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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 trial (SOFTER)

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15 **Protocol for an outcome assessor-blinded pilot randomised controlled trial of an ion-exchange**
16 **water softener for the prevention of atopic eczema in neonates, with an embedded mechanistic**
17 **study: The SOFTened waTER for eczema prevention trial (SOFTER)**
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ABSTRACT

Introduction

Atopic eczema is a common chronic inflammatory skin condition with a typical onset within the first two years of life. It affects around 20% of UK children, and environmental factors, in addition to genetic ones, are important in its aetiology. Several observational studies suggest an increased risk of atopic eczema in children living in hard compared to soft water areas. The SOFtened watER for eczema prevention (SOFTER) pilot trial aims to test the feasibility of installing domestic ion-exchange water softeners around the time of birth to reduce the risk of atopic eczema in children with a family history of atopy. A further aim is to explore the pathophysiological mechanisms for this in an embedded mechanistic study.

Methods and analysis

Multi-centre parallel group assessor-blinded randomised controlled pilot trial. Participants are newborn babies (n=80) living in a hard water (>250 mg/L calcium carbonate) area at risk of developing atopic eczema because of a family history of atopy. Participants will be randomised prior to birth in a 1:1 ratio. The intervention group will have an ion-exchange water softener installed prior to birth. The control group will receive their usual domestic hard water supply. Follow up will be until six months of age. Data will be collected at birth (baseline), 1, 3 and 6 months of age. The main outcome is the proportion of eligible families screened who are willing and able to be randomised. Several secondary feasibility and clinical endpoints will also be evaluated, alongside mechanistic outcomes. Data will be analysed on an intention-to-treat basis. There will be no hypothesis testing for the clinical outcomes. Study acceptability will be evaluated through semi-structured interviews.

Ethics and dissemination

This study has been reviewed and given a favourable opinion by the North West – Liverpool East Research Ethics Committee (Ref: 17/NW/0661).

Registration details

Clinicaltrials.gov: NCT03270566.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study addresses a gap in research around the primary prevention of atopic eczema.
- In addition, this study aims to explore the likely mechanisms by which water softeners might prevent atopic eczema.
- SOFTER uses a commercially available, low-maintenance ion-exchange water softener as the intervention
- The study is outcome-assessor blinded as participant blinding is not possible to achieve.
- SOFTER is a pilot trial and so will not definitively answer the question of whether installation of a domestic water softener will prevent eczema in babies at high risk of developing atopic eczema, but will provide information as to how a large multicentre definitive trial on this topic can be undertaken.

INTRODUCTION

Background & Rationale

Atopic eczema (syn. atopic dermatitis, hereinafter referred to as 'eczema') is a common inflammatory skin condition affecting around 20% of UK children.¹ It is associated with significant morbidity and affects health-related quality of life. The cause of eczema is not fully understood. It is likely to be multifactorial, and several genetic and environmental factors have been identified.² No primary prevention strategy has been established.³ However, several approaches have been proposed such as probiotics during pregnancy, dietary supplementation, house dust mite avoidance, intensive emollient use and domestic water softening.⁴

Relationship between hard water and atopic eczema

Hard water is the result of dissolved minerals, mainly calcium carbonate and magnesium carbonate, from the percolation of water through rock in the environment. England, especially in the south, has very hard (>250 mg/L calcium carbonate) domestic water. A cross-sectional study conducted in the 1990s 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 around Nottingham, UK.⁵ Two further cross-sectional studies among school children conducted in Japan and Spain confirmed this association.^{6,7} Subsequently, a cross-sectional analysis from a cohort study among over 1,300 infants in England and Wales has also confirmed this relationship in early life, even after adjusting for likely confounders.⁸ The same study suggested a possible interaction with loss-of-function mutations in the skin barrier gene, filaggrin (*FLG*). Most recently, a large study from a Danish birth cohort found a 5% increase in prevalence of eczema within the first 18 months of life for each 5 unit increase in domestic water hardness (range, 6.60-35.90 German degrees of hardness [118-641 mg/L calcium carbonate]).⁹

Potential mechanisms for hard water causing atopic eczema

Several potential mechanisms have been proposed for the way in which hard water may lead to eczema development: 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, resulting in increased protease activity, could all have a detrimental effect on skin barrier function.⁸ Experimental work has demonstrated an increased deposition of SLS in skin washed with hard water vs. softened water.¹⁰ In animal studies using 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.¹¹

Eczema is associated with a preponderance of *Staphylococcus aureus* and a reduction in microbial diversity.¹² Recent work has identified a synergistic relationship between the human cathelicidin-related antimicrobial peptide LL-37 and antimicrobial peptides produced by coagulase negative staphylococcal species that selectively kill *Staphylococcus aureus*.¹³ Human LL-37 activity against some bacterial species is decreased by the presence of calcium, but not magnesium, ions.¹⁴

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.¹⁵ Early life is likely to be an important time in the development of eczema, particularly as most eczema develops before 2 years of age. 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.¹⁶ 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).¹⁷ To date, there are no published studies examining the role of water softeners in the prevention of eczema.¹⁸

Rationale

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

This pilot trial builds on the experience gained from the NIHR-funded SWET trial¹⁵ and a trial of emollients in early life (Barrier Enhancement for Eczema Prevention; BEEP),¹⁹ both of which were coordinated by Prof K Thomas and Dr J Chalmers, our collaborators on this trial, who are based at the Centre of Evidence Based Dermatology in Nottingham. This is a pilot trial, as defined by the UK National Institute for Health Research in that it is a 'version of the main study that is run in miniature to test whether the components of the main study can all work together'.²⁰ This pilot trial will therefore not definitively answer the question of whether installation of a domestic water softener will prevent eczema. The rationale for performing this pilot study is 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 will be important to determine whether the planned trial recruitment and assessment procedures are possible and workable, or whether they require adapting or changing.²¹

Trial Objectives

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 eczema. A further aim is to explore the pathophysiological mechanisms for this in an embedded mechanistic study.

METHODS AND ANALYSIS

Trial design

This is a multi-centre parallel group assessor-blinded randomised 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. Eighty newborn infants will be enrolled into the trial for a period of 6 months. The end of study is defined as the final assessment visit of the last participant to enter the trial. Recruitment commenced in February 2018 and is due to end in Spring 2019.

Pregnant women will be 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 flowchart in **Figure 1**.

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 will join the independent trial steering committee. Once the trial has been published, participants will be sent details of the results in a study newsletter suitable for a non-specialist audience.

Trial population

The study will recruit pregnant women living in hard water areas who will be identified from antenatal services at local NHS Trust sites. Women will be approached at the 20 week anomaly scan and asked if they would be interested in participating. We will also publicise the study through posters and by making clinical midwifery teams aware of the study. Full inclusion and exclusion criteria are given in **Table 1**.

Intervention

A domestic ion-exchange water softener will be 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, amongst other divalent cations, for monovalent sodium cations, typically reducing downstream water hardness to close to zero. The sodium ions come from common salt which needs to be topped up every 3-4 weeks. The water softeners to be used in this trial will be supplied and funded by Harvey Water Softeners Ltd., Woking, UK. Standard procedure will be to soften all water in the home and provide unsoftened mains drinking water 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 will be given the option to purchase the water softener from Harvey Water Softeners Ltd. at a reduced price. Alternatively, it will be removed.

Potential risks

This is a low risk trial as the intervention involves a commercially available ion exchange water softener that has no known clinical side effects. There is a potential, but low risk, of damage to participants' properties during installation of the water softening unit. However, these will be installed by a qualified water engineer according to the Code of Practice produced by British Water and in accordance with the recommendations of the WRAS (Water Regulations Advisory Service).

Concomitant medications and skincare

No restrictions will be placed on the use of concomitant treatment a child might receive. All concomitant medication will be 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 will not provide specific skincare advice to participants. However, data on such use will be sought from parents via monthly online questionnaires.

Co-enrolment guidelines

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

Participant compliance

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

Primary outcome

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).

Secondary feasibility outcomes

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

1. Pregnant women approached who agree to be screened
2. Families eligible on screening that cannot have a water softener installed (e.g. 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

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5. Participants in control arm who 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)
 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 & 6 months

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 protocol²²
3. Severity of eczema (if present) using Eczema Area and Severity Index (EASI)
4. Patient-reported eczema symptoms (Patient-Orientated Eczema Measure – POEM) score
5. Time to onset of patient-reported doctor-diagnosed eczema

Additional mechanistic outcomes

The following will be assessed as change from baseline until follow up at 6 months in the intervention compared to the control group:

1. Transepidermal water loss (TEWL)
2. Cutaneous cytokine profiles (e.g. interleukin-1 levels)
3. Natural moisturising factor (NMF) 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

Adverse events

This trial involves use of a commonly available domestic water softening unit with provision for separate mains drinking water during the time when the water softening unit is installed. Accordingly, we do not anticipate any adverse events or adverse reactions related to the trial intervention. A contact number for the service department at Harvey Water Softeners will be provided and an engineer will be sent to resolve the issue, if needed. Details of technical issues reported to the service department will be reported to the study principal investigators. Events of particular relevance such as plumbing difficulties, floods or difficulties with the units will be logged and reported to the Research Ethics Committee and relevant Research and Development departments annually.

Sample size

This is a pilot study and therefore not powered to establish the efficacy of the intervention. 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.

Findings from the Enquiring About Tolerance (EAT) study⁸ and the Barrier Enhancement Eczema Prevention (BEEP) feasibility study²³, 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% 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.¹⁵

Informed consent

Written informed antenatal consent will be obtained at the enrolment visit from the mother. The consent form will be signed and dated before entering the trial and participants are reminded that they may withdraw from the trial at any time. Mothers will be re-consented postnatally to ensure they agree to their child taking part in the study.

Randomisation and blinding

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

Participants will be randomised in a 1:1 ratio to one of the two treatment arms based on a computer-generated code. 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 researchers at King's College London who are not involved in the study, via telephone.

Experience from the SWET trial has shown that the effects of a functional water softener are too noticeable to allow participants to be blinded¹⁵. Skin examinations and measurements will be performed by research team members who will be blinded to treatment allocation. Participants will be encouraged not to disclose allocation. Results will be analysed based on treatment code, using a statistical analysis plan finalised prior to database lock and any data analysis. Only after the analyses are completed will the actual treatment arms corresponding to treatment codes be revealed. Study team members in direct contact with study participants will be trained in the study protocol and importance of demonstrating equipoise.

Visit schedule and study procedures

The schedule for assessments during the study are shown in **Table 2**.

Enrolment visit (up until 36 weeks gestation)

- Confirm eligibility (see Table 1)
- Answer questions about study
- Written consent is taken
- Antenatal history (including antibiotic exposure, probiotic and omega 3 use), family medical history and environment questionnaire
- Arrange water softener installation

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

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 eligible, the engineer will telephone a given number at King's College London to determine the randomisation group.
- Install water softener and provide water sample materials if randomised to the intervention arm.

