A pilot randomised controlled trial investigating a Mediterranean diet intervention in pregnant women for the primary prevention of allergic diseases in infants

A pilot RCT investigating a Mediterranean Diet intervention in pregnant women for the primary prevention of allergic diseases in infants.

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Abstract

Background

Observational studies suggest a potentially protective role of the Mediterranean Diet (MD) in allergic diseases, including asthma. Large scale randomised controlled trials (RCTs) are needed to test the hypothesised allergy-prevention benefits of a MD during pregnancy. This two-arm pilot RCT in pregnant women at high-risk of having a child who would develop allergic disease investigated maternal recruitment, retention and acceptability of an MD dietary intervention in the UK. The trial also assessed the effect of the intervention on MD adherence scores at 12 and at 24 weeks post-randomisation.

Methodology and Results

Thirty women were recruited at around 12 weeks gestation. Retention was high (28 out of 30; 93%). The intervention was acceptable to participants. Adherence to the MD at baseline was 12.4±2.9 in the intervention arm (n=14) and 13.0±1.9 in the control arm (n=16), where 24 represents maximal adherence. There was a favourable short-term change in MD score - the adjusted mean difference (intervention-control) in the change in MD score from baseline to 12 weeks post-randomisation was 2.4 (95%CI, 0.6 to 4.2, P=0.012).

Principal conclusions

The trial provides important insights into recruitment, retention and sustaining the dietary intervention which will be used in the design of a large RCT.

Trial registration: ClinicalTrials.gov: NCT01634516

Introduction

Given increasing evidence to suggest that prenatal and early life exposures affect the development of allergy and asthma, there is considerable interest in the possible role of diet during pregnancy and early life. The prevalence of immunoglobulin-E (IgE)-mediated organ-specific allergic diseases such as atopic eczema/dermatitis, allergic rhino-conjunctivitis and asthma and of systemic allergic disorders such as food allergy and anaphylaxis are increasing[1; 2; 3]. Asthma is one of the most common non-communicable diseases, estimated to affect around 235-300 million people, especially in high-income countries[4; 5]. In the UK, allergy and asthma are highly prevalent and are responsible for considerable morbidity, healthcare utilisation and cost to the NHS[6; 7; 8].

Maternal diet during pregnancy could hypothetically modulate the development of allergy and asthma by influencing airway and/or immune development of the foetus[9]. Associations between aspects of maternal diet during pregnancy, and childhood allergic outcomes, have been reported in birth cohort studies (e.g. Erkkola et al, 2012)[10], and in a cohort study that evaluated maternal dietary intake (i.e. Mediterranean diet (MD) adherence) with follow-up of the children who were born[11].

The MD is a cultural, healthy-eating model characterised by the abundant intake of fruits and vegetables; other plant foods such as legumes, nuts, seeds, and olive oil; fish; and a low intake of red and processed meats, and of wine consumed with meals. Evidence-based health applications of the MD have been described.[12]

In an examination of associations between food and nutrient intake by pregnant women and children and the risk of children developing allergy and asthma,[13] vitamins A, D and E, zinc, fruit and vegetables, and the MD were found to have potentially substantial protective roles. Of these, vitamins A, D and E and possibly others are under investigation e.g. Litonjua et al[14].

Investigating dietary patterns represents a more complex approach to food and nutrient consumption compared with studying single item consumption[15]. It allows for synergy between individual items that might foster favourable changes in biological mechanisms, such as in oxidative stress and inflammation, which are involved in allergy and asthma.

A review by Nurmatov et al[13] included five, observational studies of the MD, of which the study by Chatzi et al[11] was assessed to be the highest quality with only a 'moderate' risk of bias. These authors suggested that a high MD score during pregnancy was protective for persistent wheeze (OR 0.22; 95% CI 0.08 to 0.58), atopic wheeze (OR 0.30; 95% CI 0.10 to 0.90) and atopy (OR 0.55; 95% CI 0.31 to 0.97) at age 6.5 years.

The MD could offer an effective primary prevention strategy that needs to be investigated through formal experimental studies, however, there are currently no RCTs testing the hypothesis that enhancing MD adherence in the mother will decrease the risk of allergic disease in children[16]. There is therefore a need for a well-designed, adequately powered RCT to investigate the potential protective effects of the MD on the risk of developing allergy and asthma. We report here a pilot RCT to investigate rates of maternal recruitment and retention in the control and intervention arms, and to assess the acceptability of dietary MD advice and dietary MD modifications in the intervention arm. Additionally we aimed to estimate the effect of the intervention on MD score at 12 and 24 weeks post-randomisation, and we sought to measure any changes in urinary biomarkers of antioxidant capacity, oxidative stress and of whole-body nitric oxide production.

