2/h per mg $CaCO_3$ (95% CI -.0018, .0030; $P = .619$). Within strata of *FLG*, there were also no significant correlations between $CaCO_3$ at 12 months and TEWL at 12 months (Figure 3.7). However, after adjustment for confounders and inclusion of an interaction term in the model between water hardness and *FLG*, there was a small statistically significant increase in TEWL of 0.0081 g/m^2/h per mg/L in association with higher $CaCO_3$ levels (95% CI 0.00028, 0.016, $P_{Interaction} = .042$, Figure 3.8).

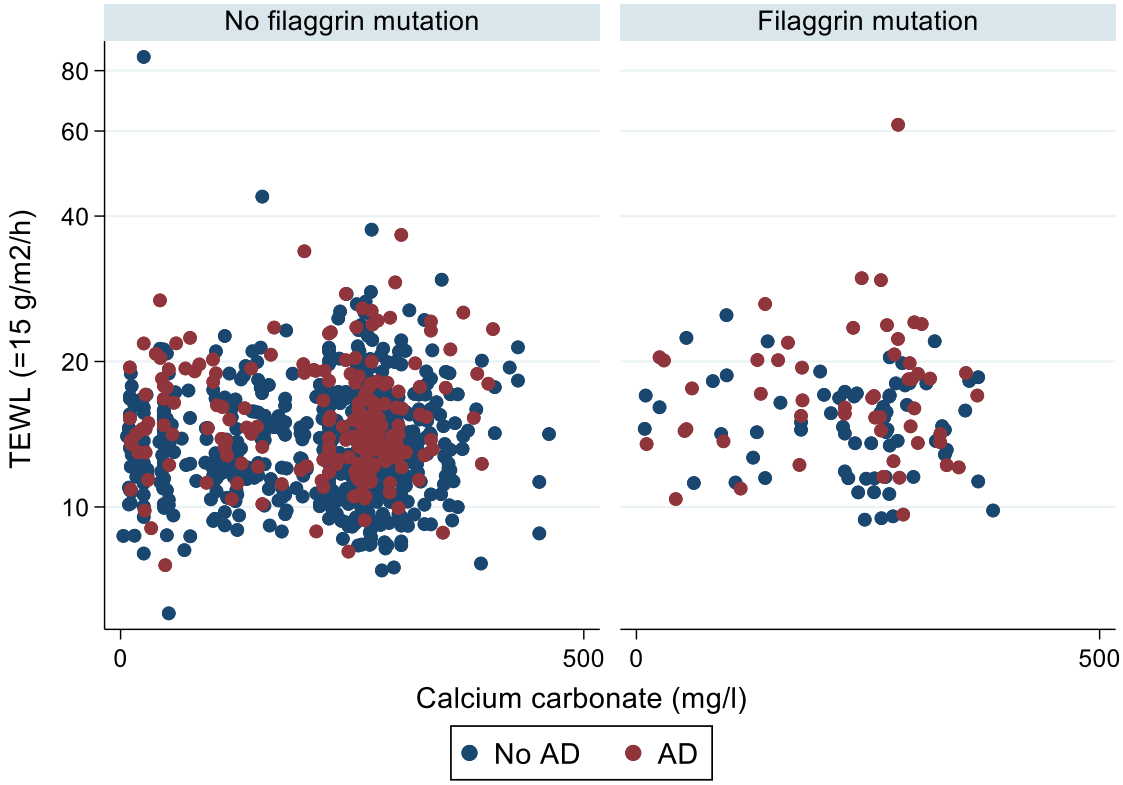

Figure 3.7 Scatterplots of transepidermal water loss at 12 months of age in infants with and without eczema, stratified by *filaggrin* mutation status

AD, eczema; TEWL, transepidermal water loss

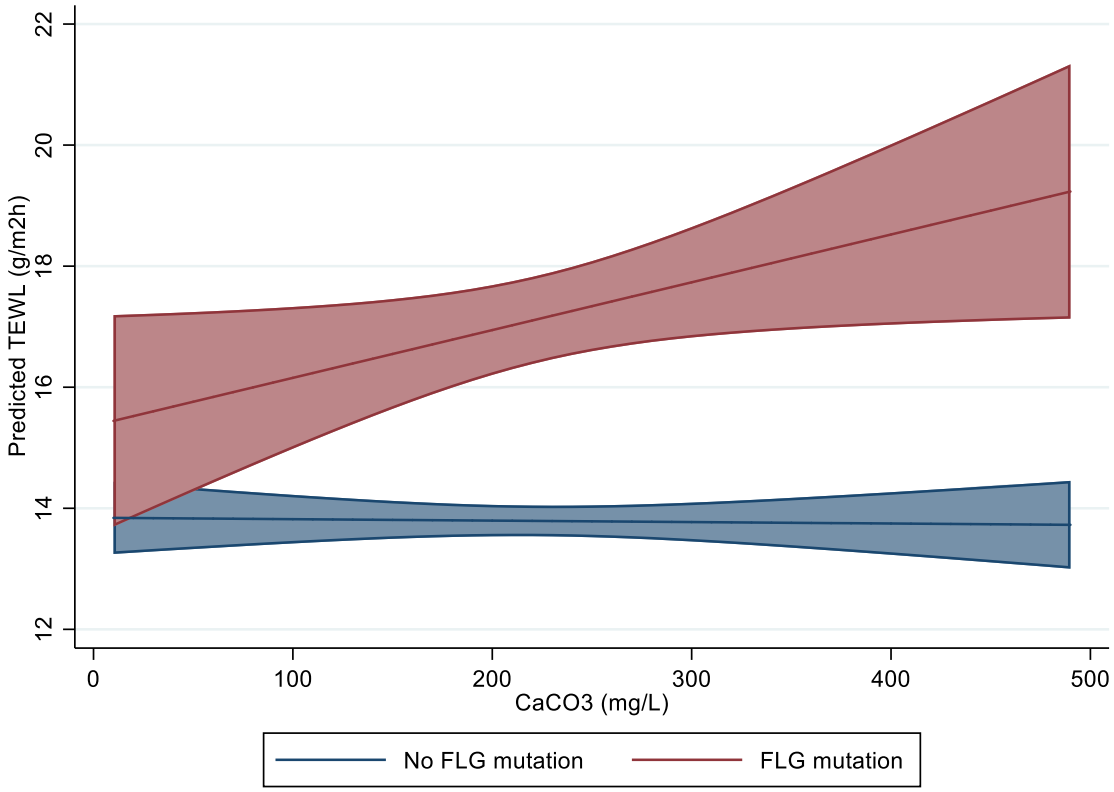

Figure 3.8 Modelled marginal effect of water calcium carbonate level and *filaggrin* mutation inheritance on predicted transepidermal water loss from 3 to 12 months of age

$CaCO_3$, calcium carbonate; *FLG*, *filaggrin*; TEWL, transepidermal water loss

3.4 Discussion

In this longitudinal analysis of the EAT study we found that infants with at least one *FLG* loss-of-function mutation exposed to harder water have a three-fold increased risk of developing eczema compared to infants with WT *FLG* exposed to softer water. This risk equates to a PAF of 3% overall with water hardness exposure and 23.2% in those with *FLG* and hard water co-exposure. Combined exposure to hard water and *FLG* mutations was also associated with a slight increase in skin barrier dysfunction, as measured by TEWL, between 3-12 months of age. These results support the growing body of evidence for the multifactorial aetiology of eczema, and provide a plausible insight into how a commonly encountered exposure, hard water, might interact with a genetically weakened skin barrier in early life to lead to further deterioration in skin barrier function, loss of epidermal water, and the initiation of eczematous skin inflammation.(78)

This was a hypothesis-driven analysis of a large, well-characterized cohort of children with comprehensive assessment of known confounders. Our findings are likely to be representative of the population in England and Wales as the study population was drawn from the general population. The measurement of *FLG* LOF mutation status and TEWL in the infants along with detailed phenotyping is a further strength. As in any observational study, there is the possibility of bias. The calculated estimates represent the true causal effect of water hardness on eczema risk, assuming no model misspecification and no unmeasured confounding. The model diagnostics suggest good model specification. There may also be misclassification of the exposure as water hardness was measured just at baseline, and we did not formally capture if participants moved. However, the moves that we were aware of occurred locally, and this effect would not be expected to happen in a differential way and should therefore not lead to biased estimates.

A high proportion (58%) of infants developed parent-reported, doctor-diagnosed eczema by 36 months of age, raising the possibility of over-reporting of the outcome. However, a sensitivity analysis using just investigator-assessed visible eczema as the outcome, rather than the composite outcome of parent-reported, doctor-diagnosed eczema, yielded similar risk estimates (adjusted HR 2.57 95% 1.91, 3.46). As the dataset was from a randomized trial, we examined the effect of including randomization group in the model, which did not appreciably alter the risk estimates.

The lack of a longitudinal statistically significant association between water hardness and eczema overall is in keeping with the results of the Spanish birth cohort study by Font-Ribera *et al*.,(63) which showed no relationship between water hardness and eczema at 14 months and 4 years of age. The overall population attributable fraction of 3% in our EAT study cohort is consistent with the value (2%) reported recently by Engebretsen et al.(54) in their analysis of a Danish cohort, but the additional contribution of *FLG* LOF mutations was not examined in the Danish population. The significant interaction between *FLG* LOF mutations and water hardness we found longitudinally is consistent with the trend towards an association observed in our previous cross-sectional analysis.

The heterogeneity of observational study results may reflect differences in study design and the range of water hardness exposure, population age and ethnicity, definitions of eczema, and the changing impact of hard water on eczema risk over time, seen with maturation of the skin of the growing child. For instance, the 90th percentile of $CaCO_3$ in the Miyake et al. study from Japan was 76 mg/L.(65) This would be considered soft water in the UK context.

The observed effect of hard water on skin barrier function is consistent with prior mechanistic work conducted using washing experiments in adults with and without *FLG* LOF mutation, which demonstrated that hard water leads to an increase in skin barrier dysfunction (raised TEWL and erythema), partly mediated by an increase in SLS deposition on the skin.(27) The relationship between water hardness and TEWL is stronger at three months than 12 months of age, suggesting the deleterious effects of hard water on skin barrier function might lessen over time with stratum corneum maturation.
Based on our longitudinal population-based analysis of a carefully phenotyped UK population, domestic hard water is an important risk factor for the development of eczema in infants aged 3-36 months who have a *FLG* loss of function mutation.

4 PILOT TRIAL OF WATER SOFTENERS FOR THE PREVENTION OF ECZEMA IN HIGH-RISK NEONATES

4.1 Introduction

4.1.1 Background & Rationale

The systematic review reported in Chapter 2 identified increased odds of eczema in non-infant children living in harder versus softer water areas with an OR of 1.27 (95% 1.03, 1.57). The review also confirmed a gap in randomised controlled trial evidence on the use of water softeners for the prevention of eczema. Given the high burden of eczema, being able to prevent the disease using a simple, non-invasive low-risk intervention such as a domestic water softener is very appealing. Randomised controlled trial (RCT) data are considered the highest level of evidence as they provide unbiased estimates of causal effects through control of known and unknown confounders in the study design itself.(79) The epidemiological analysis conducted in the EAT cohort, reported in Chapter 3, found that overall, there was no statistically significant association between living in a hard water area and developing atopic eczema by 36 months of age after adjusting for known confounders. However, there was evidence of an increased risk of atopic eczema with hard water exposure in infants with *FLG* loss-of-function mutations. This suggested that the prevention RCT should focus on high-risk individuals, i.e. to select for those with *FLG* loss-of-function mutations, who may gain the most from avoiding hard water exposure.

4.1.2 Trials of water softeners in atopic eczema

The multicentre Softened Water Eczema Trial (SWET), completed in 2011, examined the role of water softeners in treating children with established, moderate to severe eczema and found no overall benefit in terms of eczema severity reduction (26). However, the factors that drive the development of eczema may not be the same as those that determine disease exacerbations and severity and the negative result in the SWET trial therefore does not exclude a role for water softeners in prevention trials. Early life is likely to be an important time in the development of eczema, particularly as most eczema develops before 5 years of age (80). Early interactions between genes and the environment may be

crucial in instigating the cycle of inflammation and skin barrier dysfunction seen in eczema. Indeed, skin barrier dysfunction at just 1 week of age, as measured by transepidermal water loss (TEWL), is a predictor of subsequent eczema risk.(81) A small pilot randomised controlled double-blind cross-over trial of 12 patients aged 3–6 years with mild to moderate eczema compared ultrapure soft water with tap water. After 6 weeks, no statistically significant differences in Eczema Area and Severity Index (EASI) or TEWL were observed between the groups, although there was a statistically significant improvement in pruritus as measured by visual analogue score (−2.10, 95% CI −4.14 to -0.063) (36). To date, there are no published studies examining the role of water softeners in the prevention of eczema (38).

4.1.3 Rationale

The overall rationale was that by installing a domestic water softener around the time of birth, infants would be exposed to softened water rather than hard water for bathing and that this would be less irritating to the skin than hard water and so associated with a lower risk of eczema development.

This pilot trial built on the experience gained from the National Institute for Health Research (NIHR)-funded Softened Water Eczema Trial (SWET) (26) and a trial of emollients in early life, the Barrier Enhancement for Eczema Prevention, (BEEP) trial.(82) This was a pilot trial, as defined by the UK NIHR in that it was a ‘version of the main study run in miniature to test whether the components of the main study can all work together.’(83) This pilot trial was therefore not designed to definitively answer the question of whether installation of a domestic water softener will prevent eczema. The rationale for performing this pilot study was that a definitive trial on eczema prevention using domestic water softening devices would require a much larger number of participants and so prior to embarking on a larger multicentre trial it would be important to determine whether the planned trial recruitment and assessment procedures are possible and workable, or whether they require adapting or changing.(84)

4.1.4 Trial objectives

The aim of this pilot trial was to determine the feasibility of undertaking a large-scale definitive trial to determine whether installation of domestic ion-exchange water softeners around the time of birth reduces the risk of high-risk children developing eczema. A further aim was to explore the pathophysiological mechanisms for this in an embedded mechanistic study.

4.2 Methods

4.2.1 Trial design

This was a multi-centre parallel-group assessor-blinded randomised (1:1) controlled pilot trial of an ion-exchange water softener for the prevention of eczema in neonates at high risk of developing eczema, with an embedded mechanistic study.