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

- Confirm infant eligibility criteria
- Postnatal written consent
- Skin examination
- Infant skincare questions
- Record concomitant infant medications including systemic antibiotic use
- Systemic antibiotic use in mother during pregnancy, including prophylactic antibiotic use during delivery
- Infant co-morbidities
- Topical medication use in infant
- Delivery questions
- Pregnancy outcomes & birth details
- Additional mechanistic assessments (procedures marked ‡ will be performed only at the Guy's & St Thomas' Hospital site):
 - Tape stripping (forearm)
 - 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) ‡

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3 ➤ Microbiome swabs from skin (antecubital fossa & cheek) and nares
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5 *4-week visit (+/- 1 week)*
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- 7 • Skin examination including eczema severity assessment (EASI), if appropriate
8 • Mechanistic assessments (procedures marked † will only be performed at the Guy's & St
9 Thomas' Hospital site):
10 ○ Tape stripping (forearm)
11 ○ TEWL measurement (forearm)[†]
12 ○ ATR-FTIR measurement (forearm)[†]
13 ○ Skin pH measurement (forearm)[†]
14 ○ Skin surface hydration (forearm)[†]
15 ○ Microbiome swabs
16 ○ Skin (antecubital fossa & cheek)
17 ○ Nares
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19 • Buccal swab for DNA for *FLG* mutation analyses
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24 *3-month visit (+/- 2 weeks)*
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- 26 • Skin examination including eczema severity assessment (EASI), if appropriate
27 • Mechanistic assessments (procedures marked † will be performed only at the Guy's & St
28 Thomas' Hospital site):
29 ○ Tape stripping (forearm)
30 ○ TEWL measurement (forearm)[†]
31 ○ ATR-FTIR measurement (forearm)[†]
32 ○ Skin pH measurement (forearm)[†]
33 ○ Skin surface hydration (forearm)[†]
34 ○ Microbiome swabs
35 ○ Skin (antecubital fossa & cheek)
36 ○ Nares
37
38 • Buccal swab for DNA for *FLG* mutation analyses if unable to collect at 4 week visit
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43 *6-month visit (+/- 2 weeks)*
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- 45 • Acceptability questionnaire
46 • Skin examination including eczema severity assessment (EASI), if appropriate
47 • Mechanistic assessments (procedures marked † will be performed only at the Guy's & St
48 Thomas' Hospital site):
49 ○ Tape stripping (forearm)
50 ○ TEWL measurement (forearm)[†]
51 ○ ATR-FTIR measurement (forearm)[†]
52 ○ Skin pH measurement (forearm)[†]
53 ○ Skin surface hydration[†]
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- Microbiome swabs
 - Skin (antecubital fossa & cheek)
 - Nares
- Buccal swab for DNA for *FLG* mutation analyses if unable to collect at 3 month visit
- Arrange date for removal of water softener within 1 month if not planning to purchase (intervention group) or installation of water softener for 6 month period, if desired, in control arm
- Invite to participate in semi-structured qualitative interview about study (via telephone or in person) and seek verbal consent for this

Monthly email messages (intervention group)

- Remind participants to complete questionnaires
- Remind participants' parents to refill the unit with salt
- Invite to contact Harvey Water Softeners Ltd. if any issues with water softener.

Monthly email messages (control group)

- Remind participants to complete questionnaires

Monthly electronic questionnaires

A secure web-based questionnaire link using the Snap Surveys platform will be 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, hygiene, confirm residence/time away from main residence and infant general health. A paper copy of the questionnaire can be posted to participants if they prefer or if they do not have internet access.

At the end of the study, i.e. 6 month visit, a date will be arranged for removal of the water softener within 1 month if the participating family does not want to purchase the device (intervention group). Control arm participants can purchase the same water softener at the end of the study. Participants will also be invited to participate in a semi-structured qualitative interview about the study.

Embedded mechanistic sub-study

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

Attenuated Total Reflectance (ATR) Fourier-Transform Infrared (FTIR) spectroscopy measurements will be performed using the Agilent 4300 ATR-FTIR device (Agilent Technologies, USA) to measure the deposition of detergents, such as sodium lauryl sulphate, on the skin surface.²⁴

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3 Transepidermal water loss (TEWL) provides a standardised measure of skin barrier dysfunction²⁵ and
4 there is evidence to suggest that elevated TEWL in neonates is predictive of subsequent eczema.^{16 26}
5 We will use the AquaFlux AF200 condensing chamber probe (Biox Systems Ltd, London, UK) to
6 measure TEWL. Epidermal stratum corneum hydration will be measured using a CM 825
7 Corneometer (Courage and Khazaka, Cologne, Germany).
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10 Skin surface pH has an important role in the regulation of epidermal and microbial cellular process
11 and will be measured using a PH905 Skin-Surface-pH probe fitted with a Mettler and Toledo flat
12 surface electrode (Courage and Khazaka, Cologne, Germany).²⁷
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14 Skin protein samples will be collected from the skin by applying and removing eight consecutive D-
15 Squame cutaneous stripping discs (CuDerm Corporation, Dallas, USA). Samples will be collected to
16 perform the protein and cytokine analyses, such as IL-1 α levels.²⁸
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19 The filaggrin gene (*FLG*) encodes profilaggrin protein and its subsequent breakdown products, are
20 key to normal barrier function of the skin. Loss-of-function sequence variants in *FLG* are associated
21 with an increased risk of eczema (Palmer, 2006). Enquiring About Tolerance study suggests that
22 there might be an interaction between *FLG* and water hardness.⁸ We will examine the relationship
23 between *FLG* mutation status and risk of eczema in a sub-group analysis and explore the relationship
24 between *FLG* mutation status and skin microbiome diversity, TEWL and cutaneous cytokine
25 expression. DNA will be extracted by standard techniques and *FLG* whole gene sequencing will be
26 used for genotyping *FLG* loss-of-function sequence variants and the presence of intragenic copy
27 number variations (Wong et al JACI, 2017). Buccal swab for DNA will be collected once, during the 4
28 week visit if consent is given. If unable to collect at the 4 week visit, collection will be attempted at 3
29 month visit or 6 month visit.
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35 We will sample the antecubital fossa and the cheek, using Isohelix® bacterial DNA swabs (Cell
36 Projects, Harrietsham, UK). A further swab will be taken from each nostril using an Ultra Minitip
37 flocked swab (Copan Flock Technologies Srl, Brescia, Italy). A control sample is taken for each
38 participant. We will use Illumina MiSeq V1-V3 Amplicon sequencing of the 16S ribosomal subunit to
39 characterise both skin and nasal bacteria communities with regard to species diversity. We will
40 examine within-participant changes in microbial diversity over time and explore the relationship
41 between water hardness, microbial diversity and *FLG* gene mutational profile as well as taking into
42 account environmental factors such as mode of delivery and antibiotic use in the mother and infant.
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46 **Statistical analysis**

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48 The feasibility parameters of this pilot trial will not be formally statistically tested. The focus will
49 therefore be on descriptive statistics and confidence intervals. A separate and full statistical analysis
50 plan (SAP) for the feasibility trial and analysis of mechanistic data will be developed prior to unblinding
51 and any analysis of the data.
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54 Randomisation will be in a 1:1 ratio using randomly permuted blocks. Participants' data will be
55 analysed on an intention-to-treat basis. There will be no hypothesis testing for the clinical outcomes
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except in relation to the embedded mechanistic study. Results will be presented as estimates with 95% confidence intervals, where appropriate.

For peer review only

ETHICS AND DISSEMINATION

This study has been reviewed and given a favourable opinion by the North West – Liverpool East Research Ethics Committee (Ref: 17/NW/0661). It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. We will send participating families a summary of the pilot trial results.

For peer review only

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AUTHORS' CONTRIBUTIONS

CF is the Chief Investigator with overall responsibility for the SOFTER pilot trial. He conceived the idea for the study, contributed to the writing of the trial protocol, and reviewed this manuscript. ZKJ-L is the Senior Co-investigator responsible for designing and running the SOFTER pilot trial. He led on the writing of the trial protocol and drafted the manuscript. JLP is the Senior Statistical Advisor and contributed to the writing of the trial protocol and reviewed the manuscript. NG coordinates the study, reviewed the trial protocol and drafted the manuscript. DG, AB, JC, KT, TF, SK, HHK, JAS, JEAC, SD, MJC were involved in finalising the study protocol and reviewing of the manuscript.

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- Supply and installation of the water softening units.
- Salt supplies
- Testing of the water samples

DISCLAIMER

The views expressed are those of the authors and not necessarily those of the UK National Health Service, the UK Department of Health and Social Care or the National Institute of Health Research.

COMPETING INTERESTS STATEMENT

C.F. has received investigator-led research funding from Sanofi. His department has received clinical trial funding from Sanofi and AbbVie to test novel therapeutics in paediatric atopic eczema patients. M.J.C. is an Investigator and Consultant for Regeneron, Sanofi Genzyme, Pfizer, Leo, Galapagos, Novartis, Boots, L'Oreal, Dermavant, Menlo, Reckitt Benckiser, Oxagen, Johnson&Johnson, Hyphens, Astellas, Amlar, Abbvie, Galderma, Procter&Gamble. The other authors declare that they have no competing interests.