Methods

Ethical approval and trial registration

The trial was carried out according to the guidelines laid down in the Declaration of Helsinki and received a favourable ethical opinion from the NHS Lothian South East Scotland Research Ethics Committee 03 (REC reference 12/SS/0052) and management approval from the NHS Lothian Research and Development (project no. 2012/SJ/DN/01). Written informed consent was obtained from all participants. The trial was registered at ClincialTrials.gov (registration no. NCT01634516; Protocol registration receipt date 07/03/2012).

Trial design

We carried out a two-arm pilot parallel group RCT. To follow good practice[17], a study protocol manuscript was submitted prior to completion of participant recruitment and a detailed protocol has been published[16]. Two maternity service sites were used, with dating scan appointment rates of circa 100 per month. Eligible participants were randomised to receive either; diet advice and support, with a supporting MD resource booklet, in addition to standard care (the intervention arm), or, standard care with no additional advice dietary advice or support or materials (the control arm). Enrolment was for a period of ~ 6 months (i.e. from 12 through to 36 weeks of pregnancy.

Recruitment

Inclusion and exclusion criteria have previously been described[16].One hospital and one community treatment centre was used to enrol women into the study when they attended for their dating scan. Women had responded to the invitation sent out with their dating scan appointment, contacted the researcher, and were eligible for the pilot study (i.e. at high allergy/asthma risk for the foetus) based on a positive answer to the question 'Do you (the mother), or the father, or sibling of the baby have an allergic disease; eczema, a food allergy, hay fever, or asthma?' Baseline data collection was by Food Frequency questionnaire (FFQ), baseline MD questionnaire and urine specimens obtained in 100ml sample containers, refrigerated, and stored frozen at -80^{o}C in 2ml aliquots within 12-24hrs for subsequent analysis.

Randomisation and Intervention

Participating women were randomised 1:1 to the intervention or control arm. Allocation was stratified by site, using pre-randomised sealed envelopes prepared by an independent statistician.

Intervention

The intervention and the intervention arm protocol have been described previously[16]. The intervention took place at the dating scan clinic, after the scan and when the pregnancy was confirmed viable and healthy. It was a single 15 minute structured dietary advice session encouraging the consumption of foods consistent with the MD, developed with a hospital dietitian and administered face-to-face by a researcher (VSH or DAS) or dietitian using an agreed protocol and a booklet for consistency. The booklet contained text and pictures, with ideas for modifying the diet such as eating more fruit and vegetables, using olive oil, and eating more fish. No energy restrictions were suggested, and a target of at least five portions of fruit and vegetables per day was emphasised. Participants' use of supplements e.g. folic acid, vitamin D, was recorded. The researcher/dietician also discussed ideas for participants to reach the goals of eating more fruit, vegetables and fish in the context of their current portion consumption. The use of olive oil for cooking and dressings was encouraged, and a shopping voucher given (£10) at baseline and 12 weeks post-baseline, recommended for purchasing olive oil.

The initial intervention session was followed by supportive telephone calls to the women by the researcher or dietitian at four, eight and 18 weeks post-randomisation for the personalised MD goals to be reviewed and modified (e.g. increase fruit and/or vegetable target).

Control arm

Control arm participants followed the same protocol as the Intervention arm, except they did not receive the structured dietary advice session or supportive follow-up telephone calls. Control arm participants, like intervention arm participants also received supermarket vouchers, but without accompanying advice about how to spend them.

Measuring MD score

The MD score (possible range 0-24) was measured by the MD questionnaire pre-randomisation (around 12 weeks of pregnancy) and at 12 and 24 weeks post-randomisation[16]. The number of times participants consumed particular food groups in the previous week was classified into never, one or two times, or three or more times. For beneficial components (for example vegetables, legumes, fruits, cereals, fish) the frequency scoring was higher than for components considered less beneficial (meat, fast food, confectionery). As this trial involved pregnant women, we assumed dairy products to be protective (increased need for calcium), and we did not include alcohol consumption in the score[11].

Biomarker analysis

Stored urine samples were analysed at the end of the trial, such that batch and participant paired-sample analyses could be carried out. Levels of the stable metabolic products of nitrous oxide (NO), nitrite/nitrate (NOx^-) (markers of whole-body NO production, and nitrate intake), ferric reducing antioxidant potential (FRAP; a measure of total antioxidant activity), and urinary 8-hydroxy-2'-deoxyguanosine (8-OHgG; a marker of oxidative DNA damage and oxidative stress) were determined. Urinary biomarker sample values are expressed per µmol creatinine (Crn). Urinary Crn was measured using high-performance liquid chromatography based on Dunnett et al[18]. Sample Crn values were determined from peak height by reference to a Crn standard curve.