The study recruited pregnant women living in hard water areas ($CaCO_3$ >250 mg/L) identified from antenatal services at two public hospitals in London, UK: a teaching hospital with secondary and tertiary care maternity services located in urban central London; and a community hospital with secondary care maternity services serving a mixed urban and rural area in south-west London. Participants were recruited between February 2018 and October 2019. Participants with a domestic water softener already installed were not eligible. Infants needed to be born at term (≥37 weeks' gestation) and have a parent or sibling with a history of doctor-diagnosed atopy (eczema, asthma or hay fever) and were excluded if they had a significant inflammatory skin disease at birth that would make the detection and assessment of eczema difficult, or any other serious health issue that would interfere with their ability to participate in the study.

Pregnant women were randomised prior to delivery to one of two groups in a 1:1 ratio:

- Control group: usual domestic hard water supply.
- Intervention group: softened domestic water through installation of an ion-exchange water softener.

The study design is summarised as a flow chart in Figure 4.1.

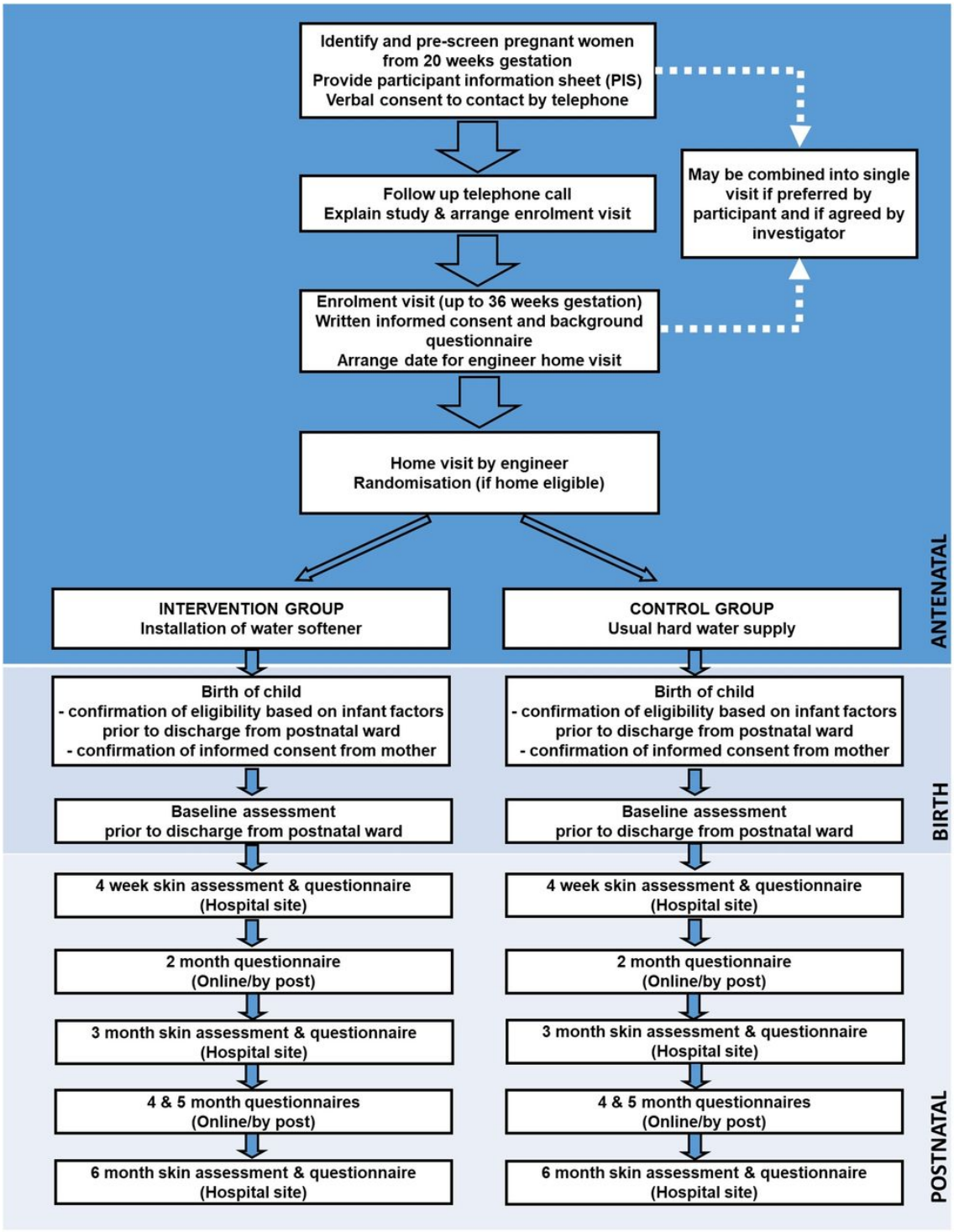

Figure 4.1 Study flow chart

4.2.2 Patient involvement

Patients were involved in the design of this study. Members of the Patient Panel at the Centre of Evidence-Based Dermatology reviewed the participant material and provided

feedback on the online questionnaires. During the trial, a parent of a child with eczema joined the independent Trial Steering Committee.

4.2.3 Trial population

The study recruited pregnant women living in hard water areas who were identified from antenatal services at local National Health Service Trust sites. Women were approached at the 20-week anomaly scan and asked if they were interested in participating. The study was also publicised through posters and by making clinical midwifery teams aware of the study. Full inclusion and exclusion criteria are given in Table 4.1.

Table 4.1 Inclusion and exclusion criteria

Inclusion criteria		
	Antenatal-maternal	
	1	History of doctor-diagnosed atopy (atopic eczema, asthma or hay fever) in the woman, her partner or other child the couple have parented
	2	Woman aged 18 years or older
	3	Woman able to understand English
	4	Lives in a hard water area (>250 mg/L calcium carbonate)
	5	Lives in a property suitable to have a water softener fitted
	6	If in a rented property – agrees to seek consent of landlord for fitting of water softener device
	7	Agrees to have water softener +/- additional tap for drinking water fitted at home
	8	Agrees to researchers accessing pregnancy and pregnancy outcome data for the mother and child
	9	Able and willing to give informed consent
	Postnatal-maternal	
	10	Maternal consent for her neonate to participate in the study
	Exclusion criteria	
	11	Preterm birth (defined as birth prior to 37 weeks gestation)
	12	Significant inflammatory skin disease at birth not including seborrheic dermatitis (“cradle cap”)

13	Sibling (including twin) previously randomised to this trial. If multiple birth, the first child will be randomised into the trial.
14	Any immunodeficiency disorder or severe genetic skin disorder
15	Child has any other serious health issue which, at parent or investigator discretion, would make it difficult for the family to take part in the trial.
16	Planned stays away from home for a continuous period of more than 2 weeks or a total of 1 month out of the 6 month follow up period
17	Water softening or filtration device already installed
18	Concurrent enrolment in any other skin-related intervention study
19	Other medical condition that in the opinion of the Chief Investigator could interfere with the conduct of the trial

4.2.4 Intervention

A domestic ion-exchange water softener was installed in the homes of participants randomised to the intervention group after enrolment and before the child's birth. Ion-exchange water softeners exchange calcium and magnesium, among other divalent cations, for monovalent sodium cations, typically reducing downstream water hardness to close to zero. The sodium ions came from common salt, which needed to be topped up every 3–4 weeks. The water softeners used in this trial were supplied and funded by Harvey Water Softeners, Woking, UK. Standard procedure was to soften all water in the home except the drinking water tap, as the water softening process exchanges calcium with sodium ions and will therefore increase the water sodium concentration, making the water unsuitable for drinking purposes. Unsoftened mains drinking water was delivered through the existing kitchen tap wherever possible, or otherwise through an extra (faucet-style) tap installed at the side of the kitchen sink. At the end of the study, all participants were given the option to purchase the water softener from Harvey Water Softeners at a reduced price of £399.00 inclusive of value-added tax, installation and warranty; this is approximately a quarter of the full retail price of £1678.80. Alternatively, the water softener was removed.

4.2.5 Potential risks

This was a low-risk trial as the intervention involves a commercially available ion-exchange water softener that has no known clinical side effects. There was a potential, but low risk, of damage to participants' properties during installation of the water softening unit. To reduce the risk of damage, the water softeners were installed by qualified water engineers according to the Code of Practice produced by British Water and in accordance with the recommendations of the Water Regulations Advisory Service.

4.2.6 Concomitant medications and skincare

No restrictions were placed on the use of concomitant treatment a child could receive. All concomitant medications were recorded at baseline and updated at follow-up visits. Given the lack of a consistent approach to neonatal skincare, and the possible interaction between some wash products and hard water, we did not provide specific skincare advice to participants. However, data on such use was sought from parents via monthly online questionnaires.

4.2.7 Co-enrolment guidelines

To avoid potentially confounding issues, neonates were not to be recruited into other prevention of eczema or allergy intervention trials.

4.2.8 Participant compliance

Compliance with treatment was not expected to represent a large problem for this trial if the participants were not absent from home for long periods of time and remembered to replenish the salt. Reminders about salt replenishment were sent. To check that the units were working correctly, participants were asked to send weekly water samples to Harvey Water Softeners using prepaid envelopes. Any samples with a reading of >20 mg/L calcium carbonate were referred to the water softener engineer for investigation. If participants moved home during the trial, attempts were made to reinstall the device in the new property. It was anticipated that loss to follow-up would be <15%.

4.2.9 Primary outcome

The primary purpose of this study was to determine the feasibility and acceptability of installing a water softener prior to birth of the baby to inform the design of a definitive multi-centre prevention RCT. The primary endpoint was the proportion of eligible families screened who were willing and able to be randomised.

4.2.10 Secondary feasibility outcomes

The secondary objectives were designed to further facilitate the design of a larger, definitive multicentre RCT. Namely, to determine (proportion, unless stated):

1. Pregnant women approached who agree to be screened.
2. Families eligible on screening that cannot have a water softener installed (eg, due to landlord or local authority refusal, technical (plumbing) reasons).
3. Families randomised that withdraw due to infant ineligibility.
4. Families in intervention arm who found the intervention acceptable.
5. Participants in control arm who become exposed to softened water (eg, by moving to a new home in a soft water area or moving to a home with an active water softener installed, before the end of follow-up).
6. Participants that have the water softening unit removed or disabled prior to end of follow-up.
7. Participants with visible eczema status (yes/no) recorded at each time point: baseline, 4 weeks, 3 and 6 months.
8. Water samples with hardness >20 mg/L calcium carbonate in the intervention arm.
9. Participants who withdraw from the trial prior to end of follow-up.
10. Median number of nights spent away from the participant's main home during follow-up.
11. Clinical outcome assessments that have remained blinded at 4 weeks, 3 and 6 months.

4.2.11 Secondary-clinical outcomes

1. Proportion with patient-reported, doctor-diagnosed atopic eczema by 6 months of age.
2. Proportion with visible eczema according to the UK diagnostic criteria-based photographic protocol (85) (Appendix 7.6.2)
3. Severity of eczema (if present) using the Eczema Area and Severity Index (EASI) (86) (Appendix 7.6.1)
4. Patient-reported eczema symptoms (Patient-Oriented Eczema Measure) score (87) (Appendix 7.6.3)
5. Time to onset of patient-reported doctor-diagnosed eczema.

4.2.12 Additional mechanistic outcomes

The following were assessed as change from baseline until follow-up at 6 months in the intervention compared with the control group:

1. Transepidermal Water Loss (TEWL).
2. Cutaneous cytokine profiles (eg, interleukin-1 (IL-1) levels).
3. Natural moisturising factor levels.
4. Shannon Diversity Index and other skin and upper respiratory microbiota parameters.
5. Median domestic water hardness level (calcium carbonate concentration).
6. Skin surface hydration.

4.2.13 Adverse events

This trial involved the use of a commonly available domestic water softening unit with provision for separate mains drinking water during the time when the water softening unit was installed. Accordingly, we did not anticipate any adverse events or adverse reactions related to the trial intervention. A contact number for the service department at Harvey Water Softeners was provided and an engineer was sent to resolve the issue, if needed. Details of technical issues reported to the service department were reported to the study principal investigators. Events of relevance such as plumbing difficulties, floods or

difficulties with the units were to be logged and reported to the Research Ethics Committee and relevant Research and Development departments annually.

4.2.14 Sample size

This was a pilot study and therefore not powered to establish the efficacy of the intervention. A total of 80 families (40 per group) was judged to provide a sufficiently precise (within 10 percentage points for a 95% CI) estimate of the proportion of families who are willing and able to be randomised.

Findings from the Enquiring About Tolerance (EAT) study(24) and the BEEP feasibility study(88) allowed us to make a conservative estimate that approximately 70% of families screened will have a history of atopy that predisposes to a high risk of eczema in their offspring. Of these, 40%–60% were expected to be willing and able to participate. Home factors also needed to be considered: in SWET, 27% of eligible families could not participate because their home was not suitable for installation.(26)

4.2.15 Informed consent

Written informed antenatal consent was obtained at the enrolment visit from the mother. The consent form was to be signed and dated before entering the trial and participants were reminded that they could withdraw from the trial at any time. Mothers were reconsented postnatally to ensure they agreed to their child taking part in the study.

4.2.16 Randomisation, blinding and allocation concealment

Participants were randomised antenatally at the time of the engineer home visit to receive either a water softener or their usual water supply, once:

- Antenatal eligibility criteria were fulfilled.
- Fully informed written consent was obtained.
- The engineer was satisfied that the softener could be installed.

Participants were randomised in a 1:1 ratio to one of the two treatment arms based on a computer-generated code. The independent online randomisation service was provided by Guy's and St Thomas' Biomedical Research Centre (BRC) and used the MedSciNet

database system (MedSciNet AB, Stockholm, Sweden). When a patient was recruited, an independent BRC administrator who was not involved in patient assessment, obtained the allocation from the online system. This information was relayed by telephone to the water softener installation engineer, so they knew whether to install a softener or not in that participant's home.

The randomisation result was relayed to the installation engineer by telephone as either an 'INSTALL' or 'DO NOT INSTALL' instruction. The randomisation service was provided by researchers at King's College London who were not involved in the study, via telephone.

Experience from the SWET had shown that the effects of a functional water softener are too noticeable to allow participants to be blinded (26). Skin examinations and measurements were performed by research team members who were blinded to treatment allocation. Participants were encouraged not to disclose allocation. Results were analysed according to a predefined statistical analysis plan (SAP). Study team members in direct contact with study participants were trained on the study protocol and the importance of demonstrating equipoise.

4.2.17 Visit schedule and study procedures

The schedule for assessments during the study is shown in

Table 4.2.