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TABLE 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 | |
| 11 | Maternal consent for her neonate to participate in the study |
| Exclusion criteria | |
| 12 | Preterm birth (defined as birth prior to 37 weeks gestation) |
| 13 | Significant inflammatory skin disease at birth not including seborrheic dermatitis (“cradle cap”) |
| 14 | Sibling (including twin) previously randomised to this trial. If multiple birth, the first child will be randomised into the trial. |
| 15 | Any immunodeficiency disorder or severe genetic skin disorder |
| 16 | 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. |
| 17 | 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 |
| 18 | Water softening or filtration device already installed |
| 19 | Concurrent enrolment in any other skin-related intervention study |
| 20 | Other medical condition that in the opinion of the Chief Investigator could interfere with the conduct of the trial |

TABLE 2 – Schedule of study assessments and procedures

| | Screening# | Enrolment# | 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) score | | | | | | From 4 weeks to 6 months of age | | |
| Weekly water samples (in intervention arm) | | | | From installation to 6 months of age | | | | |

*Any other concomitant illnesses that develop 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

Abbreviations: DNA – deoxyribonucleic acid; EASI – Eczema Area and Severity Index; m – month; POEM – Patient Oriented Eczema Measure; TEWL – transepidermal water loss; Wk – week

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3 **FIGURE LEGEND**
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5 **FIGURE 1 – Study flowchart**
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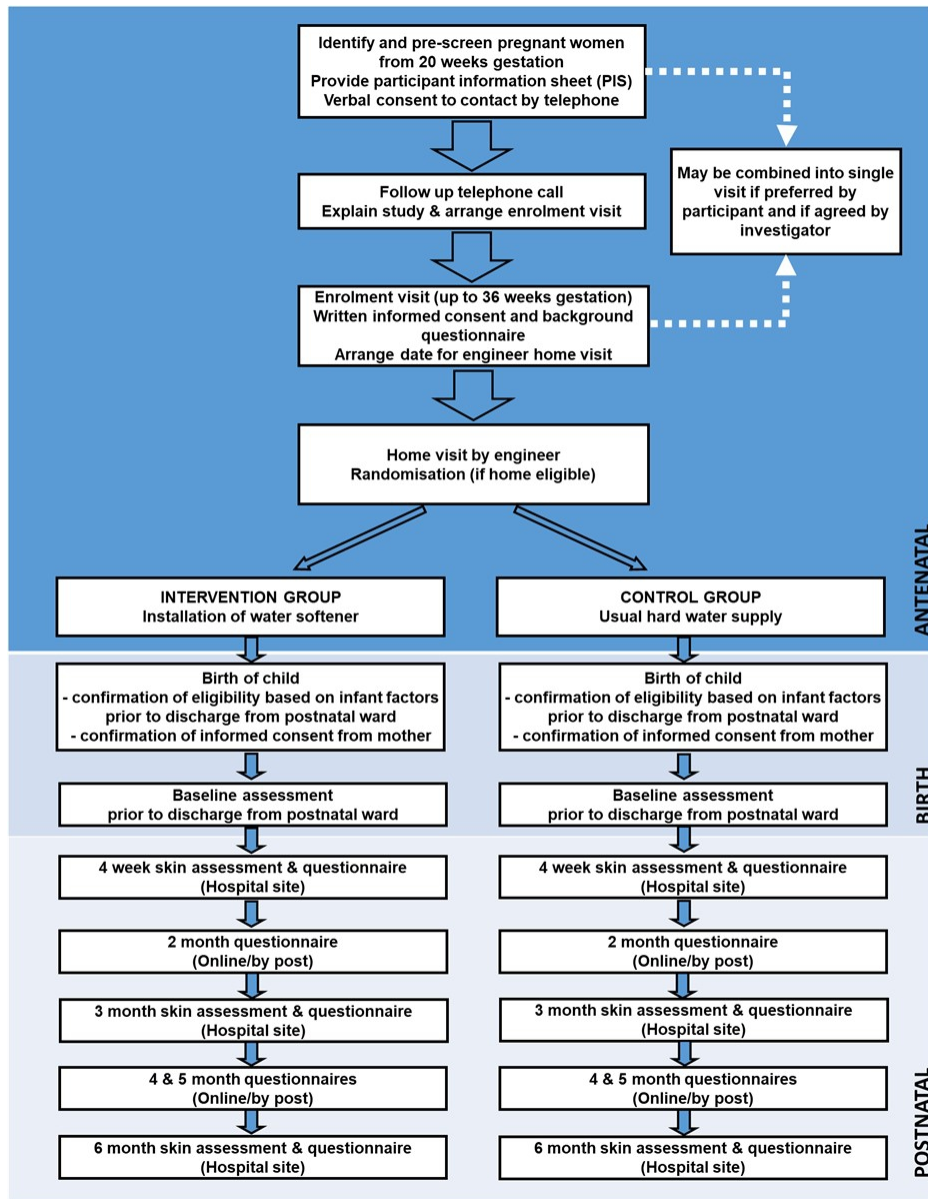


FIGURE 1 - Study flowchart

245x317mm (96 x 96 DPI)

BMJ Open

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 trial (SOFTER)

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| Primary Subject Heading: | Dermatology |

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| | |



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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 trial (SOFTER)

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ABSTRACT

Introduction

Atopic eczema affects 20% of UK children, and environmental factors are important in its aetiology. Several observational studies suggest an increased risk of atopic eczema in children living in hard water areas. The SOFTened water for eczema prevention (SOFTER) pilot trial tests the feasibility of installing domestic ion-exchange water softeners around the time of birth to reduce the risk of atopic eczema in children with a family history of atopy. A further aim is to explore the pathophysiological mechanisms for this in an embedded mechanistic study.

Methods and analysis

Multi-centre parallel group assessor-blinded randomised controlled pilot trial. Participants are newborn babies (n=80) living in a hard water (>250 mg/L calcium carbonate) area at risk of developing atopic eczema because of a family history of atopy. Participants will be randomised prior to birth in a 1:1 ratio. The intervention group will have an ion-exchange water softener installed prior to birth. The control group will receive their usual domestic hard water supply. Follow up will be until six months of age. Data will be collected at birth (baseline), 1, 3 and 6 months of age. The main outcome is the proportion of eligible families screened who are willing and able to be randomised. Several secondary feasibility and clinical endpoints will also be evaluated, alongside mechanistic outcomes. Data will be analysed on an intention-to-treat basis. There will be no hypothesis testing for the clinical outcomes. Study acceptability will be evaluated through semi-structured interviews.

Ethics and dissemination

This study has been reviewed and given a favourable opinion by the North West – Liverpool East Research Ethics Committee (Ref: 17/NW/0661). The results of the study will be reported at international conferences and in peer-reviewed scientific journals. We will send participating families a summary of the pilot trial results.

Registration details

Clinicaltrials.gov: NCT03270566.

ARTICLE SUMMARY

Strengths and limitations of this study

- This pilot study comprehensively tests the design of a a randomised controlled trial for the primary prevention of atopic eczema using a mixture of quantitative and qualitative methods.
- In addition, various mechanistic measurements will further our understanding of the mechanisms by which water softeners might prevent atopic eczema.
- This study is being performed in two centres to gain a wider understanding of potential barriers and facilitators to recruitment and retention in a multicentre definitive trial.
- The study is outcome-assessor blinded as participant blinding is not possible to achieve.
- Follow up is only for 6 months and so may not provide full information on post-randomisation losses to follow up that could occur in a future definitive trial with a longer duration of follow up.

INTRODUCTION

Background & Rationale

Atopic eczema (syn. atopic dermatitis, hereinafter referred to as 'eczema') is a common inflammatory skin condition affecting around 20% of UK children.¹ It is associated with significant morbidity and affects health-related quality of life. The cause of eczema is not fully understood. It is likely to be multifactorial, and several genetic and environmental factors have been identified.² No primary prevention strategy has been established.³ However, several approaches have been proposed such as probiotics during pregnancy, dietary supplementation, house dust mite avoidance, intensive emollient use and domestic water softening.⁴

Relationship between hard water and atopic eczema

Hard water is the result of dissolved minerals, mainly calcium carbonate and magnesium carbonate, from the percolation of water through rock in the environment. England, especially in the south, has very hard (>250 mg/L calcium carbonate) domestic water. A cross-sectional study conducted in the 1990s 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 around Nottingham, UK.⁵ Two further cross-sectional studies among school children conducted in Japan and Spain confirmed this association.^{6 7} Subsequently, a cross-sectional analysis from a cohort study among over 1,300 infants in England and Wales has also confirmed this relationship in early life, even after adjusting for likely confounders.⁸ The same study suggested a possible interaction with loss-of-function mutations in the skin barrier gene, filaggrin (*FLG*). Most recently, a large study from a Danish birth cohort found a 5% increase in prevalence of eczema within the first 18 months of life for each 5 unit increase in domestic water hardness (equivalent to 89.2 mg/L calcium carbonate)⁹ over a range of 6.60-35.90 German degrees of hardness [118-641 mg/L calcium carbonate]).¹⁰

Potential mechanisms for hard water causing atopic eczema

Several potential mechanisms have been proposed for the way in which hard water may lead to eczema development: 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, resulting in increased protease activity, could all have a detrimental effect on skin barrier function.⁸ Experimental work has demonstrated an increased deposition of SLS in skin washed with hard water vs. softened water.¹¹ In animal studies using a hairless mouse model, low extracellular concentrations of calcium ions in the

1
2
3 upper epidermis led to exocytosis of lamellar bodies, required for skin barrier repair, independent of
4 skin barrier disruption.¹²

5
6 Eczema is associated with a preponderance of *Staphylococcus aureus* and a reduction in microbial
7 diversity.¹³ Recent work has identified a synergistic relationship between the human cathelicidin-related
8 antimicrobial peptide LL-37 and antimicrobial peptides produced by coagulase negative staphylococcal
9 species that selectively kill *Staphylococcus aureus*.¹⁴ Human LL-37 activity against some bacterial
10 species is decreased by the presence of calcium, but not magnesium, ions.¹⁵

17 18 **Trials of water softeners in atopic eczema**

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20 The multicentre Softened Water Eczema Trial (SWET), completed in 2011, examined the role of water
21 softeners in treating children with established, moderate-to-severe eczema and found no overall
22 benefit in terms of eczema severity reduction.¹⁶ However, the factors that drive the development of
23 eczema may not be the same as those that determine disease exacerbations and severity and the
24 negative result in the SWET trial does therefore not exclude a role for water softeners in prevention
25 trials. Early life is likely to be an important time in the development of eczema, particularly as most
26 eczema develops before 2 years of age. Early interactions between genes and the environment may
27 be crucial in instigating the cycle of inflammation and skin barrier dysfunction seen in eczema. Indeed,
28 skin barrier dysfunction at just one week of age, as measured by transepidermal water loss, is a
29 predictor of subsequent eczema risk.¹⁷ A small pilot randomised controlled double-blind crossover trial
30 of 12 patients aged 3-6 years with mild-moderate eczema compared ultra-pure soft water to tap
31 water. After 6 weeks, no statistically significant differences in eczema area severity index (EASI) or
32 transepidermal water loss (TEWL) were observed between the groups, although there was a
33 statistically significant improvement in pruritus as measured by visual analogue score (-2.10, 95% CI -
34 4.14, -0.063).¹⁸ To date, there are no published studies examining the role of water softeners in the
35 prevention of eczema.¹⁹

52 **Rationale**

53
54 The overall rationale is that by installing a domestic water softener around the time of birth, the infant
55 will be exposed to softened water rather than hard water for bathing and that this will be less irritating
56 to the skin than hard water and so associated with a lower risk of eczema development.