The stable breakdown products of nitrous oxide (NO), nitrite ($^-NO_2$) and nitrate ($^-NO_3$) were measured using a colorimetric assay kit (Arbor Assays, catalogue no. K023-H1). Samples were prepared by filtering through a molecular weight cut-off filter (Corning Spin-X UF 500, catalogue no. 431478) according to the kit manufacturer instructions. Samples were diluted 1 in 10 with assay buffer and 50 µL analysed in duplicate. 'Total NO' determination was preceded by $^-NO_2$ determination, both using a wavelength of 548nm. Sample $^-NO_3$ concentrations were obtained by subtracting the $^-NO_2$ concentration from the 'Total NO' concentration. Sample values were expressed per µmol creatinine (Crn). The between assay coefficient of variation (CV) was 3.3% (mean 206 µmol $^-NO_3$; n=5).

The antioxidant potential of urine was determined using a colorimetric FRAP method that estimates the reduction of ferric tripyridyl triazine (Fe(III) TPTZ) complex to ferrous form, based on Benzie and Strain[19]. The change in absorbance is directly related to the combined or 'total' reducing power of the electron donating antioxidants present in the reaction mixture. Iron (II) sulphate was used to construct a standard curve. Samples were diluted 1 in 20 (or repeated at 1 in 40 if out of range) with distilled water. Samples were measured in duplicate and results expressed as µmol Fe(II)/µmol Crn. Within-assay CV was 1.1% (mean 255.8 µmol Fe(II); n=10).

Urinary 8-OHdG was determined using an in-vitro enzyme-linked immunosorbent assay kit (JaICA, Catalogue no. KOG-200S/E). Urine samples were thawed shortly before analysis and centrifuged at 4,000g for 10 minutes. Fifty microlitres of clear, undiluted urine was analysed in triplicate according to the assay kit manufacturer instructions. Urine sample values (ng/ml) were extrapolated from a standard curve generated for each assay, and expressed per µmol creatinine (Crn). Within assay precision was assured by constructing a standard curve for each batch of participant paired (baseline/end of study) samples analysed, and the (CV) was 9.3% (mean 8.86 ng/ml; n=10).

Between-assay precision for the all of the biomarkers was monitored using a freshly thawed aliquot of urine, stored at minus 80^{o}C for quality control purposes.

Nutrient intake estimation

The Scottish Collaborative Group FFQ (v6.6), a self-administered, 169-item FFQ, was used to estimate nutrient intake and to compare the intervention and control arms.[20]

Health economic data

We recorded health economic data to assess the feasibility of pilot procedures for reporting intervention costs.

Qualitative evaluation of the trial

A sample of participants were contacted by telephone at the end of the trial period for a recorded semi-structured telephone interview by a researcher who had not been involved in

meeting the participants. These aimed to evaluate the process of the pilot trial from the perspective of participants i.e. to explore views regarding the acceptability of the intervention, any concerns, and any suggestions for improving the trial procedures.. The interview structure was developed by the Project Management Team, particularly the psychologist (AR) and one of the clinical triallists (AW).

Statistical analysis and calculations

Data analysis using Statistical Package for the Social Sciences (SPSS) v22 was initially carried out blind to the allocation arm. The data related to the participant characteristics, MD score and biomarker values are presented as mean values and standard deviations (SD). The statistical analysis plan (descriptive analysis, recruitment and retention and MD score) has been described[16].

Measurement of potential confounders

Data relating to potential confounders were collected: eczema, food allergy, allergic rhino-conjunctivitis and asthma in the mother, father and siblings as reported by the participant; exposure to smoking during pregnancy (by the mother, partner, or in the household); mothers' dietary pattern; folic acid and vitamin D supplementation; maternal education; maternal and paternal employment; body mass at booking-in and at end of trial; the baby's birth weight, and gender. Given the small sample size of this pilot trial, it was not possible to adjust for these confounders in the analysis.

Qualitative data analysis

Telephone interviews were transcribed verbatim and were analysed for key emerging themes, using a thematic content analysis[21].

Results

Recruitment and retention

Details of the trial were sent to 848 pregnant women, of whom we anticipated 25% were likely to have been eligible. Thirty one women responded to the invitation and met the eligibility criteria (3.7%; Figure 1). Of these, 30 (11 nulliparous) were recruited into the trial between June and December 2012, at a gestational age of around 12 weeks. Twenty-eight (93%) participants completed the trial. Both participants who withdrew were in the intervention arm. No reason was offered for their withdrawal.

Participant and eligibility characteristics

All women required only routine low risk antenatal care. The mean age ±SD of participants in each arm were: Intervention arm 32.2±5.2 years, range 17-38 years, n=14; Control arm 33.9±4.2 years, range 27-39 years, n=16. Socio-demographic characteristics of the women were recorded. Most had none or 1 child, did not smoke, were employed and had a high educational attainment. Eligibility qualifying criteria can be seen in Table 1.