Table 4.2 Schedule of study assessments and procedures

	Screening#	Enrolment#	Home screen & installation	Birth	Baseline (+/- 1 wk)	4 wk (+/- 1 wk)	3 m (+/- 2 wk)	6 m (+/- 2 wk)
Confirm eligibility	X	X			X			
Verbal consent to collect contact details and access antenatal records	X							
Written informed consent		X			X			
Demographic data	X				X			
Engineer home assessment			X					
Install water softener			X					
Randomisation			X					
Visible eczema status						X	X	X
Blinded eczema severity assessment (EASI)						X	X	X
DNA collection from buccal swab						X		
Antenatal factors questionnaire		X						
Acceptability & feedback questionnaire								X
Invite to participate in semi-structured interview about study								X
Collection of skin and nasal microbiome swabs					X	X	X	X
TEWL measurement‡					X	X	X	X
Cutaneous tape stripping‡					X	X	X	X
Skin pH measurement‡					X	X	X	X
ATR-FTIR measurement‡					X	X	X	X
Skin surface hydration‡					X	X	X	X
Monthly infant skin and health* questionnaire, including Patient Orientated Eczema Measure (POEM) score						From 4 weeks to 6 months of age		
Weekly water samples (in intervention arm)			From installation to 6 months of age					

ATR-FTIR, attenuated total reflectance-Fourier transform infra red ; EASI, eczema area and severity index; POEM, patient—oriented eczema measure; TEWL, transepidermal water loss; wk, week

*Any other concomitant illnesses that developed during the study including episodes of respiratory, gastrointestinal and other acute illnesses. #Screening and enrolment may occur at the same visit if participant prefers & if investigator agrees. ‡At Guy's & St Thomas' Hospital site only.

4.2.17.1 Enrolment visit (up until 36 weeks' gestation)

- Confirmed eligibility.
- Answered questions about study.
- Written consent was obtained.
- Antenatal history solicited (including antibiotic exposure, probiotic and omega 3 use), family medical history and environment questionnaire.
- Water softener installation arranged.

The enrolment visit could occur at the same time as the screening visit, if preferred by the participant and deemed appropriate by the investigator.

4.2.17.2 Water softener engineer home visit (up until 40 weeks' gestation)

- Assessment by the engineer to check the home's suitability for water softener installation.
- If home was eligible, the engineer telephoned a given number at King's College London to determine the randomisation group.
- Water softener installed and participants provided with water sample materials (if randomised to the intervention arm).

4.2.17.3 Baseline visit (on postnatal ward or within 1 week of birth)

- Confirmed infant eligibility criteria.
- Postnatal written consent was obtained.
- Skin examination.
- Infant skincare questions.
- Recorded concomitant infant medications including systemic antibiotic use.
- Systemic antibiotic use in mother during pregnancy, including prophylactic antibiotic use during delivery.
- Infant comorbidities.
- Topical medication use in infant.
- Delivery questions.
- Pregnancy outcomes and birth details.

- Additional mechanistic assessments (procedures marked ‡ were performed only at the Guy's and St Thomas' Hospital site):
    - Tape stripping (forearm).
    - TEWL measurement (forearm)‡.
    - Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy measurement (forearm)‡.
    - Skin pH measurement (forearm)‡.
    - Skin surface hydration (forearm) ‡.
    - Microbiome swabs from skin (antecubital fossa and cheek) and nares.

4.2.17.4 Four-week visit (±1 week)

- As per baseline, plus optional buccal swab for DNA for *FLG* mutation analyses.

4.2.17.5 Three-month visit (±2 weeks)

- As per baseline, plus optional buccal swab for DNA for *FLG* mutation analyses (if unable to collect at 4-week visit).

4.2.17.6 Six-month visit (±2 weeks)

- As per baseline, plus:
- Acceptability questionnaire.
- Optional buccal swab for DNA for *FLG* mutation analyses (if unable to collect at 3-month visit).
- Arranged date for removal of water softener within 1 month if not planning to purchase (intervention group) or installation of water softener for 6 months period, if desired, in control arm.
- Invited to participate in semi-structured qualitative interview about study (via telephone or in person) and seek verbal consent for this.

4.2.17.7 Monthly email messages (intervention group)

- Reminded participants to complete questionnaires.
- Reminded participants to refill the unit with salt.

- Invited participants to contact Harvey Water Softeners for any issues with water softener.

4.2.17.8 Monthly email messages (control group)

- Reminded participants to complete questionnaires.

4.2.18 Monthly electronic questionnaires

A secure web-based questionnaire link using the Snap Surveys platform was emailed to participants' parents monthly to determine whether the child has received a diagnosis of eczema from a healthcare professional and to check current skincare and hygiene and confirm residence/time away from main residence and infant general health. A paper copy of the questionnaire could be posted to participants if they preferred, or if they did not have internet access.

At the end of the study, that is, at the 6-month visit, a date was arranged for removal of the water softener within 1 month if the participating family did not want to purchase the device (intervention group). Control arm participants could also purchase the water softener at the end of the study. Participants were also invited to participate in a semi-structured qualitative interview about the study.

4.2.19 Embedded mechanistic sub-study

The embedded mechanistic sub-study was intended to help elucidate the mechanisms by which softened water might affect skin barrier function and therefore eczema risk. It was also an opportunity to provide more general insights into the pathogenesis of eczema in young infants with resulting opportunities for hypothesis generation.

TEWL provides a standardised measure of skin barrier dysfunction (89) and there is evidence to suggest that elevated TEWL in neonates is predictive of subsequent eczema.(81) The AquaFlux AF200 condensing chamber probe (Biox Systems, London, UK) was used to measure TEWL. Epidermal stratum corneum hydration was measured using a CM 825 Corneometer (Courage and Khazaka, Cologne, Germany).

Skin surface pH has an important role in the regulation of epidermal and microbial cellular process and was measured using a PH905 Skin-Surface-pH probe fitted with a Mettler and Toledo flat surface electrode (Courage and Khazaka).(90)

Additional mechanistic outcomes were also evaluated in a sub-study and the analyses of these will be reported separately.

4.2.20 Statistical analysis

The feasibility parameters of this pilot trial were not formally statistically tested. The focus was therefore on descriptive statistics and CIs. A Statistical Analysis Plan (SAP) for the feasibly trial was developed prior to unblinding and any analysis of the data. The SAP can be found in Appendix 7.7.

Randomisation was done in a 1:1 ratio using randomly permuted blocks. Participants' data were analysed on an intention-to-treat basis. There was no hypothesis testing for the clinical outcomes. Results were presented as estimates with 95% CIs, where appropriate. Confidence intervals for proportions were calculated using Stata version 15 (StataCorp, College Station, TX, USA) using the *proportion* command using the logit method.(91)

4.3 RESULTS

Baseline characteristics are given in Table 4.3 and were mostly well-balanced between the randomised groups. The mean overall level of water hardness at baseline was 272 mg/L CaCO3 and was similar in the two arms. Levels of parental atopy were also similar by study arm. A higher proportion of female infants was seen in the water softener arm. Just over half of all participants lived in flats and 93% lived in urban locations.

Table 4.3 Baseline characteristics of the infant trial population

Characteristic of the infant		Water softener % (n/N)	Usual hard water % (n/N)
Number in group		40	40
Sex:	Female	64 (25/39)	47 (18/38)
	Male	36 (14/39)	53 (20/38)
Ethnicity:	White British	33 (13/39)	34 (13/38)

	Other White	15 (6/39)	18 (7/38)
	White and Asian	18 (7/39)	16 (6/38)
	Other Mixed	15 (6/39)	13 (5/38)
	Chinese	10 (4/39)	5 (2/38)
	Other	8 (3/39)	13 (5/38)
Birth history			
Birth weight, kg, mean (SD)		3.5 (0.51) (n=37)	3.4 (0.40) (n=35)
Mode of delivery:	Vaginal	67 (26/39)	74 (28/38)
	C-section	33 (13/39)	26 (10/38)
Born in a bathing pool		0 (0/26)	0 (0/28)
Maternal antibiotic exposure during pregnancy		62 (23/37)	46 (17/37)
Family atopy status (self-reported)			
Maternal			
Eczema		45 (18/40)	45 (18/40)
Atopy*		80 (32/40)	82 (33/40)
Paternal			
Eczema		37 (14/38)	26 (10/39)
Atopy*		61 (23/38)	67 (26/39)
Home environment			
Property type:	House	38 (15/40)	45 (18/40)
	Flat	62 (25/40)	55 (22/40)
Home location type:	Urban	92 (37/40)	92 (37/40)
	Rural non-farm	8 (3/40)	8 (3/40)
Domestic water $CaCO_3$ mg/L, median (IQR)		265.5 (259, 280.5)	266 (259, 273)
Skin physiological parameters			
Skin surface hydration, arbitrary units, median (IQR)		15.3 (11.2, 22.7) (n=31)	15.9 (12.4, 17.8) (n=29)
Transepidermal water loss, $g \cdot m^{-2} \cdot h^{-1}$ median (IQR)		13.1 (10.9, 14.6) (n=27)	14.0 (11.2, 16.1) (n=27)
Skin pH, mean (SD)		5.7 (0.6) (n=30)	5.9 (0.7) (n=28)

$CaCO_3$, calcium carbonate; IQR, interquartile range, SD, standard deviation

*Atopy defined as a history of eczema, asthma or hay fever

4.3.1 Feasibility endpoints

A total of 500 pregnant women were approached who expressed an interest in the study and were pre-screened, of which 231 (46%, 95% CI 42, 51%) were potentially eligible. Of those potentially eligible on pre-screening, 154 agreed to consider the study further and had a mean gestation of 31 weeks (SD 6 weeks). 152 women then signed an informed consent form at an enrolment visit, of these 149 women were fully eligible for the study. Of the 149 eligible women, 80 (54%, 95% CI: 45, 62%) were randomized, the primary endpoint of the study. The most common reason for ineligibility on pre-screening was no history of atopy (49%). The Consolidated Standards of Reporting Trials (CONSORT) flow diagram adapted for pilot and feasibility trials is shown in Figure 4.2.(37, 92) Of the 69 confirmed as eligible, but who were not subsequently randomised, 36 (52%) had a home that was not suitable for installation of a water softener due to technical (plumbing) reasons. 11 (16%) participants gave birth before the installation visit could take place. Four (5.8%) were unable to have a water softener installed due to landlord or local authority refusal or subsequently discovered technical problems. Of those randomised, two were immediately lost in each group: one in the water softener arm where the device could not be installed, and one in the hard water control arm who withdrew, leaving 39 in each arm of the trial (78 participants). No participants in the intervention arm had the water softening unit removed before the end of follow-up.

Potential contamination of the intervention, based on the mean (SD) number of nights spent away from the main residence in the 6 months of follow up, was 12 (12) and was similar in both groups (Table 4.4).

Out of 708 analysed water samples received from the 39 participants in the intervention arm, 56 samples (7.9%) were above 20 mg/L CaCO3. Sixteen participants (41%) had at least 1 water sample with increased water hardness levels (>20 mg/L CaCO3). No faults were found with the units, other than a lack of salt. One participant in the intervention arm experienced water hardness exposure >100mg/L (104.5 mg/L CaCO3).

By 6 months postpartum, 4 participants in the water softener arm and 8 in the control arm were lost to follow-up or withdrew (15% attrition). A total of 69/78 (88%) families completed the study acceptability questionnaire, all of whom reported that they found the study acceptable and 67/69 (97%) said that they would take part in the same study again.

Table 4.4 Feasibility outcomes

Outcome	Estimate n/N
Proportion of eligible families screened who are willing and able to be randomised (95% CI)	55% (47, 63%) (80/146)
Proportion of pregnant women approached who agree to be screened (95% CI)	45% (41, 49%) (225/500)
Proportion of families eligible on screening that cannot have a water softener installed (e.g. due to landlord or local authority refusal, technical (plumbing) reasons) (95% CI)	28% (21, 35%) (41/149)
Proportion of families randomised that withdraw due to infant ineligibility	0% (0/80)
Proportion of families in intervention arm who found the study acceptable	90% (36/40)
Proportion of participants in control arm that become exposed to softened water (e.g. by moving to a new home in a soft water area, or moving to a home with an active water softener installed, before the end of follow up)	Unable to determine from available data
Proportion of participants that have the water softening unit removed or disabled prior to end of follow up	0% (0/40)
Proportion of participants (randomised) with visible eczema status recorded at each time point (95% CI): • 4 weeks • 3 months • 6 months	 93% (84, 97%) (74/80) 89% (80, 94%) (71/80) 85% (75, 91%) (68/80)
Proportion of water samples with hardness >20 mg/L calcium carbonate in the intervention arm	7.9% (56/708)
Proportion of subjects in the intervention arm with at least 1 water sample with hardness >20 mg/L calcium carbonate	41% (16/39)
Proportion of participants that withdraw from the trial prior to end of follow up (95% CI)	16% (9.7, 26%) (13/79)
Mean number of nights spent away from the participant's main home during follow up (SD)	12 (12) (n=36)
Proportion of clinical outcome assessments that have remained blinded at 4 weeks, 3 & 6 months (95% CI), [N#]: • 4 weeks • 3 months • 6 months	 96% (88, 99%) (72/75) 96% (88, 98%) (69/72) 100% (69/69%)

[N#], Number of assessments completed; CI, Confidence interval; N, Number of water samples received; SD, standard deviation.