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3 This pilot trial builds on the experience gained from the NIHR-funded SWET trial¹⁶ and a trial of
4 emollients in early life (Barrier Enhancement for Eczema Prevention; BEEP),²⁰ both of which were
5 coordinated by Prof K Thomas and Dr J Chalmers, our collaborators on this trial, who are based at the
6
7 Centre of Evidence Based Dermatology in Nottingham. This is a pilot trial, as defined by the UK National
8
9 Institute for Health Research in that it is a 'version of the main study that is run in miniature to test
10
11 whether the components of the main study can all work together'.²¹ This pilot trial will therefore not
12
13 definitively answer the question of whether installation of a domestic water softener will prevent eczema.
14
15 The rationale for performing this pilot study is that a definitive trial on eczema prevention using domestic
16
17 water softening devices would require a much larger number of participants and so prior to embarking
18
19 on a larger multicentre trial it will be important to determine whether the planned trial recruitment and
20
21 assessment procedures are possible and workable, or whether they require adapting or changing.²²
22
23

24 **Trial objectives**

25
26 The aim of this pilot trial is to determine the feasibility of undertaking a large-scale definitive trial to
27
28 determine whether installation of domestic ion-exchange water softeners around the time of birth
29
30 reduces the risk of high-risk children developing eczema. A further aim is to explore the
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32 pathophysiological mechanisms for this in an embedded mechanistic study.
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METHODS AND ANALYSIS

Trial design

This is a multi-centre parallel group assessor-blinded randomised 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. Eighty newborn infants will be enrolled into the trial for a period of 6 months. The end of study is defined as the final assessment visit of the last participant to enter the trial. Recruitment commenced in February 2018 and is due to end in autumn 2019.

Pregnant women will be 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 flowchart in **Figure 1**.

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 will join the independent trial steering committee. Once the trial has been published, participants will be sent details of the results in a study newsletter suitable for a non-specialist audience.

Trial population

The study will recruit pregnant women living in hard water areas who will be identified from antenatal services at local NHS Trust sites. Women will be approached at the 20 week anomaly scan and asked if they would be interested in participating. We will also publicise the study through posters and by making clinical midwifery teams aware of the study. Full inclusion and exclusion criteria are given in

Table 1.

Intervention

A domestic ion-exchange water softener will be 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, amongst other divalent cations, for monovalent sodium cations, typically reducing downstream water hardness to close to zero. The sodium ions come from common salt which

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3 needs to be topped up every 3-4 weeks. The water softeners to be used in this trial will be supplied and
4 funded by Harvey Water Softeners Ltd., Woking, UK. Standard procedure will be to soften all water in
5 the home except the drinking water tap. Unsoftened mains drinking water will be delivered through the
6 existing kitchen tap wherever possible, or otherwise through an extra (faucet-style) tap installed at the
7 side of the kitchen sink. At the end of the study, all participants will be given the option to purchase the
8 water softener from Harvey Water Softeners Ltd. at a reduced price of £399.00 inclusive of VAT,
9 installation and warranty; this is approximately a quarter of the full retail price (£1,678.80).. Alternatively,
10 it will be removed.

11 *Potential risks*

12 This is a low risk trial as the intervention involves a commercially available ion exchange water softener
13 that has no known clinical side effects. There is a potential, but low risk, of damage to participants'
14 properties during installation of the water softening unit. However, these will be installed by a qualified
15 water engineer according to the Code of Practice produced by British Water and in accordance with the
16 recommendations of the WRAS (Water Regulations Advisory Service).

17 **Concomitant medications and skincare**

18 No restrictions will be placed on the use of concomitant treatment a child might receive. All concomitant
19 medication will be recorded at baseline and updated at follow up visits. Given the lack of a consistent
20 approach to neonatal skincare, and the possible interaction between some wash products and hard
21 water, we will not provide specific skincare advice to participants. However, data on such use will be
22 sought from parents via monthly online questionnaires.

23 **Co-enrolment guidelines**

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

26 **Participant compliance**

27 Compliance with treatment does not represent a large problem for this trial as long as the participants
28 are not absent from home for long periods of time and remember to replenish the salt. Reminders about
29 salt replenishment will be sent. To check that the units are working correctly, participants will be asked
30 to send weekly water samples to Harvey Water Softeners using prepaid envelopes. Any samples with
31 a reading of >20 mg/L calcium carbonate will be referred back to the engineer for investigation. If
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3 participants move home during the trial, attempts will be made to reinstall the device in the new property.

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5 It is anticipated that loss to follow-up will be <15%.

6 7 **Primary outcome**

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9 Proportion of eligible families screened who are willing and able to be randomised. This is key to the
10 determination of the likely success of a future, large-scale definitive randomised controlled trial (RCT).

11 12 **Secondary feasibility outcomes**

13
14 The secondary objectives are designed to further facilitate the design of a larger, definitive multi-centre
15 RCT. Namely, to determine the proportion of:

- 16
17 1. Pregnant women approached who agree to be screened
- 18
19 2. Families eligible on screening that cannot have a water softener installed (e.g. due to landlord
20 or local authority refusal, technical (plumbing) reasons)
- 21
22 3. Families randomised that withdraw due to infant ineligibility
- 23
24 4. Families in intervention arm who found the intervention acceptable
- 25
26 5. Participants in control arm who become exposed to softened water (e.g. by moving to a new
27 home in a soft water area, or moving to a home with an active water softener installed, before
28 the end of follow up)
- 29
30 6. Participants that have the water softening unit removed or disabled prior to end of follow up
- 31
32 7. Participants with visible eczema status (yes/no) recorded at each time point: baseline, 4 weeks,
33 3 and 6 months
- 34
35 8. Water samples with hardness >20 mg/L calcium carbonate in the intervention arm
- 36
37 9. Participants who withdraw from the trial prior to end of follow up
- 38
39 10. Median number of nights spent away from the participant's main home during follow up
- 40
41 11. Clinical outcome assessments that have remained blinded at 4 weeks, 3 & 6 months

42 43 **Secondary clinical outcomes**

- 44
45 1. Proportion with patient-reported, doctor-diagnosed atopic eczema by 6 months of age
 - 46
47 2. Proportion with visible eczema according to the UK diagnostic criteria-based photographic
48 protocol²³
 - 49
50 3. Severity of eczema (if present) using Eczema Area and Severity Index (EASI)
 - 51
52 4. Patient-reported eczema symptoms (Patient-Orientated Eczema Measure – POEM) score
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5. Time to onset of patient-reported doctor-diagnosed eczema

Additional mechanistic outcomes

The following will be assessed as change from baseline until follow up at 6 months in the intervention compared to the control group:

1. Transepidermal water loss (TEWL)
2. Cutaneous cytokine profiles (e.g. interleukin-1 levels)
3. Natural moisturising factor (NMF) 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

Adverse events

This trial involves use of a commonly available domestic water softening unit with provision for separate mains drinking water during the time when the water softening unit is installed. Accordingly, we do not anticipate any adverse events or adverse reactions related to the trial intervention. A contact number for the service department at Harvey Water Softeners will be provided and an engineer will be sent to resolve the issue, if needed. Details of technical issues reported to the service department will be reported to the study principal investigators. Events of particular relevance such as plumbing difficulties, floods or difficulties with the units will be logged and reported to the Research Ethics Committee and relevant Research and Development departments annually.

Sample size

This is a pilot study and therefore not powered to establish the efficacy of the intervention. 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.

Findings from the Enquiring About Tolerance (EAT) study⁸ and the Barrier Enhancement Eczema Prevention (BEEP) feasibility study²⁴, 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

1
2
3 offspring. Of these, 40-60% would be expected to be willing and able to participate. Home factors also
4 need to be considered: In SWET, 27% of eligible families could not participate because their home was
5 not suitable for installation.¹⁶
6
7

8 **Informed consent**

9
10 Written informed antenatal consent will be obtained at the enrolment visit from the mother. The consent
11 form will be signed and dated before entering the trial and participants are reminded that they may
12 withdraw from the trial at any time. Mothers will be re-consented postnatally to ensure they agree to
13 their child taking part in the study.
14
15
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18 **Randomisation and blinding**

19
20 Participants will be randomised antenatally at the time of the engineer home visit to receive either a
21 water softener or their usual water supply, once:
22
23

- 24 • Antenatal eligibility criteria have been fulfilled;
- 25 • Fully informed written consent has been obtained;
- 26 • The engineer is satisfied that the softener can be installed
27
28
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32 Participants will be randomised in a 1:1 ratio to one of the two treatment arms based on a computer-
33 generated code. The randomisation result will be relayed to the installation engineer by telephone as
34 either an 'INSTALL' or 'DO NOT INSTALL' instruction. The randomisation service will be provided by
35 researchers at King's College London who are not involved in the study, via telephone.
36
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38

39 Experience from the SWET trial has shown that the effects of a functional water softener are too
40 noticeable to allow participants to be blinded¹⁶. Skin examinations and measurements will be performed
41 by research team members who will be blinded to treatment allocation. Participants will be encouraged
42 not to disclose allocation. Results will be analysed based on treatment code, using a statistical analysis
43 plan finalised prior to database lock and any data analysis. Only after the analyses are completed will
44 the actual treatment arms corresponding to treatment codes be revealed. Study team members in direct
45 contact with study participants will be trained in the study protocol and importance of demonstrating
46 equipoise.
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57 **Visit schedule and study procedures**

58 The schedule for assessments during the study are shown in **Table 2**.
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3 *Enrolment visit (up until 36 weeks gestation)*
4

- 5 • Confirm eligibility (see Table 1)
- 6
- 7 • Answer questions about study
- 8
- 9 • Written consent is taken
- 10
- 11 • Antenatal history (including antibiotic exposure, probiotic and omega 3 use), family medical
12 history and environment questionnaire
- 13
- 14 • Arrange water softener installation
- 15

16 The enrolment visit may occur at the same time as the screening visit, if preferred by the participant
17 and deemed appropriate by the investigator.
18

19
20 *Water softener engineer home visit (up until 40 weeks gestation)*
21

- 22 • Assessment by the engineer to check the home's suitability for water softener installation.
- 23
- 24 • If home eligible, the engineer will telephone a given number at King's College London to
25 determine the randomisation group.
- 26
- 27
- 28 • Install water softener and provide water sample materials if randomised to the intervention arm.
29
- 30

31
32 *Baseline visit (on postnatal ward or within 1 week of birth)*
33

- 34 • Confirm infant eligibility criteria
- 35
- 36 • Postnatal written consent
- 37
- 38 • Skin examination
- 39
- 40 • Infant skincare questions
- 41
- 42 • Record concomitant infant medications including systemic antibiotic use
- 43
- 44 • Systemic antibiotic use in mother during pregnancy, including prophylactic antibiotic use during
45 delivery
- 46
- 47 • Infant co-morbidities
- 48
- 49 • Topical medication use in infant
- 50
- 51 • Delivery questions
- 52
- 53 • Pregnancy outcomes & birth details
- 54
- 55 • Additional mechanistic assessments (procedures marked ‡ will be performed only at the Guy's
56 & St Thomas' Hospital site):
57
 - 58 ➤ Tape stripping (forearm)
 - 59
 - 60

- 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 from skin (antecubital fossa & cheek) and nares

4-week visit (+/- 1 week)

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

3-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)

6-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)
- Arrange date for removal of water softener within 1 month if not planning to purchase (intervention group) or installation of water softener for 6 month period, if desired, in control arm
- Invite to participate in semi-structured qualitative interview about study (via telephone or in person) and seek verbal consent for this

Monthly email messages (intervention group)

- Remind participants to complete questionnaires
- Remind participants' parents to refill the unit with salt
- Invite to contact Harvey Water Softeners Ltd. if any issues with water softener.