Change in MD score

Baseline MD score was around 50-55% of the potential maximum (Table 2). The adjusted mean difference (Intervention-Control) in the change in MD score from baseline to 12 weeks was 2.4 (95%CI, 0.6 to 4.2, P=0.012) and from baseline to 24 weeks was 1.4 (95%CI, -0.4 to 3.3, P=0.13).

Biomarker analysis

No nitrite ($^{-}NO_2$) was detected in any of the samples, as would be expected in urine from healthy participants. There was no significant difference in urinary nitrate ($^{-}NO_3$) between baseline and 24-weeks post-randomisation (Table 3) in either Intervention or Control arm (P=0.172 and P=0.069 respectively). The adjusted mean difference (Intervention-Control) in the change in urinary $^{-}NO_3$ from baseline to 24 weeks was not significant (0.011 µmol/µmol Crn; 95%CI, -0.017 to 0.039, P=0.431).

There were no adjusted mean differences (Intervention-Control) in urinary FRAP (0.086 µmol Fe(II)/µmol Crn; 95%CI, -0.199 to 0.371, P=0.539), or in urinary 8-OHdG (0.092 ng 8-OHdG/µmol Crn (95%CI, -0.199 to 0.384, P=0.519).

Estimation of nutrient intake

Based on FFQ completion, total energy intake was 11.1±3.2 MJ at baseline (range 5.3 to 19.6 MJ) and 11.1±3.3 MJ 24 weeks post-randomisation (range 6.4 to 19.6 MJ). Saturated fatty acid intake was unchanged in both Intervention and Control arms from baseline to 24 weeks, as was monounsaturated and polyunsaturated fatty acid intake. Vitamin C intake in the Intervention arm at baseline was 158.3±75.6 mg compared with 24 weeks post-randomisation (193.8±68.6 mg; P=0.051), however, the adjusted mean difference (Intervention-Control) in the change in estimated vitamin C intake from baseline to 24 weeks was 25.9 mg (95%CI, -14.3 to 66.0, P=0.195). There were no significant differences in the estimated intake of vitamins A, D or E between arms or significant changes from baseline to 24 weeks post-intervention in either arm.

Health economic data

The mean (±SD) time taken to deliver the intervention was 18.3±6.1 minutes, range 10-35 minutes). Telephone calls to intervention arm participants at 4, 8 and 18 weeks post-enrolment had an average duration (range) of 6.3±2.3 (3-11), 5.4±1.7 (3-8) and 6.0±2.8 (4-14) minutes respectively. The mean total duration of telephone calls per participant was 16.3±4.3 minutes.

Pregnancy outcomes

Weight gain from baseline (~12 weeks of pregnancy) to 36 weeks of pregnancy was 11.6±4.1 kg (range 5-19 kg) in the intervention arm (n=11) and 11.3±4.0 kg (range 3-18kg) in the control arm (n=14). All participants successfully delivered and all were single births. There were eight females and four males born to the intervention arm participants, and nine females and seven males born to the control arm participants. Birth weights were 3.57±0.54 kg and 3.61±0.32 kg in the Intervention and Control arm respectively.

Qualitative evaluation of the trial

Thirteen participants (intervention arm n=9) were interviewed by telephone at the end of the trial. The interviews lasted 10-30 minutes and were audio-recorded. Participants believed themselves already to have been somewhat aware of healthy eating, and taking part in the pilot trial had increased awareness of diet in pregnancy for both intervention and control participants. Having a child, partner or other family member with allergy or asthma was the primary motivation for participating.. Interviewees reported no drawbacks to joining the trial, although some suggested that real or perceived additional costs for shopping for a MD could be a drawback for other women.

The intervention was highly acceptable to interviewees. The personal and flexible contact with the trial researcher and especially the follow up support calls were appreciated by interviewed participants, e.g. opportunity to ask minor questions, to be motivated and to be reminded about returning trial questionnaires. Some expressed concerns about the accuracy of retrospectively reporting diet in the FFQ; suggestions included advising participants to keep a brief weekly food record to support later completion of the questionnaire, considering intermittent, short periods of keeping a full food diary as part of the trial process, and introducing a mobile app for participants to record diet information in real time. Suggestions for improving the MD booklet were to include a wider range of recipes in the booklet itself or to give access to a webpage where further recipes as well links to more detailed information on diet, allergy, asthma and related research would be easily available for anyone interested in finding out more.

The role of midwives in the lives of pregnant women was highlighted by interviewees, who could be used to enhance trial recruitment. Interviewees suggested that the midwife could give potential recruits trial information personally during their initial meetings but recruitment should be later, when women had had time to discuss the study with other household members and to pass any first trimester 'morning sickness'. Recruitment, interviewees reported, should be proactive in order to avoid women having to take the initiative to contact the research team at a time they were likely to be feeling tired and forgetful, even if they were interested and willing to enrol in research. Other suggestions to enhance enrolment included more use of information technology (IT) to publicise the trial through existing, popular social networks, for example, Mumsnet, Some interviewees also suggested 'snowballing' from recruited participants who could use their personal networks of parents to spread information about the research.