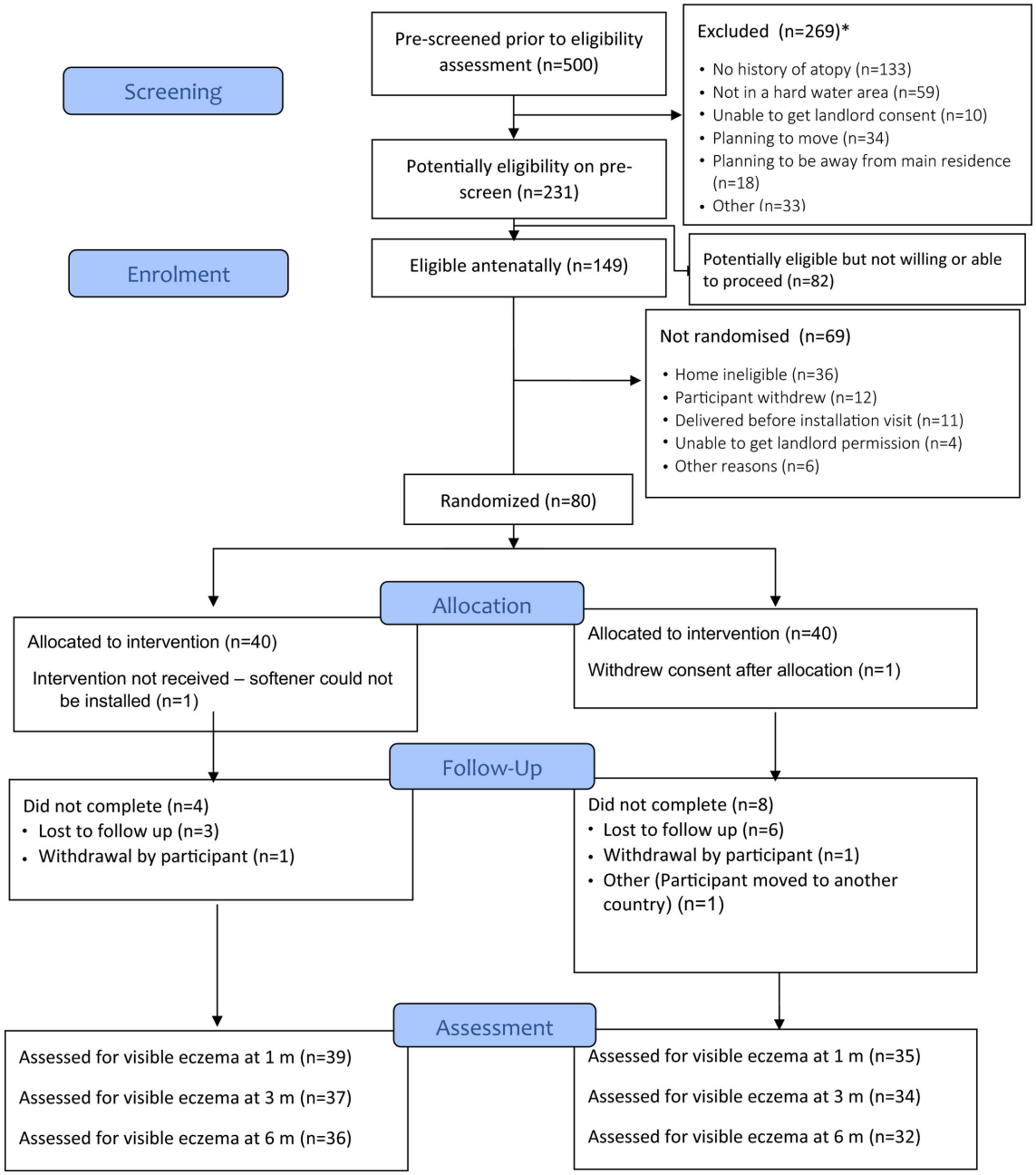

Figure 4.2 CONSORT flow diagram

*More than one reason possible per participant

4.3.2 Clinical endpoints

By 6 months of age, 27/67 infants (40%) developed visible eczema and 15/36 infants (42%) had parent-reported doctor-diagnosed eczema. Of those with parent-reported doctor-diagnosed eczema, 13/15 (87%) also had visible eczema on examination, however, only 68% (13/19) of those with visible eczema on examination had corresponding parent-reported doctor-diagnosed eczema. Blinding was maintained for 96% of completed assessments at 4 weeks and 3 months and 100% at 6 months.

A lower proportion of infants in the water softener arm (6/17, 35%) had parent-reported, doctor-diagnosed atopic eczema by 6 months of age compared to those in the control arm (9/19, 47%) exposed to hard water (difference -12%, 95% CI -44, 20%). This magnitude of effect was also observed in the proportion of infants with visible eczema by 6 months of age (difference -15%, 95% CI -38, 8.3%) (Table 4.5). Time to onset of parent-reported doctor-diagnosed eczema was similar in the two arms (Figure 4.3). Trends in EASI and POEM scores were generally consistent. Median EASI scores beyond 4 weeks were lower in the water softener arm than the control arm. Median POEM scores beyond 4 weeks were lower in the water softener arm than the control arm (Table 4.5).

Table 4.5 Clinical outcomes

Outcome	Water softener (n/N)	Usual hard water (n/N)	Difference (water softener – hard water) (95% CI)*
Parent-reported, doctor-diagnosed atopic eczema by 6 months of age,	35% (6/17)	47% (9/19)	−12% (−44, 20%) (*n* = 36)
Time to onset of patient-reported doctor-diagnosed eczema (weeks), mean (SD)	24.0 (4.9) (*n* = 37)	23.5 (5.7) (*n* = 34)	0.55 (−1.9, 3.1) (*n* = 71)
Visible eczema at 4 weeks	2.6% (1/39)	17% (6/35)	−15% (−28, −1.1%) (*n* = 74)
Visible eczema at 3 months of age	24% (9/37)	8.8% (3/34)	−16% (−1.29, 32%) (*n* = 71)
Visible eczema at 6 months of age,	8.3% (3/36)	28% (9/32)	−20% (−38, −1.8%) (*n* = 68)
Visible eczema by 6 months of age	33% (12/36)	48% (15/31)	−15% (−38, 8.3%) (*n* = 67)
EASI at 4 weeks, median (IQR)[#]	17 (0) (*n* = 1)	1.2 (1.8) (*n* = 5)	16 (*n* = 6)
EASI at 3 months, median (IQR)[#]	0.8 (0.4) (*n* = 9)	1.3 (12.5) (*n* = 3)	−0.5 (*n* = 12)
EASI at 6 months, median (IQR)[#]	0.8 (0.4) (*n* = 2)	2.0 (1.0) (*n* = 9)	−1.2 (*n* = 11)
POEM at 4 weeks, median (IQR)[#]	16 (0) (*n* = 1)	10 (0) (*n* = 1)	6 (*n* = 2)
POEM at 2 months, median (IQR)[#]	1.0 (0) (*n* = 1)	4.5 (1.0) (*n* = 2)	−3.5 (*n* = 3)
POEM at 3 months, median (IQR)[#]	4.0 (0) (*n* = 6)	8.5 (9.5) (*n* = 4)	−4.5 (*n* = 10)
POEM at 4 months, median (IQR)[#]	3.0 (1.0) (*n* = 3)	16 (15) (*n* = 2)	−13 (*n* = 5)
POEM at 5 months, median (IQR)[#]	2.0 (4.0) (*n* = 2)	8.5 (8.5) (*n* = 8)	−6.5 (*n* = 10)
POEM at 6 months, median (IQR)[#]	1.0 (1.0) (*n* = 2)	10 (9.0) (*n* = 7)	−9 (*n* = 9)

*Only calculated for differences in means.
[#]Only completed when the mother reported that the infant had eczema.
Differences shown are for the values of the respective outcomes in the water softener group minus the hard water group
EASI—Eczema Area and Severity Index, IQR—interquartile range, N: total number of participants, [N] number of participants with complete data, POEM—patient-oriented eczema measure, SD standard deviation.

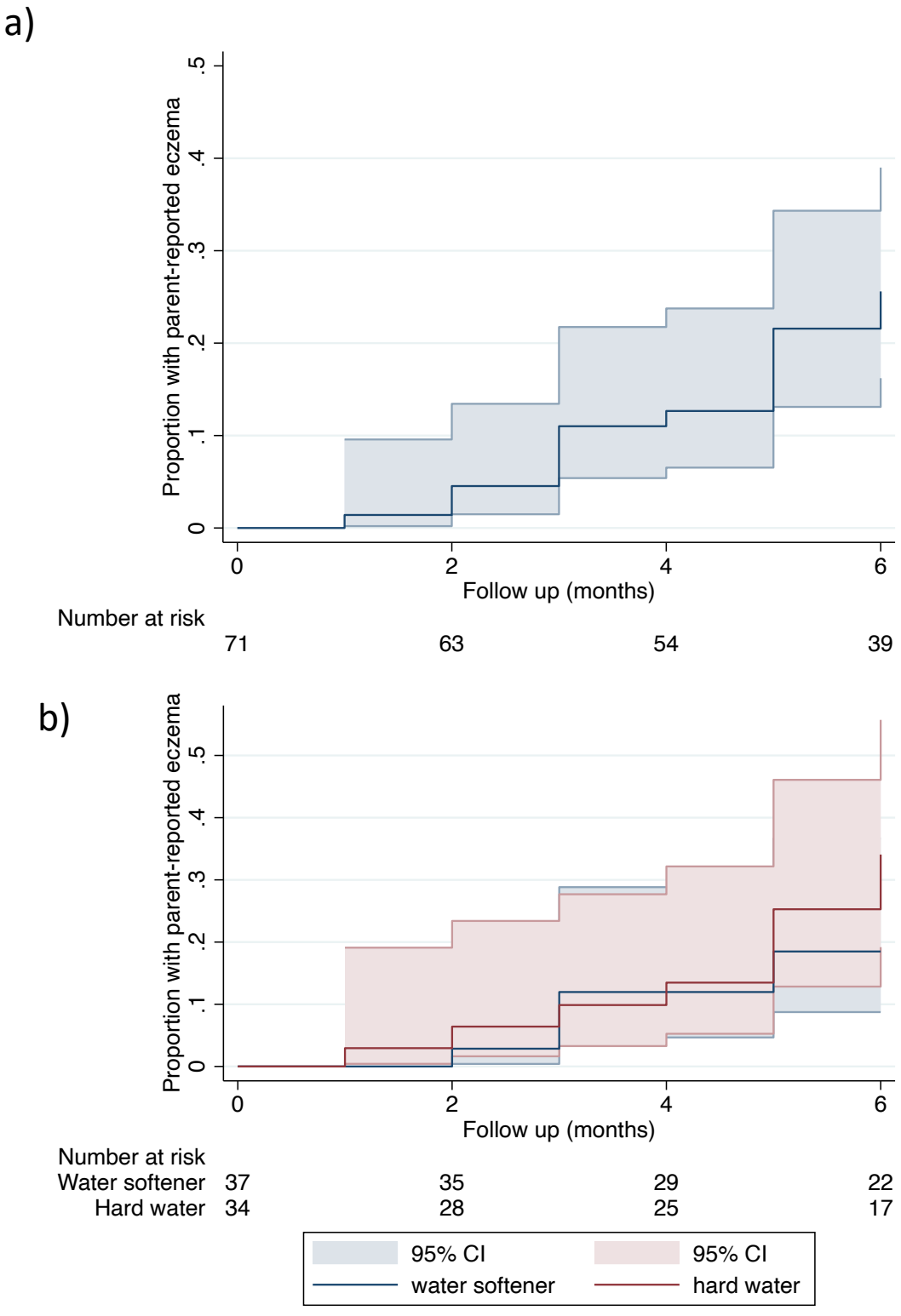

Figure 4.3 Kaplan-Meier curves for time to onset of parent-reported doctor-diagnosed eczema

Upper panel a) shows risk of parent-reported eczema overall in infants followed up in the study. Lower panel b) shows risk of parent-reported eczema by intervention group (water softener versus ususal hard water)

4.3.3 Bathing and skincare practices

A post-hoc analysis of bathing and skincare practices was performed. Almost all infants were bathed at the 4-week, 3- and 6-month timepoints and the proportions of infants bathed were similar in the two groups (Table 4.6). Most infants were bathed using an immersion bath. Top-and-tail washing was used less commonly compared to immersion bathing, particularly at timepoints beyond the first 4 weeks. The majority of infants in either group were bathed without the use of bath products at the 4-week timepoint, although from 3-months onwards two-thirds of infants in both groups used bathing products (Table 4.6).

Table 4.6 Bathing practices

Outcome Timepoint	**Water softener (n/N)**	**Usual hard water (n/N)**
Infant bathed		
4 weeks	97% (38/39)	100% (35/35)
3 months	97% (36/37)	100% (34/34)
6 months	100% (37/37)	100% (32/32)
Immersion bath		
4 weeks	84% (32/38)	86% (30/35)
3 months	97% (35/36)	91% (31/34)
6 months	95% (35/37)	94% (30/32)
'Top and tail' wash		
4 weeks	16% (6/38)	14% (5/35)
3 months	3% (1/36)	9% (3/34)
6 months	5% (2/37)	6% (2/32)
Any bathing products used		
4 weeks	41% (16/39)	34% (12/35)
3 months	70% (26/37)	68% (23/34)
6 months	68% (25/37)	72% (23/34)

Leave-on skincare products were used for the majority of infants at all timepoints in the hard water group. In the water softener arm the majority used leave-on skincare products from 3 months onwards (Table 4.7). The proportions were similar in the two groups (68% versus 69% in the water softener and hard water groups, respectively, at 6-months). Vegetable oil was the most common type of leave-on product at 4-weeks. At 3- and 6-months, emollient cream or ointment was the most common type of product used, with a similar proportion of infants using these products in each arm (Table 4.7). Steroid cream or ointment was only used on infants in the hard water group.

Table 4.7 Skincare practices

Outcome Timepoint	Water softener (n/N)	Usual hard water (n/N)
Leave-on skin products used		
4 weeks	38% (15/39)	57% (20/35)
3 months	62% (23/37)	68% (23/34)
6 months	68% (25/37)	69% (22/32)
Emollient cream/ointment		
4 weeks	13% (5/39)	11% (4/35)
3 months	46% (17/37)	38% (13/34)
6 months	57% (21/37)	47% (15/32)
Vegetable oil		
4 weeks	16% (6/38)	14% (5/35)
3 months	16% (6/37)	21% (7/34)
6 months	3% (1/37)	22% (7/32)
Mineral oil		
4 weeks	0% (0/39)	3% (1/35)
3 months	3% (1/37)	3% (1/34)
6 months	3% (1/37)	3% (1/32)
Steroid cream/ointment		
4 weeks	0% (0/39)	0% (0/35)
3 months	0% (0/37)	6% (2/34)
6 months	0% (0/37)	3% (1/32)

4.4 DISCUSSION

This pilot study assessed the feasibility of installing home water softeners for the prevention of eczema in high-risk neonates. Overall, around half of eligible pregnant women were willing and able to be randomised and this is consistent with the proportion (42%) of eligible families who were randomised into the Barrier Enhancement Eczema Prevention (BEEP) pilot study (88) that informed the feasibility of the design of the full-scale BEEP study. The most common reason for failure to proceed to randomisation was that the participant's home was not suitable for the installation of a water softener. Adherence with the intervention was generally good, with a low number of nights (mean 12 nights, SD 12) on average spent away from the main residence over the 6-month follow-up period. Overall, 41% (n=39) of participants in the water softener arm had at least one water sample out of the softer water range (>20 mg/L CaCO3) with a low

proportion of total water samples (7.9%, n=708) out of the soft water range, despite the need for participants to top-up the unit with salt.