Monthly email messages (control group)

- Remind participants to complete questionnaires

Monthly electronic questionnaires

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3 A secure web-based questionnaire link using the Snap Surveys platform will be emailed to participants'
4 parents monthly to determine whether the child has received a diagnosis of eczema from a healthcare
5 professional and to check current skincare, hygiene, confirm residence/time away from main residence
6 and infant general health. A paper copy of the questionnaire can be posted to participants if they prefer
7 or if they do not have internet access.
8

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12 At the end of the study, i.e. 6 month visit, a date will be arranged for removal of the water softener within
13 1 month if the participating family does not want to purchase the device (intervention group). Control
14 arm participants can purchase the same water softener at the end of the study. Participants will also be
15 invited to participate in a semi-structured qualitative interview about the study.
16
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19 **Embedded mechanistic sub-study**

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21
22 The embedded mechanistic sub-study will help to elucidate the mechanisms by which softened water
23 might affect skin barrier function and therefore eczema risk. It will also provide more general insights
24 into the pathogenesis of eczema in young infants with resulting opportunities for hypothesis generation.
25
26 Attenuated Total Reflectance (ATR) Fourier-Transform Infrared (FTIR) spectroscopy measurements
27 will be performed using the Agilent 4300 ATR-FTIR device (Agilent Technologies, USA) to measure the
28 deposition of detergents, such as sodium lauryl sulphate, on the skin surface.²⁵
29

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32
33 Transepidermal water loss (TEWL) provides a standardised measure of skin barrier dysfunction²⁶ and
34 there is evidence to suggest that elevated TEWL in neonates is predictive of subsequent eczema.^{17 27}
35
36 We will use the AquaFlux AF200 condensing chamber probe (Biox Systems Ltd, London, UK) to
37 measure TEWL. Epidermal stratum corneum hydration will be measured using a CM 825 Corneometer
38 (Courage and Khazaka, Cologne, Germany).
39

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43 Skin surface pH has an important role in the regulation of epidermal and microbial cellular process and
44 will be measured using a PH905 Skin-Surface-pH probe fitted with a Mettler and Toledo flat surface
45 electrode (Courage and Khazaka, Cologne, Germany).²⁸
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48
49 Skin protein samples will be collected from the skin by applying and removing eight consecutive D-
50 Squame cutaneous stripping discs (CuDerm Corporation, Dallas, USA). Samples will be collected to
51 perform the protein and cytokine analyses, such as IL-1 α levels.²⁹
52

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54
55 The filaggrin gene (*FLG*) encodes profilaggrin protein and its subsequent breakdown products, are key
56 to normal barrier function of the skin. Loss-of-function sequence variants in *FLG* are associated with an
57 increased risk of eczema (Palmer, 2006). Enquiring About Tolerance study suggests that there might
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3 be an interaction between *FLG* and water hardness.⁸ We will examine the relationship between *FLG*
4 mutation status and risk of eczema in a sub-group analysis and explore the relationship between *FLG*
5 mutation status and skin microbiome diversity, TEWL and cutaneous cytokine expression. DNA will be
6 extracted by standard techniques and *FLG* whole gene sequencing will be used for genotyping *FLG*
7 loss-of-function sequence variants and the presence of intragenic copy number variations (Wong et al
8 JACI, 2017). Buccal swab for DNA will be collected once, during the 4 week visit if consent is given. If
9 unable to collect at the 4 week visit, collection will be attempted at 3 month visit or 6 month visit.

10
11 We will sample the antecubital fossa and the cheek, using Isohelix® bacterial DNA swabs (Cell Projects,
12 Harrietsham, UK). A further swab will be taken from each nostril using an Ultra Minitip flocced swab
13 (Copan Flock Technologies Srl, Brescia, Italy). A control sample is taken for each participant. We will
14 use Illumina MiSeq V1-V3 Amplicon sequencing of the 16S ribosomal subunit to characterise both skin
15 and nasal bacteria communities with regard to species diversity. We will examine within-participant
16 changes in microbial diversity over time and explore the relationship between water hardness, microbial
17 diversity and *FLG* gene mutational profile as well as taking into account environmental factors such as
18 mode of delivery and antibiotic use in the mother and infant.

31 **Statistical analysis**

32
33 The feasibility parameters of this pilot trial will not be formally statistically tested. The focus will therefore
34 be on descriptive statistics and confidence intervals. A separate and full statistical analysis plan (SAP)
35 for the feasibility trial and analysis of mechanistic data will be developed prior to unblinding and any
36 analysis of the data.

37
38 Randomisation will be in a 1:1 ratio using randomly permuted blocks. Participants' data will be analysed
39 on an intention-to-treat basis. There will be no hypothesis testing for the clinical outcomes except in
40 relation to the embedded mechanistic study. Results will be presented as estimates with 95%
41 confidence intervals, where appropriate.

ETHICS AND DISSEMINATION

This study has been reviewed and given a favourable opinion by the North West – Liverpool East Research Ethics Committee (Ref: 17/NW/0661). It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. We will send participating families a summary of the pilot trial results.

For peer review only

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AUTHORS' CONTRIBUTIONS

CF is the Chief Investigator with overall responsibility for the SOFTER pilot trial. He conceived the idea for the study, contributed to the writing of the trial protocol, and reviewed this manuscript. ZKJ-L is the Senior Co-investigator responsible for designing and running the SOFTER pilot trial. He led on the writing of the trial protocol and drafted the manuscript. JLP is the Senior Statistical Advisor and contributed to the writing of the trial protocol and reviewed the manuscript. NG coordinates the study, reviewed the trial protocol and drafted the manuscript. DG, AB, JC, KT, TF, SK, HHK, JAS, JEAC, SD, MJC were involved in finalising the study protocol and reviewing of the manuscript.

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- Supply and installation of the water softening units.
- Salt supplies
- Testing of the water samples

DISCLAIMER

The views expressed are those of the authors and not necessarily those of the UK National Health Service, the UK Department of Health and Social Care or the National Institute of Health Research.

COMPETING INTERESTS STATEMENT

1
2
3 C.F. has received investigator-led research funding from Sanofi. His department has received clinical
4 trial funding from Sanofi and AbbVie to test novel therapeutics in paediatric atopic eczema patients.
5
6 M.J.C. is an Investigator and Consultant for Regeneron, Sanofi Genzyme, Pfizer, Leo, Galapagos,
7
8 Novartis, Boots, L'Oreal, Dermavant, Menlo, Reckitt Benckiser, Oxagen, Johnson&Johnson, Hyphens,
9
10 Astellas, Amlar, Abbvie, Galderma, Procter&Gamble. The other authors declare that they have no
11
12 competing interests. Harvey Water Softeners contributed to the design and operational running of the
13
14 study (supply and installation of water softeners, testing of water samples). Final decisions around
15
16 design and conduct were made independently by investigators. HWS will not be involved in the analysis
17
18 or interpretation of the results.
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TABLE 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 | |
| 11 | Maternal consent for her neonate to participate in the study |
| Exclusion criteria | |
| 12 | Preterm birth (defined as birth prior to 37 weeks gestation) |
| 13 | Significant inflammatory skin disease at birth not including seborrheic dermatitis (“cradle cap”) |
| 14 | Sibling (including twin) previously randomised to this trial. If multiple birth, the first child will be randomised into the trial. |
| 15 | Any immunodeficiency disorder or severe genetic skin disorder |
| 16 | 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. |
| 17 | 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 |
| 18 | Water softening or filtration device already installed |
| 19 | Concurrent enrolment in any other skin-related intervention study |
| 20 | Other medical condition that in the opinion of the Chief Investigator could interfere with the conduct of the trial |

TABLE 2 – Schedule of study assessments and procedures

| | Screening# | Enrolment# | 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) score | | | | | | From 4 weeks to 6 months of age | | |
| Weekly water samples (in intervention arm) | | | | | From installation to 6 months of age | | | |

*Any other concomitant illnesses that develop 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
Abbreviations: DNA – deoxyribonucleic acid; EASI – Eczema Area and Severity Index; m – month; POEM – Patient Oriented Eczema Measure; TEWL – transepidermal water loss; Wk - week

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3 **FIGURE LEGEND**
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6 **FIGURE 1 – Study flowchart**
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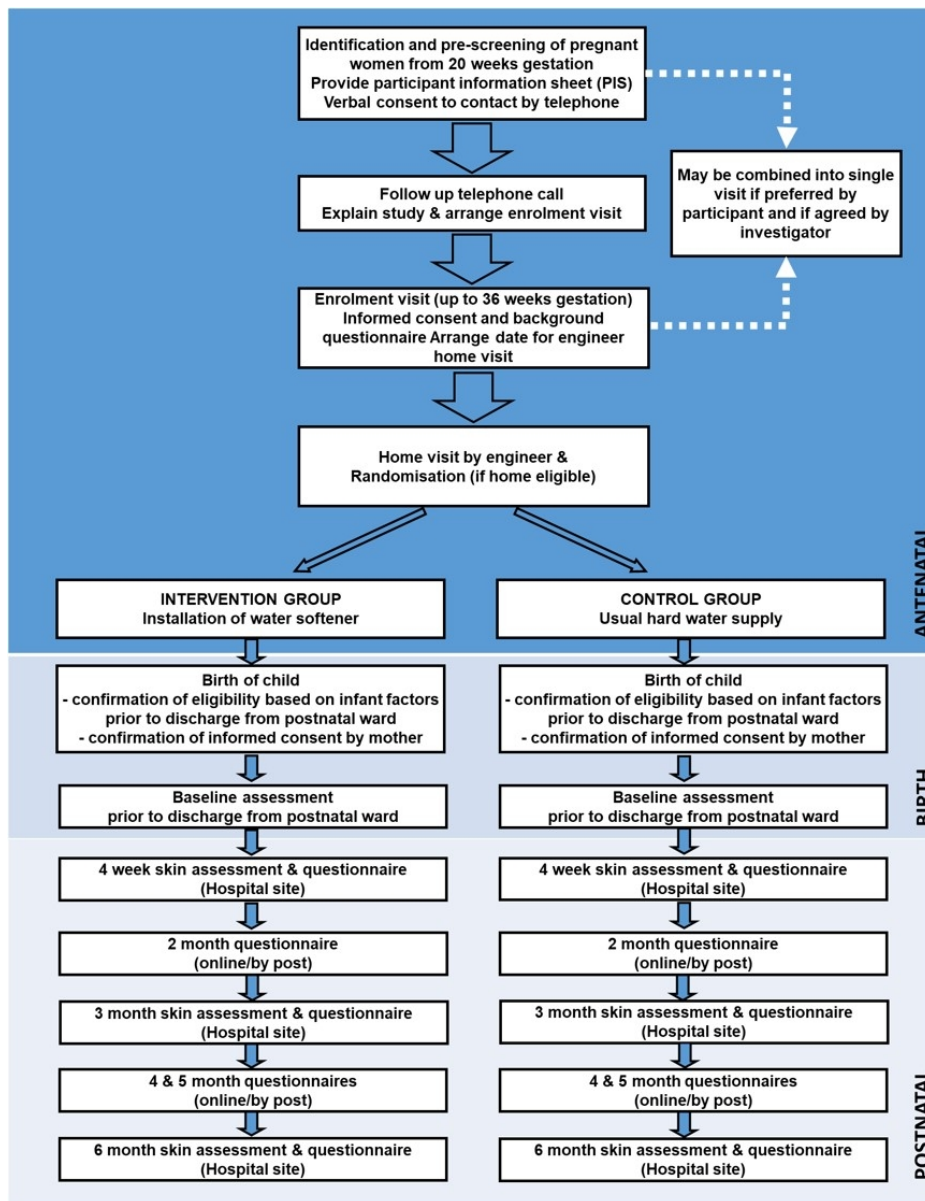