Discussion

This pilot trial demonstrated the feasibility of retaining a group of pregnant women over a period of 24 weeks and demonstrated an increase in MD score after 12 weeks of the intervention compared with control. Such an increase, if equating to a move from a low-quality maternal MD score into a higher range may potentially be protective for wheeze and atopy in the children born, as was suggested by the cohort study of Chatzi et al[11]. Our trial intervention apparently encouraged an increased intake of fruit and vegetables, potentially increasing the intake of the antioxidant vitamins C and E, however, analysis of the FFQ data did not reveal any significant difference. It can be hypothesised that an increased fruit and vegetable intake would result in the urinary excretion of a water-soluble, antioxidant vitamin such as vitamin C, but we did not see indication of an increase in urinary FRAP in the intervention arm. Fruit and vegetables, particularly vegetables, are a major source of dietary nitrate[22], however, there was no adjusted mean difference between trial arms of urinary nitrate. A small sample size and therefore a lack of statistical power is likely to be a limitation to the biomarker analysis. In this pilot trial we were also able to collect health economic and pregnancy outcome data that is necessary to inform a larger trial where health economic analysis is intended and confounding variables need to be adjusted for.

This pilot trial, of an intervention aimed at increasing adherence to an unrestricted MD in pregnant women, is a pre-requisite for informing the design of a large-scale trial to test the hypothesis that greater adherence to a MD during pregnancy will reduce the risk of allergy in children. The available epidemiological evidence is supportive of a link between the MD and the prevention of allergic disease[13], however, this is only testable in a large-scale primary prevention RCT with follow-up of the infants for several years.

Our pilot trial provided the opportunity to model the potential intervention and to refine its practicality (e.g. recruitment, retention, sample size determinants). Having considered the range of options for recruitment, we chose a dating scan clinic recruitment strategy as a feasible and cost-effective one which has the potential for future scaling-up. We were able to recruit and retain a small sample of women at high-risk of their children developing allergic disease. Recruitment was, however, slower than anticipated. The recruitment period was extended, but was also limited by the funding opportunity (maximum project duration of 12 months). We recruited through a centralised NHS booking system operated from the major hospital in the region, meaning that the number of invitees was high in proportion to the

number that were eligible and might respond to such an invitation. Prior to the trial we estimated that around 800 invitations to participate would be sent and that one-quarter (n=200) would fulfil the eligibility criteria[16], based on the epidemiology of allergic disorders in Scotland, systematic reviews of primary prevention trials[23; 24], and a dietary intervention trial in pregnant women[25]. From our discussions with consumer representatives which informed the trial, we anticipated that around 50 eligible women would be willing to take part, and, after some participant and data attrition, we anticipated that 40 participants would complete the study. Whilst our maternal recruitment rate was lower than anticipated, the retention rate of participants into the study was higher than envisaged. Any future recruitment strategy should, we suggest, include prior engagement with community midwives in an attempt to have them introduce the possibility of taking part in the study at an earlier stage, in order to increase the contemplative phase of participation. Furthermore, engagement with Children and Family Centres and other organisations with a role in improving maternal nutrition, through national frameworks (e.g. The Scottish Government[26]) and greater use of IT social networks could also be utilised to disseminate trial information and prepare potential participants for a letter of invitation. These wider strategies should also help to address any lack of diversity in participant characteristics - the sample recruited for this trial was largely well-educated and employed, and older than the Scottish average (29.7yrs; NRS[27]). Only 4 out of the 30 women in this pilot RCT were less than 30 years of age. Evidence from a systematic review of socioeconomic position (SEP) in the development of allergy and asthma suggests that allergy is associated with higher SEP, and asthma with lower SEP[28], emphasising the need to recruit from a broad socioeconomic spectrum, as well as considering underlying dysfunction ('endotype'). The recruitment rate and recruitment duration will be used to calculate how many centres will be required to carry out a large RCT.

We incorporated behavioural change techniques (BCTs; [29]) into the pilot intervention, such as goal-setting, and, provided information on how to perform the behaviour. Our aim is to continue to develop evidence-based BCTs, and their timing, for implementation in the dietary intervention for a large-scale RCT, with the aim of maintaining an increased adherence to the MD through to the end of pregnancy and postnatally. Although the MD score increased significantly in the intervention arm from baseline to 12 weeks, this was not sustained, with the increase in MD score being insignificantly higher at 24 weeks compared with baseline. After week 8 and before week 18 post-baseline, MD goals were not reviewed and revised.