These findings suggest that the current study design could be successfully scaled-up in a fully powered prevention study. The question is then how large such a study would need to be. Approximately one-third of infants developed eczema over the first 6 months of life in this high-risk population and this is consistent with other estimates in the literature, for example, the Cork Babies After Scope birth cohort (35). Fewer infants in the water softener group developed visible or parent-reported eczema by 6 months of age, demonstrating proof-of-concept. The use of bathing products and the use of leave-on skincare products were similar across the two groups over the 6-month period, suggesting that these factors probably did not explain the observed difference in eczema risk between the two groups. Even though parents were not blinded to the intervention, it was reassuring that there was consistency between the risk differences obtained for parent-reported doctor-diagnosed eczema and for visible eczema on blinded skin examination, which opens up the possibility of using virtual visits for follow up that could further reduce participant burden and attrition.

This study is the first randomised controlled trial testing the effect of water softeners on infant eczema and provides useful information on the likely magnitude of the effect and therefore sample size requirements that an adequately statistically powered prevention study would need. There are limitations to the approach of using pilot data to inform sample size calculations for the main trial as such estimates might lack both precision and clinical meaningfulness.(93) A further limitation is that formal criteria were not specified for progression from the pilot to the main trial.(37) However, as a single intervention for the prevention of eczema, the observed difference in visible eczema of 15% would represent a clinically meaningful reduction in eczema risk, consistent with effect size used to power the BEEP study of emollients for the primary prevention of eczema.(82) Assuming a difference of 15% between the water softener and control arms and attrition of 16%, the sample size requirement for a study with 80% power is likely to be >860 participants, allowing for attrition. Based on the data generated in this study, roughly 6 pregnant women had to be approached and pre-screened for every randomized

participant, suggesting around 5,200 pregnant women would need to be approached about the study.

During 2018-2019 there were 6,510 deliveries recorded at Guy's and St Thomas' NHS Foundation Trust and 4,845 deliveries at Kingston Hospital NHS Foundation Trust (94). Extrapolating these annual figures across both sites to the 20-month recruitment period used in this pilot study, there would have been approximately 19,000 babies delivered during the study recruitment period where 80 infants were randomised, i.e. 0.42%. On average, in 2018/19 there were 4,600 births per maternity unit (NHS hospital trust) (94), and so an "average" site would be estimated to yield around 20 randomised infants. Therefore, to achieve 860 infants randomised, approximately 40 maternity units would be needed, i.e. around 1/6 of all maternity units in England. There may be challenges in finding enough research-equipped maternity sites located in very hard water areas. The recruitment period could be extended and/or the number of sites increased, however, the latter would probably require additional countries to be added to the study.

There were 27 infants who developed visible eczema by 6 months. The magnitude of the point estimate of the relative risk (softened water/control) is 0.68 (95% CI 0.38, 1.2), which is consistent with the magnitude of risk reduction that might be expected by softening water based on the increased odds identified with hard water exposure in children in the systematic review and meta-analysis described in Chapter 2 (OR 1.28).(38) However, in the absence of longer-term follow up there is the possibility that use of a water softener in early life simply delays the onset of eczema rather than preventing it. Given that approximately 80% of eczema cases occur before 2 years of age, this would seem an appropriate follow-up period for a definitive prevention trial.

Infants in the water softener arm who developed eczema appeared to have lower severity scores, both in terms of clinician-assessed (EASI) and parent-assessed (POEM) measures, compared to those in the hard water arm. Lower POEM scores were seen with the addition of a water softener to usual care versus usual care alone in the SWET trial. However, in this study as with the SWET trial, there is a high risk of biased POEM assessments as parents were unblinded as to the intervention status.

The results from this pilot RCT indicate that a definitive RCT to assess the prevention of atopic eczema in high-risk infants may be feasible in a mixed urban and suburban setting in England. However, a large number of clinical sites (approximately 40) would be needed to recruit a sufficient number of pregnant women in a 1-year period. The outcome, eczema, is a binary variable and as such requires a considerably larger sample size to detect differences than would a continuous outcome, if one were to be available. The mechanistic outcomes are currently being analysed in various laboratories in readiness for these data to be examined in depth. It may be that one of these may provide a suitable proxy continuous outcome that could be used in conjunction with visible eczema to design a more efficient definitive trial. Overall, pregnant women found the study design acceptable. Adjustments to the study design may help to reduce the proportion of eligible pregnant women who do not go on to be randomised, in particular, around the timing and organisation of the water softener installation visit and so improve the efficiency of the trial.

5 GENERAL DISCUSSION

Several studies have suggested that water hardness may play a role in the development of atopic eczema. The objective of this thesis was to comprehensively evaluate the role of water hardness on the development of eczema in children, specifically by addressing the following questions:

- What is the effect of domestic water hardness on atopic eczema and skin barrier function?
- How is the effect of hard water on the skin modified by *filaggrin* mutation status?
- Is it feasible to conduct a trial of the installation of water softeners prior to birth for the prevention of eczema in infants?

5.1 Summary of findings

5.1.1 What is the effect of domestic water hardness on atopic eczema and skin barrier function?

The systematic literature review identified that there was a positive association between living in a hard-water (range: 76 to >350 mg/L $CaCO_3$) area and eczema in children overall, although there were differences in exposure and outcome classification between studies and a lack of high-quality evidence on the effect of water hardness exposure on eczema risk in adults and very young children. Subsequently, in the longitudinal analysis of a well-characterised cohort of infants in the EAT study there was no overall association between exposure to harder (>255 mg/L calcium carbonate [$CaCO_3$]) versus softer (≤255 mg/L $CaCO_3$) water with an HR of 1.07 (95% CI 0.92, 1.24) after adjustment for confounders.

The pilot RCT findings of a numerically lower absolute and relative risk of eczema in neonates exposed to softened versus hard water provide the first randomized controlled trial evidence that by replacing domestic hard water exposure with softened water may reduce the risk of infants with a family history of atopy developing eczema, however, as expected with the small sample size, the results were not statistically significant and so no firm conclusions can be drawn at this stage. As the water softener used in the pilot

study removed various types of polyvalent cations, the use of an ion-exchange water softener as an intervention does not provide proof that it's the concentration of calcium ions specifically drives eczema risk, however, it does show that that the concentration of polyvalent metal cations in bathing water might be more important than the ratio of different ions such as calcium and magnesium.

5.1.2 How is the effect of hard water on the skin modified by *filaggrin* mutation status?

In a longitudinal analysis of the EAT study we found that infants with at least one FLG loss-of-function mutation exposed to harder water have a three-fold increased risk of developing eczema compared to infants with WT *FLG* exposed to softer water. This risk equates to a PAF of 3% overall with water hardness exposure and 23.2% in those with *FLG* and hard water co-exposure. Combined exposure to hard water and *FLG* mutations was also associated with a slight increase in skin barrier dysfunction, as measured by TEWL, between 3-12 months of age. These results support the growing body of evidence for the multifactorial aetiology of eczema, and provide a plausible insight into how a commonly encountered exposure, hard water, might interact with a genetically weakened skin barrier in early life to lead to further deterioration in skin barrier function, loss of epidermal water, and the initiation of eczematous skin inflammation.(78)

5.1.3 Is it feasible to conduct a trial of the installation of water softeners prior to birth for the prevention of eczema in infants?

The results from this pilot RCT indicate that a definitive RCT to assess the prevention of atopic eczema in high-risk infants may be feasible in England. However, a large number of clinical sites (approximately 40) would be needed to recruit a sufficient number of pregnant women in a 1-year period. Overall, pregnant women found the study design acceptable. Adjustments to the study design may help to reduce the proportion of eligible pregnant women who do not go on to be randomised, in particular around the timing and organisation of the water softener installation visit and so improve the efficiency of the trial.

No primary prevention strategy for atopic eczema has been established to date.(95) However, several approaches have been proposed such as probiotics during

pregnancy, dietary supplementation, house dust mite avoidance, intensive emollient use and domestic water softening.(14) Of these approaches, so far only intensive emollient use has been rigorously evaluated in a fully-powered multi-centre prevention trial. In the Barrier Enhancement Eczema Prevention (BEEP) trial, new-borns were randomised to receive either daily emollient use plus standard skin-care advice or standard skin-care advice alone.(82) At age 2 years, eczema was present in 139 (23%) of 598 infants with outcome data collected in the emollient group and 150 (25%) of 612 infants in the control group with an adjusted relative risk of 0·95 [95% CI 0·78 to 1·16], p=0·61, providing evidence that daily emollient during the first year of life does not prevent eczema in high-risk children.(82) This result occurred despite a very promising signal from an earlier pilot version of the same study.(88) Hence the prevention of eczema through elimination of single risk factors may not be the best way to approach this multifactorial disease and a cautious approach should be taken in the interpretation of the preliminary findings of a numerically lower absolute and relative risk of eczema in babies exposed to softened versus hard water.

5.2 Practical applications/implications

Water hardness needs to be considered in the context of other risk factors for the development of eczema, both genetic and environmental, particularly in younger children. There is not enough evidence at present to recommend that water softeners be used explicitly for the prevention of eczema. However, the overall evidence generated so far suggests that there may be a benefit in terms of eczema risk reduction in targeting infants with a family history of atopy living in a hard water area and that the use of a water softener as an intervention in a prevention RCT is feasible and should be explored further. These findings may not be generalisable to other parts of the world with less extreme levels of water hardness, or where hardness is driven by cations other than calcium and magnesium.

5.3 Recommendations for further research

The next step would be to conduct a fully powered RCT of a water softener for the prevention of eczema in infants with a family history of atopy living in hard water areas. Based on the observed difference of 9.8 percentage points (26.6-16.2%) and attrition of 16%, the sample size requirement for a study with 90% power is likely to be >750

participants and follow-up would need to last for at least 2 years. This would require significant resource allocation. Further work is needed to explore the mechanisms by which hard water interacts with the skin barrier in order to understand better how calcium carbonate interacts with the skin barrier at a cellular and molecular level. Finally, combinations of interventions aimed at improving skin barrier function, such as water softening combined with regular emollient use and avoidance of harsh wash products, may conceivably have an additive effect and could be tested together in a clinical trial setting.

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7 APPENDIX

7.1 Systematic review search strategy

Original search date 03/08/2018
MEDLINE (OVID) and EMBASE search strategies
1. exp Dermatitis, Atopic/ (MESH)
2. atopic dermatitis.mp.
3. atopic eczema.mp.
4. exp neurodermatitis/ (MESH)
5. neurodermatitis.mp.
6. infantile eczema.mp.
7. childhood eczema.mp.
8. Besnier's prurigo.mp.
9. exp Eczema/ (MESH)
10. cutaneous irritation.mp
11. cutaneous reaction.mp
12. skin dryness.mp
13. xerosis.mp
14. or/1-13
15. exp Water (MESH)
16. exp Water softening/ (MESH)
17. water hardness.mp.
18. exp Drinking water/ (MESH)
19. exp Calcium/ (MESH)
20. exp Magnesium/ (MESH)
21. Chlorine/ (MESH)
22. Chloramine.mp.
23. Stearate.mp or exp Stearic acid/ (MESH)
24. Alkalinity
25. Hydrogen ion concentration/ (MESH)
26. pH
27. transepidermal water loss.mp
28. or/16-27
29. 14 and 28

Indices:
Exp: indicates that the term was exploded
Mp: indicates a free text search for a term (title, abstract, original title, subject heading word, keyword heading word; MESH: Medical subject heading

GREAT

(water) OR (calcium) OR (hardness)

CENTRAL

#1

MeSH descriptor: [Dermatitis, Atopic] explode all trees

#2

MeSH descriptor: [Water] explode all trees

#3

MeSH descriptor: [Water Softening] explode all trees

#2 or #3

#1 and #4

Web of Science Core Collection search strategy

#26	#25 AND #12 *DocType=All document types; Language=All languages;*
#25	#24 OR #23 OR #22 OR #21 OR #20 OR #19 OR #18 OR #17 OR #16 OR #15 OR #14 OR #13 *DocType=All document types; Language=All languages;*
#24	ts=transepidermal water loss *DocType=All document types; Language=All languages;*
#23	ts=pH *DocType=All document types; Language=All languages;*
#22	ts=alkalinity *DocType=All document types; Language=All languages;*
#21	ts=stearate *DocType=All document types; Language=All languages;*
#20	ts=chloramine *DocType=All document types; Language=All languages;*

#19	ts=chlorine *DocType=All document types; Language=All languages;*
#18	ts=magnesium *DocType=All document types; Language=All languages;*
#17	ts=calcium *DocType=All document types; Language=All languages;*
#16	ts=drinking water *DocType=All document types; Language=All languages;*
#15	ts=water hardness *DocType=All document types; Language=All languages;*
#14	ts=water softening *DocType=All document types; Language=All languages;*
#13	ts=water *DocType=All document types; Language=All languages;*
#12	#11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1 *DocType=All document types; Language=All languages;*
#11	ts=xerosis *DocType=All document types; Language=All languages;*
#10	ts=skin dryness *DocType=All document types; Language=All languages;*
#9	ts=cutaneous reaction *DocType=All document types; Language=All languages;*

#8	ts=cutaneous irritation *DocType=All document types; Language=All languages;*
#7	ts=Eczema *DocType=All document types; Language=All languages;*
#6	ts=Besnier's prurigo *DocType=All document types; Language=All languages;*
#5	ts=childhood eczema *DocType=All document types; Language=All languages;*
#4	ts=infantile eczema *DocType=All document types; Language=All languages;*
#3	**TOPIC:** (neurodermatitis) *DocType=All document types; Language=All languages;*
#2	TS=atopic eczema *DocType=All document types; Language=All languages;*
#1	TS=Atopic dermatitis *DocType=All document types; Language=All languages;*

7.2 Systematic review excluded studies

Author, year	Reason excluded
Anonymous, 1998[1]	Commentary - not a primary study
Adachi, 1998[2]	Wrong intervention/exposure
Akimoto, 1990[3]	Wrong intervention/exposure
Anonymous, 2011[4]	Commentary - not a primary study
Anonymous, 2012[5]	Wrong outcomes
Anveden Berglind, 2009[6]	Wrong intervention/exposure
Anveden Berglind, 2012[7]	Wrong intervention/exposure
Barthel, 1994[8]	Wrong intervention/exposure
Choi, 2013[9]	Wrong intervention/exposure
Donato, 2003[10]	Commentary - not a primary study
Fernandez-Luna, 2016[11]	Wrong intervention/exposure
Font-Ribera, 2009[12]	Wrong outcomes
Gamble, 2011[13]	Commentary - not a primary study
Giannetti, 2005[14]	Wrong intervention/exposure
Gittler, 2017[15]	Not a systematic review
Jabbar-Lopez, 2017[16]	Abstract reporting this systematic review
Jungmayr, 1998[17]	Commentary - not a primary study
Kantor, 2016[18]	Not a systematic review
Kim, 2015[19]	Wrong intervention/exposure
Kim, 2012[20]	Wrong intervention/exposure
Lacour, 1999[21]	Commentary - not a primary study
Licu, 2002[22]	Wrong intervention/exposure
Lipkin, 1965[23]	Wrong intervention/exposure
Nardi, 2003[24]	Wrong intervention/exposure

Proksch, 2005[25]	Wrong intervention/exposure
Sengupta, 2013[26]	Wrong outcomes
Simmonds, 2009[27]	Commentary - not a primary study
Tanaka, 2008[28]	Insufficient detail presented in abstract
Tanaka, 2009[29]	Insufficient detail presented in abstract; wrong outcomes
Tanaka, 2012[30]	Insufficient detail presented in abstract
Tanaka, 2012[31]	Insufficient detail presented in abstract
Tsai, 1999[32]	Not a systematic review
Walters, 2016[33]	Wrong outcomes
Yoshizawa, 2003[34]	Wrong intervention/exposure

7.2.1 References of Excluded Studies

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31 Tanaka A, Matsuda A, Takai M *et al.* Topical metallic soap on the skin induces Th2-type immune responses in a model for human atopic dermatitis, NC/Tnd mice. Allergy: European Journal of Allergy and Clinical Immunology 2012; 67: 637.