Figure 1

74x94mm (300 x 300 DPI)

BMJ Open

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 trial (SOFTER)

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| Primary Subject Heading: | Dermatology |

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|----------------------------|---|
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| | |



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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 trial (SOFTER)

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ABSTRACT

Introduction

Atopic eczema affects 20% of UK children, and environmental factors are important in its aetiology. Several observational studies suggest an increased risk of atopic eczema in children living in hard water areas. The SOFTened water for eczema prevention (SOFTER) pilot trial tests the feasibility of installing domestic ion-exchange water softeners around the time of birth to reduce the risk of atopic eczema in children with a family history of atopy. A further aim is to explore the pathophysiological mechanisms for this in an embedded mechanistic study.

Methods and analysis

Multi-centre parallel group assessor-blinded randomised controlled pilot trial. Participants are newborn babies (n=80) living in a hard water (>250 mg/L calcium carbonate) area at risk of developing atopic eczema because of a family history of atopy. Participants will be randomised prior to birth in a 1:1 ratio. The intervention group will have an ion-exchange water softener installed prior to birth. The control group will receive their usual domestic hard water supply. Follow up will be until six months of age. Data will be collected at birth (baseline), 1, 3 and 6 months of age. The main outcome is the proportion of eligible families screened who are willing and able to be randomised. Several secondary feasibility and clinical endpoints will also be evaluated, alongside mechanistic outcomes. Data will be analysed on an intention-to-treat basis. There will be no hypothesis testing for the clinical outcomes. Study acceptability will be evaluated through semi-structured interviews.

Ethics and dissemination

This study has been reviewed and given a favourable opinion by the North West – Liverpool East Research Ethics Committee (Ref: 17/NW/0661). The results of the study will be reported at international conferences and in peer-reviewed scientific journals. We will send participating families a summary of the pilot trial results.

Registration details

Clinicaltrials.gov: NCT03270566.

ARTICLE SUMMARY

Strengths and limitations of this study

- This pilot study comprehensively tests the design of a a randomised controlled trial for the primary prevention of atopic eczema using a mixture of quantitative and qualitative methods.
- In addition, various mechanistic measurements will further our understanding of the mechanisms by which water softeners might prevent atopic eczema.
- This study is being performed in two centres to gain a wider understanding of potential barriers and facilitators to recruitment and retention in a multicentre definitive trial.
- The study is outcome-assessor blinded as participant blinding is not possible to achieve.
- Follow up is only for 6 months and so may not provide full information on post-randomisation losses to follow up that could occur in a future definitive trial with a longer duration of follow up.

INTRODUCTION

Background & Rationale

Atopic eczema (syn. atopic dermatitis, hereinafter referred to as 'eczema') is a common inflammatory skin condition affecting around 20% of UK children.¹ It is associated with significant morbidity and affects health-related quality of life. The cause of eczema is not fully understood. It is likely to be multifactorial, and several genetic and environmental factors have been identified.² No primary prevention strategy has been established.³ However, several approaches have been proposed such as probiotics during pregnancy, dietary supplementation, house dust mite avoidance, intensive emollient use and domestic water softening.⁴

Relationship between hard water and atopic eczema

Hard water is the result of dissolved minerals, mainly calcium carbonate and magnesium carbonate, from the percolation of water through rock in the environment. England, especially in the south, has very hard (>250 mg/L calcium carbonate) domestic water. A cross-sectional study conducted in the 1990s 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 around Nottingham, UK.⁵ Two further cross-sectional studies among school children conducted in Japan and Spain confirmed this association.^{6 7} Subsequently, a cross-sectional analysis from a cohort study among over 1,300 infants in England and Wales has also confirmed this relationship in early life, even after adjusting for likely confounders.⁸ The same study suggested a possible interaction with loss-of-function mutations in the skin barrier gene, filaggrin (*FLG*). Most recently, a large study from a Danish birth cohort found a 5% increase in prevalence of eczema within the first 18 months of life for each 5 unit increase in domestic water hardness (equivalent to 89.2 mg/L calcium carbonate)⁹ over a range of 6.60-35.90 German degrees of hardness [118-641 mg/L calcium carbonate]).¹⁰

Potential mechanisms for hard water causing atopic eczema

Several potential mechanisms have been proposed for the way in which hard water may lead to eczema development: 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, resulting in increased protease activity, could all have a detrimental effect on skin barrier function.⁸ Experimental work has demonstrated an increased deposition of SLS in skin washed with hard water vs. softened water.¹¹ In animal studies using a hairless mouse model, low extracellular concentrations of calcium ions in the

1
2
3 upper epidermis led to exocytosis of lamellar bodies, required for skin barrier repair, independent of
4 skin barrier disruption.¹²

5
6 Eczema is associated with a preponderance of *Staphylococcus aureus* and a reduction in microbial
7 diversity.¹³ Recent work has identified a synergistic relationship between the human cathelicidin-related
8 antimicrobial peptide LL-37 and antimicrobial peptides produced by coagulase negative staphylococcal
9 species that selectively kill *Staphylococcus aureus*.¹⁴ Human LL-37 activity against some bacterial
10 species is decreased by the presence of calcium, but not magnesium, ions.¹⁵

17 18 **Trials of water softeners in atopic eczema**

19
20 The multicentre Softened Water Eczema Trial (SWET), completed in 2011, examined the role of water
21 softeners in treating children with established, moderate-to-severe eczema and found no overall
22 benefit in terms of eczema severity reduction.¹⁶ However, the factors that drive the development of
23 eczema may not be the same as those that determine disease exacerbations and severity and the
24 negative result in the SWET trial does therefore not exclude a role for water softeners in prevention
25 trials. Early life is likely to be an important time in the development of eczema, particularly as most
26 eczema develops before 2 years of age. Early interactions between genes and the environment may
27 be crucial in instigating the cycle of inflammation and skin barrier dysfunction seen in eczema. Indeed,
28 skin barrier dysfunction at just one week of age, as measured by transepidermal water loss, is a
29 predictor of subsequent eczema risk.¹⁷ A small pilot randomised controlled double-blind crossover trial
30 of 12 patients aged 3-6 years with mild-moderate eczema compared ultra-pure soft water to tap
31 water. After 6 weeks, no statistically significant differences in eczema area severity index (EASI) or
32 transepidermal water loss (TEWL) were observed between the groups, although there was a
33 statistically significant improvement in pruritus as measured by visual analogue score (-2.10, 95% CI -
34 4.14, -0.063).¹⁸ To date, there are no published studies examining the role of water softeners in the
35 prevention of eczema.¹⁹

52 **Rationale**

53
54 The overall rationale is that by installing a domestic water softener around the time of birth, the infant
55 will be exposed to softened water rather than hard water for bathing and that this will be less irritating
56 to the skin than hard water and so associated with a lower risk of eczema development.

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3 This pilot trial builds on the experience gained from the NIHR-funded SWET trial¹⁶ and a trial of
4 emollients in early life (Barrier Enhancement for Eczema Prevention; BEEP),²⁰ both of which were
5 coordinated by Prof K Thomas and Dr J Chalmers, our collaborators on this trial, who are based at the
6
7 Centre of Evidence Based Dermatology in Nottingham. This is a pilot trial, as defined by the UK National
8
9 Institute for Health Research in that it is a 'version of the main study that is run in miniature to test
10
11 whether the components of the main study can all work together'.²¹ This pilot trial will therefore not
12
13 definitively answer the question of whether installation of a domestic water softener will prevent eczema.
14
15 The rationale for performing this pilot study is that a definitive trial on eczema prevention using domestic
16
17 water softening devices would require a much larger number of participants and so prior to embarking
18
19 on a larger multicentre trial it will be important to determine whether the planned trial recruitment and
20
21 assessment procedures are possible and workable, or whether they require adapting or changing.²²
22
23

24 **Trial objectives**

25
26 The aim of this pilot trial is to determine the feasibility of undertaking a large-scale definitive trial to
27
28 determine whether installation of domestic ion-exchange water softeners around the time of birth
29
30 reduces the risk of high-risk children developing eczema. A further aim is to explore the
31
32 pathophysiological mechanisms for this in an embedded mechanistic study.
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METHODS AND ANALYSIS

Trial design

This is a multi-centre parallel group assessor-blinded randomised 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. Eighty newborn infants will be enrolled into the trial for a period of 6 months. The end of study is defined as the final assessment visit of the last participant to enter the trial. Recruitment commenced in February 2018 and is due to end in autumn 2019.

Pregnant women will be 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 flowchart in **Figure 1**.

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 will join the independent trial steering committee. Once the trial has been published, participants will be sent details of the results in a study newsletter suitable for a non-specialist audience.

Trial population

The study will recruit pregnant women living in hard water areas who will be identified from antenatal services at local NHS Trust sites. Women will be approached at the 20 week anomaly scan and asked if they would be interested in participating. We will also publicise the study through posters and by making clinical midwifery teams aware of the study. Full inclusion and exclusion criteria are given in

Table 1.