There was contact at 12 weeks to complete a MD score questionnaire. We suggest that additional contact and continuation of the BCTs used in our intervention are justified, given the arguably short amount of time spent delivering the intervention (about 18 minutes on average) and on follow-up telephone calls (about 16 min on average). The average time spent on delivering the intervention i.e. on both face to face delivery at baseline and on subsequent telephone calls, was 34 min. This represents a mean cost of £19.82 per participant (excluding telephone call charges) based on a recent estimate of the cost of hospital dietitian time[30].

The aim of measuring urinary biomarkers markers was to gauge possible shifts towards increased antioxidant capacity (FRAP), increased whole body NO production and/or fruit and vegetable intake (Nitrite/Nitrate), and decreased oxidative stress (8-OHdG). A key physiological mechanism underlying the potential effect of maternal diet on allergy outcome is based on the hypothesis that at a critical stage of foetal development there might be oxidative stress or compromised vascular development in the lung (e.g. for asthma) which causes damage, leading to allergy susceptibility. A diet rich in antioxidants for example might offset oxidative stress. Measuring a marker of both antioxidant capacity and oxidative stress may allow for better understanding/corroboration of data. Measuring the stable products of nitric oxide (e.g. nitrate) to give a marker of whole-body NO production may inform us of the status of a key metabolic regulator implicated in diverse pathological states, also related to free radical biology, and of potential influence in the vasculogenesis of organs and tissues. Vegetable consumption is also a determinant of urinary nitrate excretion. Whilst purporting to be an assay measure of whole body NO production, in the expected absence of urinary nitrite in our sample population the assay used was effectively a measure of urinary nitrate. The source of the nitrate is therefore a combination of in-vivo formation from NO, and, ingested nitrate. Approximately 80% of dietary nitrates are derived from vegetable consumption[31]. Other sources include fruit. Urinary nitrate might therefore reflect fruit and vegetable intake and this can readily be seen if a concentrated source of vegetable nitrate (beetroot juice) is ingested (Sewell, Unpublished observations). It could be hypothesised that an increase in MD score which is in part due to an increase in fruit and vegetable intake, a key part of the intervention strategy used in the present study, may result in an increase in urinary nitrate. Despite evidence of a correlation between the intake of foods with a high antioxidant content (e.g. walnuts[32], extract of *Hibiscus sabdariffa*[33], and Green Tea[34]) and plasma and urinary FRAP having been demonstrated, we were not able to demonstrate using

robust statistical analysis any differences between trial arms, most likely due to the small sample size, and a 'fade' in compliance to the advice, reflected in the smaller difference in the MD score between groups at 24 weeks compared with 12 weeks. Of the urinary biomarkers measured, urinary nitrate and FRAP in a random urine sample, or in the future in blood samples, might be ones to take forward to a larger RCT to add further credence to a short questionnaire method of assessing MD change.

No significant changes were seen between groups or over time in the measurement of urinary 8-OHd, a sensitive, stable and integral marker of oxidative stress in-vivo [35]. This was included as a biomarker because previous work relating to allergy has suggested that urinary 8-OHd was higher in patients with atopic dermatitis compared with control children free of allergic or inflammatory diseases[35]. In our hands, the within-assay CV was too high, particularly in comparison with the other biomarker measures. Furthermore, the commercially available kit is relatively expensive, and we suggest that whilst the measure may be of benefit in comparing patients with different clinical outcomes, it appears less promising as a biomarker of dietary change.

Baseline FFQ data were collected prior to randomisation to the intervention or control arm so that well-recognised limitations of dietary reporting tools (e.g. contamination of control arm) were not amplified at that stage. Intervention arm study participants would, however, have been aware of expected dietary behaviour on completion of the FFQ at the end of the trial. It might be hypothesised that the emphasis placed by the intervention on the increased use of olive oil, consumption of fish and the reduction of red meat consumption would potentially increase MUFA and PUFA intake and decrease saturated fat intake, however this was not seen in the data. Between group and differences over time of vitamin intake for which there is a potentially a protective role (vitamins A, C, D and E) were also not evident. Given the small sample size and the systematic variability in dietary assessment (e.g. problems inherent in the use of retrospective, self-reported methods of dietary intake, which lack quantitative sensitivity), one might anticipate difficulty in finding differences.

We have been able to show an increase in a MD score during the second trimester of pregnancy, however, we were not able to maintain a significant increase in MD score during the third trimester of pregnancy. Our MD score assumed dairy products to be protective because of the increased requirement for calcium during pregnancy, and the score did not include alcohol consumption because alcohol consumption is not recommended in