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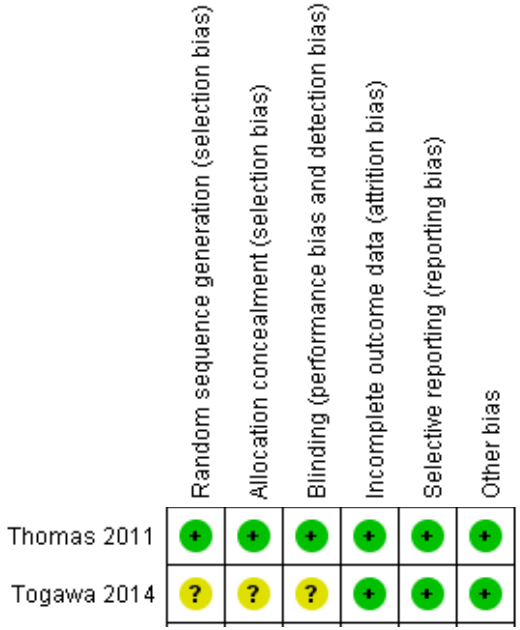
33 Walters RM, Anim-Danso E, Amato SM et al. Hard water softening effect of a baby cleanser. Clinical, Cosmetic and Investigational Dermatology 2016; **9**: 339-45.
34 Yoshizawa Y, Kitamura K, Kawana S *et al.* Water, salts and skin barrier of normal skin. *Skin Research and Technology* 2003; **9**: 31-3.

7.3 Risk of bias of included studies

a) Cochrane risk of bias assessment of RCTs



b) Newcastle-Ottawa Quality Assessment Scale – Cohort Studies

Study	Selection				Comparability	Outcome		
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts
Font-Ribera et al., 2015	1	1	1	1	1	0	1	0
Engebretsen et al., 2017	1	1	1	1	2	0	1	0

7.4 ToxTool quality assessment of Tanaka et al, 2015

Reliability assessment of in vivo toxicity studies		
Study under evaluation		
Authors:		
	Tanaka et al.	
Titel:		
	Ultra-pure soft water amerliorates atopic skin disease by preventing metallic soap deposition in NC/Tnd mice and reduces skin dryness in humans	
Testing facility, year, sponsor, study no. or bibliographic reference:		
	2015	
Explanations are available for most criteria and show up, when the cursor is moved over the criteria field. Please read carefully! Red criteria: the maximum score is needed for these criteria to achieve reliability category 1 or 2 (see worksheet Explanations): Please evaluate with special care!		
Criteria		
No.	**Criteria Group I: Test substance identification**	**Score**
1	Was the test substance identified?	1
2	Is the purity of the substance given?	1
3	Is information on the source/origin of the substance given?	0
4	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?	1
		3
	Criteria Group II: Test organism characterisation	
5	Is the species given?	1
6	Is the sex of the test organism given?	0
7	Is information given on the strain of test animals plus, if considered necessary to judge the study, other specifications (see explanation for examples)?	1
8	Is age or body weight of the test organisms at the start of the study given?	0
9	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?	1
		3
	Criteria Group III: Study design description	
10	Is the administration route given?	1
11	Are doses administered or concentrations in application media given?	1
12	Are frequency and duration of exposure as well as time-points of observations explained?	1
13	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required, see explanations for study types and their respective requirements on controls)?	1
14	Is the number of animals (in case of experimental human studies: number of test persons) per group given?	1
15	Are sufficient details of the administration scheme given to judge the study (see explanation for examples)?	1
16	For inhalation studies and repeated dose toxicity studies only (give point for other study types): Were achieved concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?	
		6
	Criteria Group IV: Study results documentation	
17	Are the study endpoint(s) and their method(s) of determination clearly described?	1
18	Is the description of the study results for all endpoints investigated transparent and complete?	1
19	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?	1
		3
	Criteria Group V: Plausibility of study design and results	
20	Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?	1
21	Are the quantitative study results reliable (see explanations for arguments)?	1
		2
		17
	A Numerical result leads to initial Category:	2
	B Checking red scores leads to revised Category:	2
	C Evaluator's proposal: Category:	
	D Justification in case evaluator deviates from B:	

7.5 SOFTER Participant Information Leaflet

Do I have to take part?
Whether you decide to take part or not is entirely up to you. Your decision will not affect the care you or your baby receive in any way. If you agree to take part, you are free to withdraw at a later stage, without giving a reason. If you withdraw from the study we will arrange for the water softener to be removed.

What are the benefits of taking part?
There may be no direct benefit from taking part in the study, but your household will have softened water for 6 months either during the study or after. You will also have the opportunity to buy the water softener after the study at a discounted price of £399, about a quarter of the full retail price of £1,678.80.

If you complete a short interview at the end of the study (by telephone or in person) about your experiences of taking part in the study you will receive a £20 Amazon voucher as a token of appreciation.

What are the possible risks or side effects of taking part?
Only the water that your baby washes and bathes in will be softened and there are no known risks from this. Obtaining the measurements from your baby's skin samples will have no side effects.

Time commitment
Taking part in the study will mean that you will have extra visits to the hospital after your baby's birth for the study and these visits may last between 1 to 2 hours. We will reimburse reasonable travel costs.

What if there is a problem?
If you have a concern about any aspect of this study, please ask to speak to the researchers who will do their best to answer your questions (call 020 7188 7188 ext. 51601/57716). If you remain unhappy and wish to complain formally, you can do this through the Patients Advice and Liaison Service (PALS) on 020 7188 8801, pals@gstt.nhs.uk. The PALS team are based in the main entrance on the ground floor at St Thomas' Hospital.

In the event that something does go wrong and you are harmed during the research you may have grounds for legal action for compensation against the NHS Trust where you/your child is being treated and/or King's College London, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

What will happen to the results of the study?
The information from this study will help us work out how best to plan further research into whether water softeners prevent eczema in larger numbers of people.

The results will be published at conferences and in professional journals but no one will be identified. We will also send a copy of the study findings to everyone who took part.

What if new information becomes available?
Should new information become available, the researchers will discuss this with you and you can decide if you want to continue in the study. It may be that the doctors and midwives decide it would be better for you to stop being part of the study. If this happens they will discuss this with you.

Who is paying for this research?
The study is funded by the National Institute for Health Research (NIHR), the Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and Harvey Water Softeners Ltd., Surrey.

Who has reviewed this study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee. This study has been reviewed and given a favourable opinion by the North West - Liverpool East Research Ethics Committee (Ref: 17/NW/0661).

What do I do if I have further questions or want to take part?

For further information please contact:

Dr Zarif Jabbar-Lopez
St Thomas' Hospital, London
Tel: 020 7188 7188 ext 57716
Email: Softer@gstt.nhs.uk

Thank you for taking time to read this leaflet and for considering taking part in this study

King's College London

NHS
Guy's and St Thomas'
NHS Foundation Trust

Do water softeners prevent eczema in newborn babies?

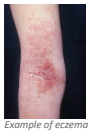
Study of Softened Water for Eczema Prevention (SOFTER)

You are being invited to take part in a research study. This study is being conducted as an educational project.

Before you decide, it is important for you to understand why the research is being done and what it will involve.

Please take time to read the following information carefully and discuss it with friends and relatives if you wish. Please ask if anything is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Background
Eczema is a common itchy skin condition that can have a big impact on people's lives. Eczema is more common in infants who live in a hard water area, and in this study we are testing whether installation of a water softener can prevent eczema.


Example of eczema

What is 'hard water'?
Hard water is caused by water picking up different minerals when it seeps through the ground. Some of these minerals cause lime scale in your kitchen kettle, for example.

What are water softeners?
Water softeners are small units that remove minerals such as calcium and magnesium and replace them with sodium from common salt.

Because water coming out of a water softener can be saltier than tap water, a regular kitchen water supply is needed for drinking water, as we want to make sure that babies do not have too much salt.

Why have I been chosen?
You have been chosen because you or someone in your immediate family have (or have had) eczema, asthma or hayfever, and you are pregnant and having your baby at a study hospital. You also live in an area that has hard water.

What do we want to find out?
Eczema has no cure, so we are trying to find a way to prevent it. We would like to see if softening water will reduce the risk of babies getting eczema.

What do I have to do if I take part?
One of the research team will call you after you have had time to read this leaflet and will answer any questions you may have. If you are interested, we will arrange to meet with you at your antenatal appointment when you are around 30-34 weeks pregnant.

If you would like to take part in SOFTER, you need to be willing to have a water softener installed in your home, usually under the kitchen sink. If you rent your home, you need to have permission from your landlord (we can give you a letter to help with this). A plumber will check that your home is suitable for installation of a water softener. You will need to have a regular kitchen drinking water supply. Where possible this will be through your existing tap. Otherwise, it may be necessary to have a separate tap fitted to your sink.

Half of homes will have a water softener installed. The other half will receive their usual water supply.
The water softener will be installed by a trained plumber at no cost to you. We will not be able to tell you in advance whether you will have a softener installed. This will be decided by a computer.

Typical under-sink water softener installation

This process is called randomisation.

If you do not have a water softener installed for the study, you will have the choice to have one at the end of the study so you can also have softened water for 6 months, if you wish. Water softeners will be removed by a trained plumber at the end of the study, or may be bought at a discounted price (see below).

What will the study involve for me and my baby?
After your baby's birth, we would like to see your baby until s/he is 6 months old, to look at your baby's skin.


Example of a skin measurement

Shortly after birth, we would come to see you and your baby in the hospital to carry out some non-invasive tests on your baby's skin. Not all hospital sites will carry out all of these tests. Your research team will tell you which are available at your hospital site.

These tests would involve looking to see how much water is in your baby's skin with a special tool, as well as looking at his/her skin and put a little bit of tape on (a bit like sellotape) to study the skin immune system in relation to hard versus soft water. This may make the skin a little red, but does not hurt your baby at all.

We may also measure changes in the surface of the skin to look for soap residues and to see how acidic the skin is. Whilst you are in hospital, we may also take a swab from your baby's arm and nose to study bacteria. We would also like to take a saliva sample to look at eczema genes.
We would invite you to bring your baby back to see us at the hospital 3 times when s/he is around 4 weeks, 3 months and 6 months old.

At monthly intervals we will ask you to complete some online questionnaires about your baby's skin and general health. We can send email reminders to help with this. We will also ask those who have the water softeners fitted to send weekly water samples to Harvey Water Softeners using prepaid envelopes so that we can check the softener is working correctly.

At the end of the study you may be invited to take part in an optional short interview (around 30 mins) about your experiences in the study. The interview can either be by telephone or in person at the hospital.

What will happen to the samples and data that you collect?
The laboratory analyses will be done at King's College London and at other universities in Europe and the US. Your baby's samples and data will be labelled with a code and will not have their name or any identifiable information on them. The samples will be disposed of after being analysed.

Data will be held in accordance with the Data Protection Act 1998. At the end of the study, the data will be stored until your child is 25 years old in a secure archiving facility. Following this it will be destroyed. This is a legal requirement for studies involving children.

Will my taking part be kept confidential?
We will keep your contact details on file so that we can contact you during the study. With your permission, your contact details will be shared with our commercial collaborator, Harvey Water Softeners Ltd., so that they can install and maintain the water softener.

All other information stored about you and your baby will have your name, address and other identifying details removed. No one will be able to identify you or your baby from anything we record. All computers used will be password protected. Only people directly involved in the study will have access to the information. With your permission, we will inform your baby's GP that s/he is taking part in the study.

7.6 SOFTER Trial Clinical Assessment Forms

7.6.1 Eczema Area and Severity Index

Eczema Area and Severity Index (EASI) Score

Was EASI performed by a blind assessor?	1	Yes
	0	N/A

Area of involvement : Each body region has potentially 100% involvement, Score 0-6 based on the following table:							
% Involvement	0	1-9	10-29	30-49	50-69	70-89	90-100
Region score	0	1	2	3	4	5	6

Severity of signs: Grade the severity of each sign on a scale of 0 to 3	
0	None
1	Mild
2	Moderate
3	Severe
-Take an average of the severity across the involved area -Half points can be used e.g. 2.5	

Body region	Erythema (0-3)	Edema/Papulation (0-3)	Excoriation (0-3)	Lichenification (0-3)	Region score (0-6)	Multiplier	Score per body region
Head and neck	(+	+	+	)	X	X0.2	
Trunk	(+	+	+	)	X	X0.3	
Upper extremities	(+	+	+	)	X	X0.2	
Lower extremities	(+	+	+	)	X	X0.3	
				The final EASI is the sum of the 4 region scores (0-72)			

7.6.2 Visible eczema

Skin Examination

1.	Has the baby got signs of visible eczema (poorly demarcated redness with surface change, i.e. fine scaling, vesicles, oozing, crusting or lichenification) in any of the following places?	
Body area	Yes	No
Around the eyes (skin crease)	1	0
Around the ears (skin crease)	1	0
Around the neck (skin crease)	1	0
Fronts of the elbows (skin crease)	1	0
Behind the knees (skin crease)	1	0
Front of the ankles (skin crease)	1	0
Cheeks (any patch involving one or both cheeks, non flexural)	1	0
Forearms (elbow to wrist), at least one patch on EACH forearm(non flexural)	1	0
Lower legs (knee to ankle), at least one patch on EACH leg (non flexural)	1	0
Any other place. Please specify:	1	0

NB: Individual patches have to be larger than 1cm to be scored positive for skin creases and greater than 2cm to be scored positive for non-flexural skin. Please still record smaller areas and other locations under 'Any other place'.
Beware of black skin. Redness may be difficult to see and is not an essential criterion in black skin, but there must be surface change (ie scaling, vesicles, oozing, crusting and/or lichenification).