Intervention

A domestic ion-exchange water softener will be 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, amongst other divalent cations, for monovalent sodium cations, typically reducing downstream water hardness to close to zero. The sodium ions come from common salt which

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3 needs to be topped up every 3-4 weeks. The water softeners to be used in this trial will be supplied and
4 funded by Harvey Water Softeners Ltd., Woking, UK. Standard procedure will be to soften all water in
5 the home except the drinking water tap, as the water softening process exchanges calcium with sodium
6 ions and will therefore increase the water sodium concentration, making the water unsuitable for
7 drinking purposes. Unsoftened mains drinking water will be delivered through the existing kitchen tap
8 wherever possible, or otherwise through an extra (faucet-style) tap installed at the side of the kitchen
9 sink. At the end of the study, all participants will be given the option to purchase the water softener from
10 Harvey Water Softeners Ltd. at a reduced price of £399.00 inclusive of VAT, installation and warranty;
11 this is approximately a quarter of the full retail price (£1,678.80).. Alternatively, it will be removed.

20 *Potential risks*

21
22 This is a low risk trial as the intervention involves a commercially available ion exchange water softener
23 that has no known clinical side effects. There is a potential, but low risk, of damage to participants'
24 properties during installation of the water softening unit. However, these will be installed by a qualified
25 water engineer according to the Code of Practice produced by British Water and in accordance with the
26 recommendations of the WRAS (Water Regulations Advisory Service).

31 **Concomitant medications and skincare**

32
33 No restrictions will be placed on the use of concomitant treatment a child might receive. All concomitant
34 medication will be recorded at baseline and updated at follow up visits. Given the lack of a consistent
35 approach to neonatal skincare, and the possible interaction between some wash products and hard
36 water, we will not provide specific skincare advice to participants. However, data on such use will be
37 sought from parents via monthly online questionnaires.

43 **Co-enrolment guidelines**

44
45 To avoid potentially confounding issues, neonates should not be recruited into other prevention of
46 eczema or allergy intervention trials.

49 **Participant compliance**

50
51 Compliance with treatment does not represent a large problem for this trial as long as the participants
52 are not absent from home for long periods of time and remember to replenish the salt. Reminders about
53 salt replenishment will be sent. To check that the units are working correctly, participants will be asked
54 to send weekly water samples to Harvey Water Softeners using prepaid envelopes. Any samples with
55 a reading of >20 mg/L calcium carbonate will be referred back to the engineer for investigation. If
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3 participants move home during the trial, attempts will be made to reinstall the device in the new property.

4
5 It is anticipated that loss to follow-up will be <15%.

6 7 **Primary outcome**

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9 Proportion of eligible families screened who are willing and able to be randomised. This is key to the
10 determination of the likely success of a future, large-scale definitive randomised controlled trial (RCT).

11 12 **Secondary feasibility outcomes**

13
14 The secondary objectives are designed to further facilitate the design of a larger, definitive multi-centre
15 RCT. Namely, to determine the proportion of:

- 16
17 1. Pregnant women approached who agree to be screened
- 18
19 2. Families eligible on screening that cannot have a water softener installed (e.g. due to landlord
20 or local authority refusal, technical (plumbing) reasons)
- 21
22 3. Families randomised that withdraw due to infant ineligibility
- 23
24 4. Families in intervention arm who found the intervention acceptable
- 25
26 5. Participants in control arm who become exposed to softened water (e.g. by moving to a new
27 home in a soft water area, or moving to a home with an active water softener installed, before
28 the end of follow up)
- 29
30 6. Participants that have the water softening unit removed or disabled prior to end of follow up
- 31
32 7. Participants with visible eczema status (yes/no) recorded at each time point: baseline, 4 weeks,
33 3 and 6 months
- 34
35 8. Water samples with hardness >20 mg/L calcium carbonate in the intervention arm
- 36
37 9. Participants who withdraw from the trial prior to end of follow up
- 38
39 10. Median number of nights spent away from the participant's main home during follow up
- 40
41 11. Clinical outcome assessments that have remained blinded at 4 weeks, 3 & 6 months

42 43 **Secondary clinical outcomes**

- 44
45 1. Proportion with patient-reported, doctor-diagnosed atopic eczema by 6 months of age
- 46
47 2. Proportion with visible eczema according to the UK diagnostic criteria-based photographic
48 protocol²³
- 49
50 3. Severity of eczema (if present) using Eczema Area and Severity Index (EASI)
- 51
52 4. Patient-reported eczema symptoms (Patient-Orientated Eczema Measure – POEM) score

5. Time to onset of patient-reported doctor-diagnosed eczema

Additional mechanistic outcomes

The following will be assessed as change from baseline until follow up at 6 months in the intervention compared to the control group:

1. Transepidermal water loss (TEWL)
2. Cutaneous cytokine profiles (e.g. interleukin-1 levels)
3. Natural moisturising factor (NMF) 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

Adverse events

This trial involves use of a commonly available domestic water softening unit with provision for separate mains drinking water during the time when the water softening unit is installed. Accordingly, we do not anticipate any adverse events or adverse reactions related to the trial intervention. A contact number for the service department at Harvey Water Softeners will be provided and an engineer will be sent to resolve the issue, if needed. Details of technical issues reported to the service department will be reported to the study principal investigators. Events of particular relevance such as plumbing difficulties, floods or difficulties with the units will be logged and reported to the Research Ethics Committee and relevant Research and Development departments annually.

Sample size

This is a pilot study and therefore not powered to establish the efficacy of the intervention. 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.

Findings from the Enquiring About Tolerance (EAT) study⁸ and the Barrier Enhancement Eczema Prevention (BEEP) feasibility study²⁴, 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

1
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3 offspring. Of these, 40-60% would be expected to be willing and able to participate. Home factors also
4 need to be considered: In SWET, 27% of eligible families could not participate because their home was
5 not suitable for installation.¹⁶
6
7

8 **Informed consent**

9
10 Written informed antenatal consent will be obtained at the enrolment visit from the mother. The consent
11 form will be signed and dated before entering the trial and participants are reminded that they may
12 withdraw from the trial at any time. Mothers will be re-consented postnatally to ensure they agree to
13 their child taking part in the study.
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17

18 **Randomisation and blinding**

19
20 Participants will be randomised antenatally at the time of the engineer home visit to receive either a
21 water softener or their usual water supply, once:
22
23

- 24 • Antenatal eligibility criteria have been fulfilled;
 - 25 • Fully informed written consent has been obtained;
 - 26 • The engineer is satisfied that the softener can be installed
- 27
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32 Participants will be randomised in a 1:1 ratio to one of the two treatment arms based on a computer-
33 generated code. The randomisation result will be relayed to the installation engineer by telephone as
34 either an 'INSTALL' or 'DO NOT INSTALL' instruction. The randomisation service will be provided by
35 researchers at King's College London who are not involved in the study, via telephone.
36
37
38

39 Experience from the SWET trial has shown that the effects of a functional water softener are too
40 noticeable to allow participants to be blinded¹⁶. Skin examinations and measurements will be performed
41 by research team members who will be blinded to treatment allocation. Participants will be encouraged
42 not to disclose allocation. Results will be analysed based on treatment code, using a statistical analysis
43 plan finalised prior to database lock and any data analysis. Only after the analyses are completed will
44 the actual treatment arms corresponding to treatment codes be revealed. Study team members in direct
45 contact with study participants will be trained in the study protocol and importance of demonstrating
46 equipoise.
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57 **Visit schedule and study procedures**

58 The schedule for assessments during the study are shown in **Table 2**.
59
60

1
2
3 *Enrolment visit (up until 36 weeks gestation)*
4

- 5 • Confirm eligibility (see Table 1)
- 6
- 7 • Answer questions about study
- 8
- 9 • Written consent is taken
- 10
- 11 • Antenatal history (including antibiotic exposure, probiotic and omega 3 use), family medical
12 history and environment questionnaire
- 13
- 14 • Arrange water softener installation
- 15

16 The enrolment visit may occur at the same time as the screening visit, if preferred by the participant
17 and deemed appropriate by the investigator.
18

19
20 *Water softener engineer home visit (up until 40 weeks gestation)*
21

- 22 • Assessment by the engineer to check the home's suitability for water softener installation.
- 23
- 24 • If home eligible, the engineer will telephone a given number at King's College London to
25 determine the randomisation group.
- 26
- 27
- 28 • Install water softener and provide water sample materials if randomised to the intervention arm.
29
- 30

31
32 *Baseline visit (on postnatal ward or within 1 week of birth)*
33

- 34 • Confirm infant eligibility criteria
- 35
- 36 • Postnatal written consent
- 37
- 38 • Skin examination
- 39
- 40 • Infant skincare questions
- 41
- 42 • Record concomitant infant medications including systemic antibiotic use
- 43
- 44 • Systemic antibiotic use in mother during pregnancy, including prophylactic antibiotic use during
45 delivery
- 46
- 47 • Infant co-morbidities
- 48
- 49 • Topical medication use in infant
- 50
- 51 • Delivery questions
- 52
- 53 • Pregnancy outcomes & birth details
- 54
- 55 • Additional mechanistic assessments (procedures marked ‡ will be performed only at the Guy's
56 & St Thomas' Hospital site):
57
 - 58 ➤ Tape stripping (forearm)
 - 59
 - 60

- 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 from skin (antecubital fossa & cheek) and nares

4-week visit (+/- 1 week)

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

3-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)

6-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)
- Arrange date for removal of water softener within 1 month if not planning to purchase (intervention group) or installation of water softener for 6 month period, if desired, in control arm
- Invite to participate in semi-structured qualitative interview about study (via telephone or in person) and seek verbal consent for this

Monthly email messages (intervention group)

- Remind participants to complete questionnaires
- Remind participants' parents to refill the unit with salt
- Invite to contact Harvey Water Softeners Ltd. if any issues with water softener.