pregnancy. Six participants indicated that they consumed some alcohol during pregnancy. Assessing the potential clinical significance of the increase in MD score seen in this pilot trial is difficult, given that there have been no intervention trials of the MD on allergy. The magnitude of change seen in this pilot trial might be compared with a cohort study of the MD in pregnancy as protection for wheeze and atopy in childhood[11]. Using the 'low' level score as a reference, high MD score during pregnancy was found to be protective for persistent wheeze (OR 0.22; 95% CI 0.08 to 0.58), atopic wheeze (OR 0.30; 95% CI 0.10 to 0.90) and atopy (OR 0.55; 95% CI 0.31 to 0.97) at age 6.5 years. An increase in MD score of 3 (in a total score of 22) in the Chatzi study could potentially move a woman out of the lowest tertile MD score in pregnancy. In a large trial of the effect of a MD intervention on cardiovascular disease end-points, Estruch and colleagues[36] achieved a highly statistically significant long-term change in MD adherence, sustained for 5 years, ranging from 1.4 to 1.8 points (on a 14-point scale) which resulted in a reduction in the incidence of major cardiovascular events in participants at high cardiovascular disease risk. Our pilot trial has indicated a potential mean benefit in the MD score of between 0 and 4 points, on a 24-point scale, in the second trimester of pregnancy.

Conclusions

In this pilot RCT, recruitment was a challenge but might be improved by contact with potential participants at an earlier stage of the pregnancy and a wider recruitment strategy. Retention of participants was high. The procedures and intervention appeared highly acceptable, which is likely to have contributed to the high retention. The intervention effect might be maximised by continued BCT support during the third trimester of pregnancy. Favourable changes in MD score were achieved, but with some fall back over time, hence the need for continued reinforcement through the goal-setting and information strategies employed in the first 12 weeks of the intervention phase. The mean change in MD score would appear sufficiently promising to pursue this programme of work towards a large-scale RCT. Evidence of dietary change through urinary biomarker changes and FFQ was not seen, despite an increase in fruit and vegetable intake being a key part of the intervention and participant goal-setting reinforcement. Given that there is need for a well-designed and adequately powered RCT to investigate the potential protective effects of the MD on the risk of developing allergy and asthma, we are following a framework for developing and

evaluating complex interventions[17]. Following on from a development phase, this trial of feasibility provides important insights into recruitment and retention for a large trial. The outcome measures chosen and reported are important in the design and potential funding of an adequately powered, large-scale RCT. Through participant involvement, the qualitative work produced a number of ideas that might be considered for recruitment, retention and effect sustainability in dietary intervention studies. This pilot RCT will enable us to refine a protocol and continue the programme of work to test the hypothesis that greater adherence to a MD during pregnancy will reduce the risk of allergy in children.

Transparency declaration

The lead author (Dean A. Sewell) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned and registered (ClinicalTrials.gov: NCT01634516) have been explained. The reporting of this work is compliant with CONSORT guidelines.

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Author contributions

DAS was the Chief Investigator and with ASh obtained the funding for the trial. DAS and ASh designed the programme of work and along with VSH developed the protocol and obtained funding. VSH was the part-time project researcher who ran the trial and with the assistance of DAS carried out the recruitment, intervention and data collection. The qualitative interviews and analysis were carried out by AR. In accordance with Good Clinical Practice Guidelines and NHS Research Governance requirements, a Project Management Committee was formed including the authors DAS, ASh, VSH, GD (Clinician), AR (Psychologist), CW (Statistician), ASt (Health Economist), AW (Qualitative researcher) to enhance the protocol and support the project. DAS carried out the urinary biomarker analyses and produced the first and subsequent drafts of the paper. DAS and VSH carried out the data entry and with CW analysed the data. AW contributed to the design of the qualitative work and consumer involvement. ASt advised on health economic data collection. All authors read, contributed to and approved the final manuscript. The authors declare no conflict of interest.