7.6.3 Patient-Oriented Eczema Measure

POEM for proxy completion (e.g by parent)

Patient Details: _____

_____

_____

_____ Date: _____

Please circle one response for each of the seven questions below about your child's eczema. If your child is old enough to understand the questions then please fill in the questionnaire together. Please leave blank any questions you feel unable to answer.

1. Over the last week, on how many days has your child's skin been itchy because of their eczema?

No days | 1-2 days | 3-4 days | 5-6 days | Every day

2. Over the last week, on how many nights has your child's sleep been disturbed because of their eczema?

No days | 1-2 days | 3-4 days | 5-6 days | Every day

3. Over the last week, on how many days has your child's skin been bleeding because of their eczema?

No days | 1-2 days | 3-4 days | 5-6 days | Every day

4. Over the last week, on how many days has your child's skin been weeping or oozing clear fluid because of their eczema?

No days | 1-2 days | 3-4 days | 5-6 days | Every day

5. Over the last week, on how many days has your child's skin been cracked because of their eczema?

No days | 1-2 days | 3-4 days | 5-6 days | Every day

6. Over the last week, on how many days has your child's skin been flaking off because of their eczema?

No days | 1-2 days | 3-4 days | 5-6 days | Every day

7. Over the last week, on how many days has your child's skin felt dry or rough because of their eczema?

No days | 1-2 days | 3-4 days | 5-6 days | Every day

Total POEM Score (Maximum 28): .....

7.7 SOFTER Statistical Analysis Plan

SOFTENED WATER FOR ECZEMA PREVENTION (SOFTER) PILOT TRIAL

An outcome assessor-blinded pilot randomised controlled trial of an ion-exchange water softener for the prevention of atopic eczema in neonates, with an embedded mechanistic study

Statistical Analysis Plan

Version 2.0

28Jul2020

Clinicaltrials.gov – NCT03270566

CONTENTS

Study personnel

Chief Investigator

Senior Co-investigator

Senior Statistical Lead

A) QUANTITATIVE ANALYSIS PLAN

1. Description of the trial

See protocol publication.(1)

1.1 Principal research objectives to be addressed

The aim of this pilot trial is to determine the feasibility of undertaking a large-scale definitive trial to determine whether installation of domestic ion-exchange water softeners around the time of birth reduces the risk of high-risk children developing atopic eczema. A further aim is to explore the pathophysiological mechanisms for this in an embedded mechanistic study.

Primary objectives

(i) Proportion of eligible families* screened who are willing and able to be randomised. This is key to the determination of the likely success of a future, large-scale definitive randomised controlled trial (RCT). The primary outcome of such a trial is likely to be the cumulative incidence of eczema by 24 months of age.

*Eligible families will be defined as those who have had a pre-screening approach, or have approached the study team because they saw a poster etc., and had a family history of atopy.

Secondary objectives

The secondary objectives are designed to further facilitate the design of a larger, controlled multi-centre RCT. Namely, to determine the:

- Proportion of pregnant women approached who agree to be screened
- Proportion of families eligible on screening that cannot have a water softener installed (e.g. due to landlord or local authority refusal, technical (plumbing) reasons)
- Proportion of families randomised that withdraw due to infant ineligibility
- Proportion of families in intervention arm who found the intervention acceptable
- Proportion of participants in control arm that become exposed to softened water (e.g. by moving to a new home in a soft water area, or moving to a home with an active water softener installed, before the end of follow up)
- Proportion of participants that have the water softening unit removed or disabled prior to end of follow up
- Proportion of participants with visible eczema status (yes/no) recorded at each time point: baseline, 4 weeks, 3 and 6 months
- Proportion of water samples with hardness >20 mg/L calcium carbonate in the intervention arm
- Proportion of participants that withdraw from the trial prior to end of follow up
- Median number of nights spent away from the participant's main home during follow up

- Proportion of clinical outcome assessments that have remained blinded at 4 weeks, 3 & 6 months

We will also gather qualitative data on participants' experiences of taking part in the pilot trial through an evaluation survey given to all participants at the 6-month visit. In addition, we will invite approximately 10 participants to complete a semi-structured interview (via telephone or in person) to evaluate their views of the trial in more depth. The exact number invited may be higher or lower depending on data saturation.

Secondary Clinical Outcomes will also be assessed:

- Proportion with patient-reported, doctor-diagnosed atopic eczema by 6 months of age
- Proportion with visible eczema according to the UK diagnostic criteria-based photographic protocol at 4 weeks, 3 & 6 months of age
- Severity of eczema (if present) using Eczema Area and Severity Index (EASI) at 4 weeks, 3 & 6 months of age
- Patient-reported eczema symptoms (Patient-Orientated Eczema Measure - POEM), monthly from 4 weeks to 6 months of age
- Time to onset of patient-reported doctor-diagnosed eczema

Additional Mechanistic Outcomes will be assessed at Week 4, 3 & 6 months:

- Transepidermal water loss (TEWL)
- Cutaneous cytokine profiles (e.g. interleukin-1 levels)
- Natural moisturising factor (NMF) levels
- Shannon Diversity Index and other skin and upper respiratory microbiota parameters
- Proportion with filaggrin null mutations
- Effect of filaggrin (FLG) gene mutation status on TEWL, cytokine levels, NMF levels and skin microbiota diversity
- Median domestic water hardness level (calcium carbonate concentration)
- Skin surface hydration

1.2 Trial design including blinding

This is a multi-centre parallel group assessor-blinded randomised controlled pilot trial of an ion-exchange water softener for the prevention of atopic eczema in neonates at high risk of developing eczema, with an embedded mechanistic study. Eighty newborn infants will be enrolled into the trial for a period of 6 months. Participants will be enrolled over a period of 12 months. The end of study is defined as the final assessment visit of the last participant to enter the trial.

1.2.1 Interventions

Experimental intervention

A commercially available domestic ion-exchange water softener will be installed in the homes of participants randomised to the intervention group. Ion-exchange water softeners exchange

calcium and magnesium, amongst other divalent cations, for monovalent sodium cations using a polystyrene resin. The sodium ions come from sodium chloride (common salt). The salt needs to be topped up every 3-4 weeks and sufficient quantities of block salt will be supplied to participants (see Table 1). The water softener used in this study does not require electricity and has two cylinders of resin which are used alternately. A control valve alternates the flow between the two cylinders and ensures a constant supply of regenerated resin. Ion-exchange water softeners typically reduce downstream water hardness to close to zero.

Water-softening units will be installed in the child's principal residence. The water softeners to be used in this trial will be supplied and funded by Harvey Water Softeners Ltd., Woking, UK. Units will be installed in the participants' homes as soon as possible after enrolment and before the child's birth.

Control intervention

Usual hard water supply.

1.2.2 Study population

Pregnant women will be recruited from antenatal care settings

1 Inclusion criteria

- Participant (i.e. the neonate) must have a parent or sibling with a history of doctor-diagnosed atopy (atopic eczema, asthma or hay fever)
- Mother ≥18 years of age at enrolment
- Mother capable of giving informed consent
- Live in a hard water area (>250 mg/L calcium carbonate), as reported by local water supply company
- Occupy a property appropriate for installation of a water softener

2 Exclusion criteria

- Preterm birth (defined as birth prior to 37 completed weeks gestation)
- Significant inflammatory skin disease at birth that would make the detection and/or assessment of eczema difficult
- Sibling (including twin) previously randomised to this trial. If multiple birth, the first child will be followed up in the trial.
- Child has any other serious health issue which, at parent or investigator discretion, would make it difficult for the family to take part in the trial.
- Planned stays away from home for a continuous period of more than 2 weeks, or a total of 1 month out of the 6 month follow up period
- Water softening or filtration device already installed
- Other medical condition that in the opinion of the CI could interfere with the conduct of the trial

1.2.3. Method of allocation of groups

Participants will be randomised antenatally at the time of the engineer home visit to receive either a domestic ion-exchange water softener or their usual water supply, once:

- Antenatal eligibility criteria have been fulfilled;
- Fully informed written consent has been obtained;
- The engineer is satisfied that the softener can be installed

Randomisation will be in a 1:1 ratio using randomly permuted blocks. The randomisation result will be relayed to the installation engineer by telephone as either an 'INSTALL' or 'DO NOT INSTALL' instruction. The randomisation service will be provided by the Medical Statistics Department at King's College London .

1.2.4 Duration of the treatment period

The intervention will last for up to 6 months.

1.2.5 Frequency and duration of follow-up

Participants will complete follow up visits at birth (baseline), 1 month, 3 months, and 6 months after birth.

1.2.6 Visit windows

The visit window for the Baseline and Week 4 visits will be 1 week and +/-2 weeks for the Month 3 and Month 6 visits.

1.3 Data collection

1.3.1 Eligibility screening

Eligibility will be assessed at the initial screening visit by the inclusion and exclusion criteria described in 1.2 above.

1.3.2 Measures

Baseline

The following will be collected:

- Confirm infant eligibility criteria
- Postnatal written consent
- Skin examination
- Infant skincare questions (Has the baby been washed? Any products applied to the skin?)
- Record concomitant infant medications including systemic antibiotic use
- Systemic antibiotic use in mother during pregnancy, including prophylactic antibiotic use during delivery

- Infant co-morbidities
- Systemic medication use in infant (e.g. antibiotics)
- Topical medication use in infant (e.g. topical steroids, topical antibiotics)
- Delivery questions (e.g. mode of birth, gestation)
- Pregnancy outcomes & birth details (e.g. onset of labour, mode of birth, gestation at birth, sex of infant, birthweight, APGAR scores, admission to neonatal unit)
- Method of feeding (e.g. exclusively breast fed, fully breastfed, partially breastfed, mixed feeding, formula feeding)
- Additional mechanistic assessments (procedures marked ‡ will be performed **only** at the Guy's & St Thomas' Hospital site):
    - Tape stripping (forearm) for cutaneous cytokine work‡
    - Transepidermal water loss (TEWL) measurement (forearm)‡
    - Attenuated Total Reflectance Fourier-transform Infrared spectroscopy (ATR-FTIR) measurement (forearm)‡
    - Skin pH measurement (forearm)‡
    - Skin surface hydration (forearm) ‡
    - Microbiome swabs
        - Skin (antecubital fossa & cheek)
        - Nares

1.3.3 Primary outcome measure

Proportion of eligible families* screened who are willing and able to be randomised.

1.3.4 Secondary outcome measures

- Proportion of pregnant women approached who agree to be screened
- Proportion of families eligible on screening that cannot have a water softener installed (e.g. due to landlord or local authority refusal, technical (plumbing) reasons)
- Proportion of families randomised that withdraw due to infant ineligibility
- Proportion of families in intervention arm who found the intervention acceptable
- Proportion of participants in control arm that become exposed to softened water (e.g. by moving to a new home in a soft water area, or moving to a home with an active water softener installed, before the end of follow up)
- Proportion of participants that have the water softening unit removed or disabled prior to end of follow up
- Proportion of participants with visible eczema status (yes/no) recorded at each time point: baseline, 4 weeks, 3 and 6 months
- Proportion of water samples with hardness >20 mg/L calcium carbonate in the intervention arm
- Proportion of participants that withdraw from the trial prior to end of follow up

- Median number of nights spent away from the participant's main home during follow up
- Proportion of clinical outcome assessments that have remained blinded at 4 weeks, 3 & 6 months

- Proportion with patient-reported, doctor-diagnosed atopic eczema by 6 months of age
- Proportion with visible eczema according to the UK diagnostic criteria-based photographic protocol (85) at 4 weeks, 3 & 6 months of age
- Severity of eczema (if present) using Eczema Area and Severity Index (EASI) at 4 weeks, 3 & 6 months of age
- Patient-reported eczema symptoms (Patient-Orientated Eczema Measure - POEM), monthly from 4 weeks to 6 months of age
- Time to onset of patient-reported doctor-diagnosed eczema

- Transepidermal water loss (TEWL)
- Cutaneous cytokine profiles (e.g. interleukin-1 levels)
- Natural moisturising factor (NMF) levels
- Shannon Diversity Index and other skin and upper respiratory microbiota parameters
- Proportion with filaggrin null mutations
- Effect of filaggrin (FLG) gene mutation status on TEWL, cytokine levels, NMF levels and skin microbiota diversity
- Median domestic water hardness level (calcium carbonate concentration)
- Skin surface hydration

1.3.5 Mediators of treatment

No treatment mediators are considered

1.3.6 Moderators of treatment

No treatment moderators are considered

1.3.7 Adverse events

Adverse Event (AE):

Any untoward medical occurrence in a subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.

Adverse Reaction (AR):

Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

Unexpected Adverse Reaction (UAR):

An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the summary of product characteristics (SmPC) for the product.

Serious adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR):

Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that:

- Results in death;
- Is life-threatening;
- Required hospitalisation or prolongation of existing hospitalisation;
- Results in persistent or significant disability or incapacity;
- Consists of a congenital anomaly or birth defect.

1.4 Sample size estimation (including clinical significance)

This is a pilot study and therefore not powered to establish the efficacy of the intervention. The sample size is determined by the available resources. A total of 80 families (40 per group) is judged to provide a sufficiently precise (within 10 percentage points for a 95% confidence interval) estimate of the proportion of families who are willing to be randomised and who will go on to complete the trial.

Of infants included in the Enquiring About Tolerance (EAT) study of primary prevention of food allergy in infants (96), 81% had a parental history of atopy (eczema, asthma or hayfever). In the Barrier Enhancement Eczema Prevention (BEEP) feasibility study 79% of families identified had a history of atopy. Of these families, 42% accepted the invitation to participate (88).

Therefore, a conservative estimate would be that approximately 70% of families screened will have a history of atopy that predisposes to a high risk of eczema in their offspring. Of these, 40-60% would be expected to be willing and able to participate. Home factors also need to be considered: In SWET, 27% of eligible families could not participate because their home was not suitable for installation. The likely proportion that are ineligible due to infant factors is difficult to predict but likely to be low. For example, the stillbirth rate is around 1:1000 and a severe skin condition that might be present at birth, such as Netherton syndrome or Harlequin Ichthyosis, even rarer at 1 in 200,000 to 1 in 300,000. Accordingly, approximately 300 families will need to be approached in order to identify around 80 families at high risk of giving birth to a child with eczema who are willing and able to participate in the study.

1.8 Brief description of proposed analyses

Analyses will be carried out by the Senior Co-Investigator under the supervision of the Senior Statistical Lead. In the first instance data will be analysed under intention-to-treat assumptions (i.e. analyse all those with data in groups as randomised irrespective of treatment received).