Monthly email messages (control group)

- Remind participants to complete questionnaires

Monthly electronic questionnaires

1
2
3 A secure web-based questionnaire link using the Snap Surveys platform will be emailed to participants'
4 parents monthly to determine whether the child has received a diagnosis of eczema from a healthcare
5 professional and to check current skincare, hygiene, confirm residence/time away from main residence
6 and infant general health. A paper copy of the questionnaire can be posted to participants if they prefer
7 or if they do not have internet access.
8

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12 At the end of the study, i.e. 6 month visit, a date will be arranged for removal of the water softener within
13 1 month if the participating family does not want to purchase the device (intervention group). Control
14 arm participants can purchase the same water softener at the end of the study. Participants will also be
15 invited to participate in a semi-structured qualitative interview about the study.
16
17
18

19 **Embedded mechanistic sub-study**

20
21
22 The embedded mechanistic sub-study will help to elucidate the mechanisms by which softened water
23 might affect skin barrier function and therefore eczema risk. It will also provide more general insights
24 into the pathogenesis of eczema in young infants with resulting opportunities for hypothesis generation.
25
26 Attenuated Total Reflectance (ATR) Fourier-Transform Infrared (FTIR) spectroscopy measurements
27 will be performed using the Agilent 4300 ATR-FTIR device (Agilent Technologies, USA) to measure the
28 deposition of detergents, such as sodium lauryl sulphate, on the skin surface.²⁵
29

30
31
32
33 Transepidermal water loss (TEWL) provides a standardised measure of skin barrier dysfunction²⁶ and
34 there is evidence to suggest that elevated TEWL in neonates is predictive of subsequent eczema.^{17 27}
35
36 We will use the AquaFlux AF200 condensing chamber probe (Biox Systems Ltd, London, UK) to
37 measure TEWL. Epidermal stratum corneum hydration will be measured using a CM 825 Corneometer
38 (Courage and Khazaka, Cologne, Germany).
39

40
41
42
43 Skin surface pH has an important role in the regulation of epidermal and microbial cellular process and
44 will be measured using a PH905 Skin-Surface-pH probe fitted with a Mettler and Toledo flat surface
45 electrode (Courage and Khazaka, Cologne, Germany).²⁸
46
47

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50
51 Skin protein samples will be collected from the skin by applying and removing eight consecutive D-
52 Squame cutaneous stripping discs (CuDerm Corporation, Dallas, USA). Samples will be collected to
53 perform the protein and cytokine analyses, such as IL-1 α levels.²⁹
54

55
56
57
58 The filaggrin gene (*FLG*) encodes profilaggrin protein and its subsequent breakdown products, are key
59 to normal barrier function of the skin. Loss-of-function sequence variants in *FLG* are associated with an
60 increased risk of eczema (Palmer, 2006). Enquiring About Tolerance study suggests that there might

1
2
3 be an interaction between *FLG* and water hardness.⁸ We will examine the relationship between *FLG*
4 mutation status and risk of eczema in a sub-group analysis and explore the relationship between *FLG*
5 mutation status and skin microbiome diversity, TEWL and cutaneous cytokine expression. DNA will be
6 extracted by standard techniques and *FLG* whole gene sequencing will be used for genotyping *FLG*
7 loss-of-function sequence variants and the presence of intragenic copy number variations (Wong et al
8 JACI, 2017). Buccal swab for DNA will be collected once, during the 4 week visit if consent is given. If
9 unable to collect at the 4 week visit, collection will be attempted at 3 month visit or 6 month visit.

10
11 We will sample the antecubital fossa and the cheek, using Isohelix® bacterial DNA swabs (Cell Projects,
12 Harrietsham, UK). A further swab will be taken from each nostril using an Ultra Minitip flocced swab
13 (Copan Flock Technologies Srl, Brescia, Italy). A control sample is taken for each participant. We will
14 use Illumina MiSeq V1-V3 Amplicon sequencing of the 16S ribosomal subunit to characterise both skin
15 and nasal bacteria communities with regard to species diversity. We will examine within-participant
16 changes in microbial diversity over time and explore the relationship between water hardness, microbial
17 diversity and *FLG* gene mutational profile as well as taking into account environmental factors such as
18 mode of delivery and antibiotic use in the mother and infant.

31 **Statistical analysis**

32
33 The feasibility parameters of this pilot trial will not be formally statistically tested. The focus will therefore
34 be on descriptive statistics and confidence intervals. A separate and full statistical analysis plan (SAP)
35 for the feasibility trial and analysis of mechanistic data will be developed prior to unblinding and any
36 analysis of the data.

37
38 Randomisation will be in a 1:1 ratio using randomly permuted blocks. Participants' data will be analysed
39 on an intention-to-treat basis. There will be no hypothesis testing for the clinical outcomes except in
40 relation to the embedded mechanistic study. Results will be presented as estimates with 95%
41 confidence intervals, where appropriate.

ETHICS AND DISSEMINATION

This study has been reviewed and given a favourable opinion by the North West – Liverpool East Research Ethics Committee (Ref: 17/NW/0661). It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. We will send participating families a summary of the pilot trial results.

For peer review only

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AUTHORS' CONTRIBUTIONS

CF is the Chief Investigator with overall responsibility for the SOFTER pilot trial. He conceived the idea for the study, contributed to the writing of the trial protocol, and reviewed this manuscript. ZKJ-L is the Senior Co-investigator responsible for designing and running the SOFTER pilot trial. He led on the writing of the trial protocol and drafted the manuscript. JLP is the Senior Statistical Advisor and contributed to the writing of the trial protocol and reviewed the manuscript. NG coordinates the study, reviewed the trial protocol and drafted the manuscript. DG, AB, JC, KT, TF, SK, HHK, JAS, JEAC, SD, MJC were involved in finalising the study protocol and reviewing of the manuscript.

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- Supply and installation of the water softening units.
- Salt supplies
- Testing of the water samples

DISCLAIMER

The views expressed are those of the authors and not necessarily those of the UK National Health Service, the UK Department of Health and Social Care or the National Institute of Health Research.

COMPETING INTERESTS STATEMENT

1
2
3 C.F. has received investigator-led research funding from Sanofi. His department has received clinical
4 trial funding from Sanofi and AbbVie to test novel therapeutics in paediatric atopic eczema patients.
5
6 M.J.C. is an Investigator and Consultant for Regeneron, Sanofi Genzyme, Pfizer, Leo, Galapagos,
7
8 Novartis, Boots, L'Oreal, Dermavant, Menlo, Reckitt Benckiser, Oxagen, Johnson&Johnson, Hyphens,
9
10 Astellas, Amlar, Abbvie, Galderma, Procter&Gamble. The other authors declare that they have no
11
12 competing interests. Harvey Water Softeners contributed to the design and operational running of the
13
14 study (supply and installation of water softeners, testing of water samples). Final decisions around
15
16 design and conduct were made independently by investigators. HWS will not be involved in the analysis
17
18 or interpretation of the results.
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TABLE 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 | |
| 11 | Maternal consent for her neonate to participate in the study |
| Exclusion criteria | |
| 12 | Preterm birth (defined as birth prior to 37 weeks gestation) |
| 13 | Significant inflammatory skin disease at birth not including seborrheic dermatitis (“cradle cap”) |
| 14 | Sibling (including twin) previously randomised to this trial. If multiple birth, the first child will be randomised into the trial. |
| 15 | Any immunodeficiency disorder or severe genetic skin disorder |
| 16 | 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. |
| 17 | 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 |
| 18 | Water softening or filtration device already installed |
| 19 | Concurrent enrolment in any other skin-related intervention study |
| 20 | Other medical condition that in the opinion of the Chief Investigator could interfere with the conduct of the trial |

TABLE 2 – Schedule of study assessments and procedures

| | Screening# | Enrolment# | 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) score | | | | | | From 4 weeks to 6 months of age | | |
| Weekly water samples (in intervention arm) | | | | | From installation to 6 months of age | | | |

*Any other concomitant illnesses that develop 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
 Abbreviations: DNA – deoxyribonucleic acid; EASI – Eczema Area and Severity Index; m – month; POEM – Patient Oriented Eczema Measure; TEWL – transepidermal water loss; Wk - week

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3 **FIGURE LEGEND**
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6 **FIGURE 1 – Study flowchart**
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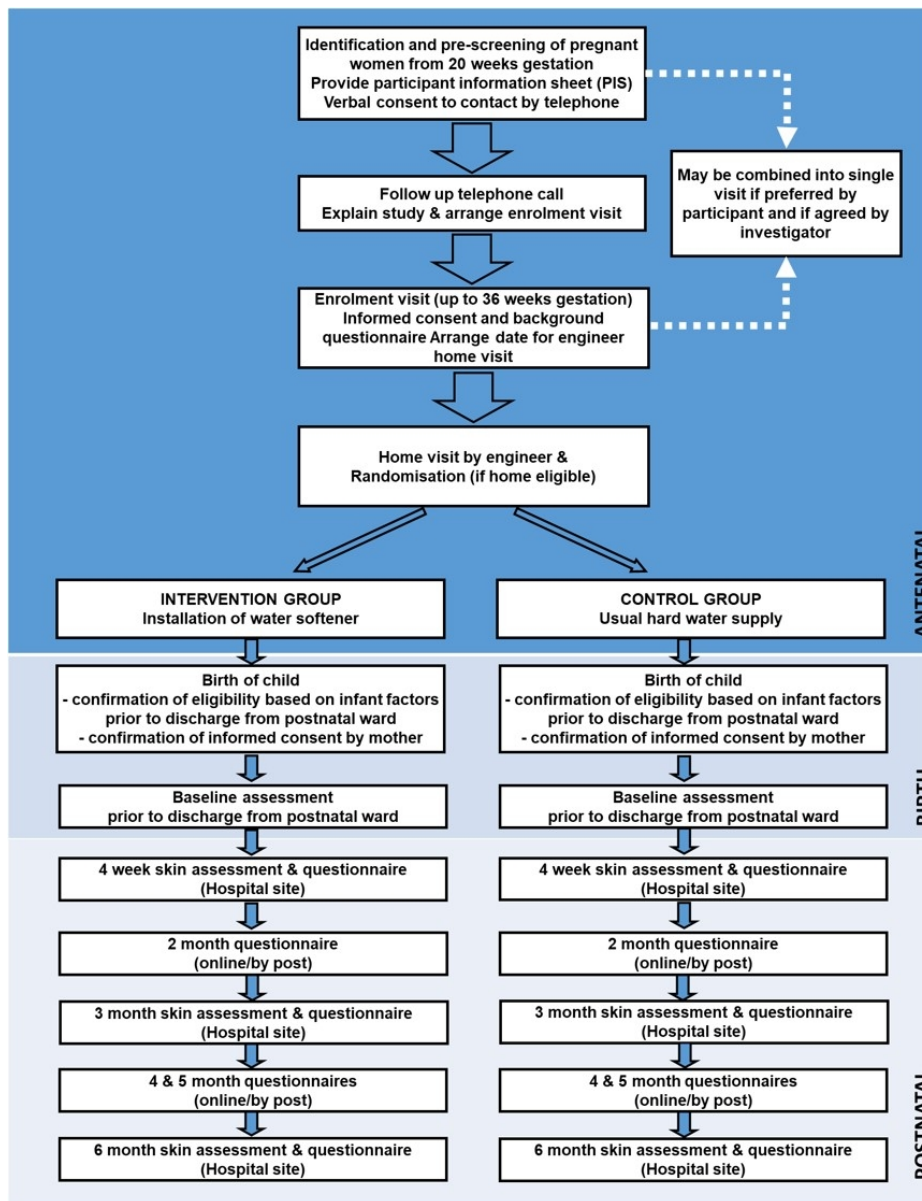


Figure 1

74x94mm (300 x 300 DPI)