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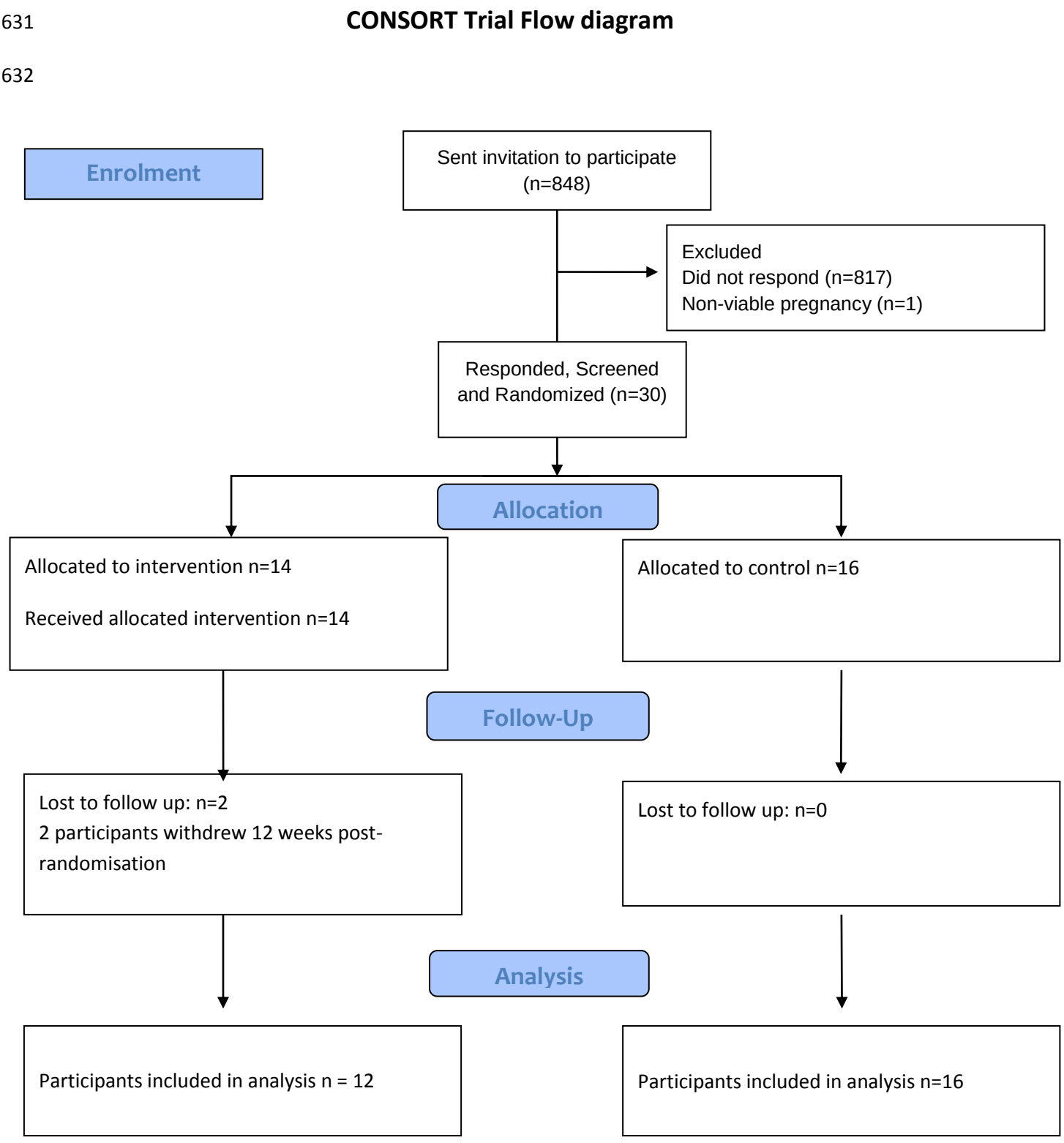
Figure. CONSORT Trial Flow diagram. Pregnant women were sent an invitation (by mail) to take part in the trial, along with their dating scan appointment confirmation and other pregnancy information.



633 Tables

634

635 Table 1. Eligibility qualifying criteria of recruited participants (n=30) based on the question
636 "Do you (the mother), or the father, or sibling of the baby have an allergic disease: eczema, a
637 food allergy, hay fever or asthma?"

638

639

Eligibility of one of eczema, food allergy, allergic rhinitis/hayfever or asthma	**n**
Mother only	7
Father only	6
Sibling only	3
Mother and father	5
Mother and sibling	3
Father and sibling	2
Mother, father and sibling	4

640

641

Table 2. Mediterranean Diet scores expressed as means (±SD). The maximum score obtainable for this current trial was 24, and included/excluded food categories appropriate in pregnancy.

	Baseline	12 weeks	24 weeks
Intervention	12.4 (2.9) n=14	*15.7 (3.0) n=10[#]	14.8 (3.0) n=12
Control	13.0 (1.9) n=16	13.6 (2.6) n=16	13.4 (1.9) n=15

*Indicates a significant increase in the adjusted mean difference (Intervention-Control) compared with Baseline (P=0.012)

[#] Two Intervention participants who completed the trial did not return the MD questionnaire at 12 weeks

Table 3. Mean values (±SD) of urinary biomarkers assessed in all samples obtained at Baseline and 24 weeks post-intervention

	Baseline				24 weeks post-intervention			
	Intervention (n=13)		Control (n=15)		Intervention (n=12)		Control (n=15)	
Biomarker	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Nitrate (µM/µM Crn)	0.052	0.018	0.083	0.045	0.071	0.037	0.068	0.027
FRAP (µM Fe(II)/µM Crn)	1.050	0.243	1.110	0.428	1.310	0.403	1.280	0.334
8-OHdG (ng/ µM Crn)	0.978	0.408	0.964	0.475	0.977	0.404	0.876	0.373
Crn (mmol/L)	*8.9*	*5.3*	*7.6*	*4.3*	*10.1*	*6.3*	*9.0*	*5.1*

FRAP, Ferric Reducing Antioxidant Potential; 8-OHdG, 8-deoxyguanosine; Crn, Creatinine

n values represent number of samples collected in each arm at each time point.