2. Data analysis plan – Data description

2.1 Recruitment and representativeness of recruited patients

CONSORT flow chart will be constructed (2) – see Figure 1. This will include the number of eligible participants, number of participants agreeing to enter the trial, number of participants refusing, then by intervention arm: the number of participants not/inadequately/adequately treated, the number continuing through the trial, the number withdrawing, the number lost to follow-up and the numbers excluded/analysed.

The reasons for withdrawal from treatment will be described and summarised.

2.2 Definition of “Not Treated”, “Inadequately Treated” and “Adequately Treated”

Not-treated is defined as not having a water softener installed (water softener arm or placebo). Inadequately treated is defined as not receiving softened water (for any reason e.g. insufficient salt use). Adequately treated is defined as all weekly samples having a reading of ≤ 20 mg/L calcium carbonate.

2.3 Baseline comparability of randomised groups

Baseline descriptions of participants (sex, age in days, gestation at birth, ethnicity, water hardness level, family history of atopy) will be presented by intervention and overall: means and standard deviation or numbers and proportions as appropriate. No significance testing will be carried out.

2.4 Adherence to allocated treatment and treatment fidelity

Absence from home will be used as a measure of treatment adherence.
Treatment fidelity will also be measured by weekly water hardness tests in the intervention arm.

Compliant versus non-compliant with the treatment will be described in each treatment arm and differences will be reported with appropriate summary measures with 95% confidence intervals.

2.5 Loss to follow-up and other missing data

The proportions of participants lost to follow-up will be summarised in each arm and at each time point.

The baseline characteristics of those missing and having completed follow up will be presented. Differences will be reported as appropriate summary measures with 95% confidence intervals.

The reasons for withdrawal from the trial will be summarised.

2.6 Protocol deviations

Numbers and percentages of subjects with protocol deviations will be summarised by deviation type:

- Inclusion/exclusion criteria
- Trial procedure not performed per protocol
- Informed consent
- Participant non-compliance with protocol
- Randomisation error
- Other

2.7 Adverse event reporting

Adverse events (AE), adverse reactions (AR), serious adverse events (SAE) and serious adverse reactions (SAR) will be listed by allocated treatment arm.

3. Data analysis plan – Inferential analysis

3.1 Analysis of treatment differences

This is a feasibility trial and is not powered to detect a treatment difference. The statistical analyses will therefore not estimate the difference in mean outcomes between participants randomised to a water softener and usual hard water.

3.1.1 Analysis of primary outcomes

Feasibility

The number of patients screened and the number and proportion that consent, are eligible, enrol and complete follow up will be reported. Consent and recruitment rates will be presented for both trial arms together. The proportion of patients who complete follow-up will be presented together and by trial arm. All binary feasibility outcomes will be presented with numbers, and proportions with 95% confidence intervals.

- Proportion of eligible families* screened who are willing and able to be randomised.
- Proportion of families screened who are randomised.

*Eligible families will be defined as those who have had a pre-screening approach, or have approached the study team because they saw a poster etc., and had a family history of atopy.

3.1.2 Analysis of secondary outcomes

The following secondary outcomes will be used to help design future studies.
All secondary outcomes will be presented with numbers, and proportions or means/SDs and 95% confidence intervals and are by trial arm and overall, unless stated.

- Proportion of pregnant women approached who agree to be screened
- Proportion of pregnant women approached who agreed to be screened
- Proportion of families eligible on screening that cannot have a water softener installed
- Proportion of families eligible on screening that cannot have a water softener installed (e.g. due to landlord or local authority refusal, technical (plumbing) reasons)
- Proportion of families randomised that withdraw due to infant ineligibility
  Proportion of families in intervention arm who found the study acceptable
- Proportion of families who received a water softener that found the intervention acceptable.
- Proportion of participants in control arm that became exposed to softened water e.g. by moving to a new home in a soft water area, or moving to a home with an active water softener installed, before the end of follow up.
- Proportion of participants that have the water softening unit removed or disabled prior to end of follow up
- Proportion of participants with visible eczema status recorded at each time point: baseline, 4 weeks, 3 and 6 months
- Proportion of participants in the intervention arm with at least one water sample with hardness >20 mg/L calcium carbonate. Median, IQR, minimum, maximum number of samples per participant that were >20mg/L.
- Proportion of participants that withdraw from the trial prior to end of follow up
- Median, IQR, minimum, maximum number of nights spent away from the participant's main home between baseline and end of follow up.
- Proportion of clinical outcome assessments that have remained blinded at 4 weeks, 3 & 6 months

- The number and percent of clinical outcome assessments that have remained blinded at 4 weeks, 3 & 6 months.

Clinical Outcomes

All outcomes will be reported with data summaries and 95% confidence intervals. No significance tests will be conducted since the study was neither designed nor powered to estimate a treatment effect.

- Proportion with patient-reported, doctor-diagnosed atopic eczema by 6 months of age
- Proportion with visible eczema according to the UK diagnostic criteria-based photographic protocol (85) at 4 weeks, 3 & 6 months of age
- Mean and standard deviation for eczema severity (if present) using Eczema Area and Severity Index (EASI) at 4 weeks, 3 & 6 months of age
- Mean and standard deviation for patient-reported eczema symptoms (Patient-Orientated Eczema Measure - POEM), monthly from 4 weeks to 6 months of age
- Kaplan-Meier curves for time to onset of patient-reported doctor-diagnosed eczema.

3.1.4 Additional Mechanistic Outcomes (to be analysed according to separate mechanistic statistical analysis plan):

- Transepidermal water loss (TEWL)
- Cutaneous cytokine profiles (e.g. interleukin-1 levels)
- Natural moisturising factor (NMF) levels
- Shannon Diversity Index and other skin and upper respiratory microbiota parameters
- Proportion with filaggrin null mutations
- Effect of filaggrin (FLG) gene mutation status on TEWL, cytokine levels, NMF levels and skin microbiota diversity
- Median domestic water hardness level (calcium carbonate concentration)
- Skin surface hydration

B) SCHEDULE OF ASSESSMENTS AND MEASURES

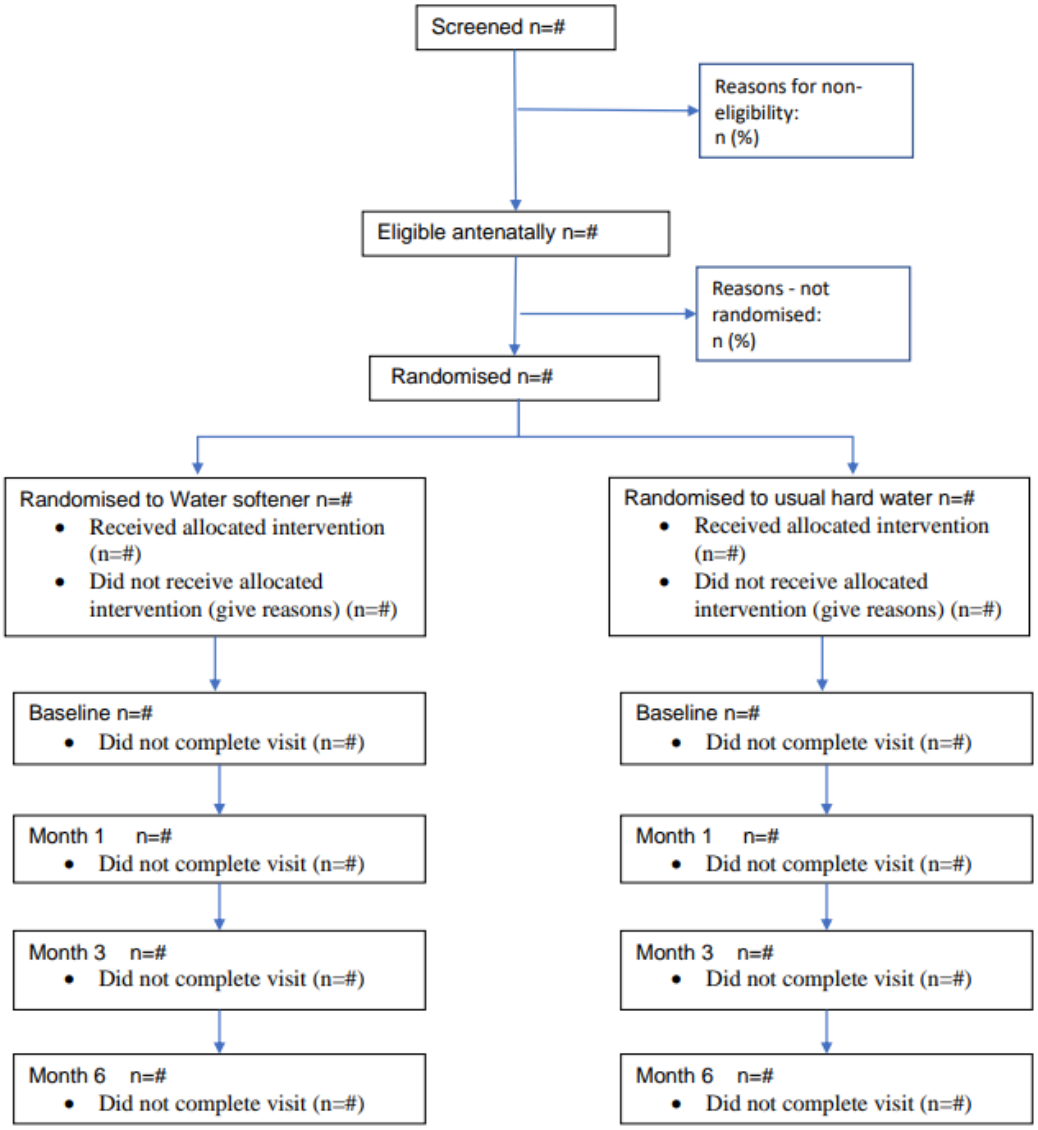
	Screen#	Enrol#	Home Screen & installation	Birth	Base-line (+/- 1 wk)	4 wk (+/- 1 wk)	3 m (+/- 2 wk)	6 m (+/- 2 wk)
Confirm eligibility	X	X			X			
Verbal consent to collect contact details and access antenatal records	X							
Written informed consent		X			X			
Demographic data	X				X			
Engineer home assessment			X					
Install water softener			X					
Randomisation			X					
Visible eczema status						X	X	X
Blinded eczema severity assessment (EASI)						X	X	X
DNA collection from buccal swab						X		
Antenatal factors questionnaire		X						
Acceptability & feedback questionnaire								X
Invite to participate in semi-structured interview about study								X
Collection of skin and nasal microbiome swabs					X	X	X	X
TEWL measurement‡					X	X	X	X
Cutaneous tape stripping‡					X	X	X	X

Skin pH measurement‡					X	X	X	X
ATR-FTIR measurement‡					X	X	X	X
Skin surface hydration‡					X	X	X	X
Monthly infant skin and health* questionnaire, including Patient Orientated Eczema Measure (POEM)						From 4 weeks to 6 months of age		
Weekly water samples (in intervention arm)			From installation to 6 months of age					

Amendments to version 1.0

1. CONSORT diagram updated
2. Adherence to allocated treatment and treatment fidelity definition updated
3. Added text that AEs will be listed by allocated treatment arm.
4. Proportion of families in intervention arm who found the intervention acceptable changed to proportion of families who found the study acceptable.
5. Shell tables added
6. Added section on protocol deviations
7. Various minor corrections of typographical errors and formatting updates

Figure 1. Template CONSORT diagram for SOFTER trial



Shell Tables

TABLE I Baseline characteristics

Characteristic	Total no.	Water softener, no. (%) n= XX	Usual hard water, no. (%), N=XX
Female	XX	XX (XX)	XX (XX)
Birth weight, mean (SD)	XX	XX (XX)	XX (XX)
Caesarean delivery	XX	XX (XX)	XX (XX)
Born in a bathing pool	XX	XX (XX)	XX (XX)
Maternal eczema	XX	XX (XX)	XX (XX)
Maternal atopy	XX	XX (XX)	XX (XX)
Paternal eczema	XX	XX (XX)	XX (XX)
Paternal atopy	XX	XX (XX)	XX (XX)
Maternal antibiotic exposure during pregnancy	XX	XX (XX)	XX (XX)
Lives in a house	XX	XX (XX)	XX (XX)
Urban home location	XX	XX (XX)	XX (XX)
Domestic water $CaCO_3$ mg/L, mean (SD)	XX	XX (XX)	XX (XX)
Ethnic origin	XX	XX (XX)	XX (XX)
Skin surface hydration, mean (SD)	XX	XX (XX)	XX (XX)
Transepidermal water loss, mean (SD)	XX	XX (XX)	XX (XX)
Skin pH, mean (SD)	XX	XX (XX)	XX (XX)

TABLE 2 Adherence to intervention

	Water softener N=XX	Usual hard water N=XX
Number of nights spent away from main residence, mean (±SD) [N}	XX (XX) [XX]	XX (XX) [XX]

N: total number of participants, [N] number of participants with complete data, SD standard deviation

TABLE 3 Clinical outcome measures

Outcome	Water softener N=XX	Usual hard water N=XX
Patient-reported, doctor-diagnosed atopic eczema by 6 months of age, No. (%) [N]	XX (XX) [XX]	XX (XX) [XX]
Visible eczema at 4 weeks, No. (%)	XX (XX) [XX]	XX (XX) [XX]
Visible eczema at 3 months of age, No. (%)	XX (XX) [XX]	XX (XX) [XX]
Visible eczema at 6 months of age, No. (%)	XX (XX) [XX]	XX (XX) [XX]
Visible eczema by 6 months of age, No. (%)	XX (XX) [XX]	XX (XX) [XX]
Time to onset of patient-reported doctor-diagnosed eczema (weeks), mean (SD)	XX (XX) [XX]	XX (XX) [XX]
EASI at 4 weeks, mean (SD) [N]	XX.X (XX) [XX]	XX.X (XX) [XX]
EASI at 3 months, mean (SD) [N]	XX.X (XX) [XX]	XX.X (XX) [XX]
EASI at 6 months, mean (SD) [N]	XX.X (XX) [XX]	XX.X (XX) [XX]
POEM at 4 weeks, mean (SD) [N]	XX.X (XX) [XX]	XX.X (XX) [XX]
POEM at 3 months, mean (SD) [N]	XX.X (XX) [XX]	XX.X (XX) [XX]
POEM at 6 months, mean (SD) [N]	XX.X (XX) [XX]	XX.X (XX) [XX]

EASI – eczema area and severity index, N: total number of participants, [N] number of participants with complete data, POEM – patient-oriented eczema measure; SD standard deviation