The PONTI Study
Prevention of Neural Tube defects by Inositol, in conjunction with folic acid

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Chief Investigator: Prof. Andrew J. Copp (AC)
Post: Professor of Developmental Neurobiology
Address: Neural Development, Institute of Child Health, 30 Guilford Street, London. WC1N 1EH
Telephone: +44 (0)207 905 2189
Fax: +44 (0)207 831 4366
E-mail: a.copp@ucl.ac.uk
**Sub-Investigator:** Dr. Lyn S. Chitty (LC)  
Post: Senior Lecturer in Genetics and Fetal Medicine  
Address: Clinical and Molecular Genetics, Institute of Child Health, 30 Guilford Street, London. WC1N 1EH  
Telephone: +44 (0)207 242 9789 ext 26  
Fax: +44 (0)207 813 8141  
E-mail: l.chitty@ucl.ac.uk

**Sub-Investigator:** Dr. Nicholas Greene (NG)  
Post: Reader in Neural Development  
Address: Neural Development, Institute of Child Health, 30 Guilford Street, London. WC1N 1EH  
Telephone: +44 (0)207 905 2217  
Fax: +44 (0)207 831 4366  
E-mail: n.greene@ucl.ac.uk

**Sub-Investigator:** Dr. Therese Hesketh (TH)  
Post: Senior Lecturer in International Child Health  
Address: Centre for International Child Health, Institute of Child Health, 30 Guilford Street, London. WC1N 1EH  
Telephone: +44 (0)207 905 2253  
Fax: +44 (0)207 404 2062  
E-mail: t.hesketh@ucl.ac.uk

**Trial Co-ordinator** Ms Victoria Shepherd  
Post: Trial Co-ordinator  
Address: Neural Development, Institute of Child Health, 30 Guilford Street, London. WC1N 1EH  
Telephone: +44 (0)207 905 2282  
Fax: +44 (0)207 831 4366  
E-mail: ich.pontistudy@ucl.ac.uk

**Sponsor Contact:** Praseeda Thaikalloor  
Post: Clinical Trials Manager  
Address: Research and Development, Institute of Child Health, 30 Guilford Street, London. WC1N 1EH  
Telephone: +44 (0)207 905 2346  
Fax: +44 (0)207 905 2201  
E-mail: Nabila.Youssouf@gosh.nhs.uk
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Summary
Neural tube defects (NTDs) are severe malformations of the central nervous system, occurring in 0.5 to 2 per 1000 pregnancies. NTDs result when the neural tube in the developing fetus fails to close in the third and fourth weeks of gestation. As pregnancy progresses, exposure to the amniotic fluid environment leads to neurodegeneration (Stiefel et al., 2003) so that, by birth, the exposed brain and/or spinal cord are destroyed. Fetuses with brain NTDs (e.g. anencephaly) are stillborn while those with spinal NTDs (e.g. myelomeningocele/open spina bifida) often survive, but are neurologically impaired below the lesion. Children with myelomeningocele often have lack of sensation, inability to walk/stand, and incontinence of urine and faeces. Associated conditions include hydrocephalus, requiring cerebrospinal fluid shunting, and vertebral deformities. Prenatal diagnosis enables termination of NTD pregnancies in the UK and some other Western countries, but this is not universal. Babies with NTDs continue to be born in the majority of countries.

Primary prevention by folic acid (FA) offers a possible universal solution to the problem of the morbidity and mortality caused by NTDs. However there is considerable variation in the estimates of the effect of FA supplementation on the prevalence of NTDs. Even after high dose (4 mg daily) therapy in the MRC trial (Wald et al., 1991), 1% of pregnancies still had recurrent NTDs. Variation between studies may relate to the differing doses of FA administered. Indeed, NTD risk is related to the serum folate level in humans (Wald et al., 2001). Nevertheless, reports of NTD pregnancies recurring in a family despite high dose folate intake (Cavalli and Copp, 2002) argue strongly that a proportion of NTD cases may be fundamentally resistant to folate. This may reflect variation in aetiology, with some subtypes of NTD being folate-sensitive and others folate-resistant. Novel therapies are needed to improve NTD prevention, by encompassing folate resistant cases which currently cannot be prevented.

Considerable evidence has now accumulated to indicate the need and appropriateness of a clinical trial to assess whether inositol is capable of preventing FA-resistant NTDs in humans, as in mice. These several lines of evidence can be summarised as:

1. Inositol deficiency is the only ‘vitamin’ deficiency that leads to NTDs in mice (Cockroft, 1988; Cockroft et al., 1992). Even FA deficiency does not cause NTDs in mice, in the absence of genetic predisposition.

2. Significantly lower inositol concentration is present in the blood of mothers carrying NTD fetuses than in normal pregnancies (Groenen et al., 2003). Hence, human NTDs may be associated with inositol deficiency.

3. Inositol supplementation during pregnancy among the NTD mutant mouse model curly tail, significantly reduces the frequency of NTDs (Greene and Copp, 1997; Cogram et al., 2002), a finding that has been replicated recently in another laboratory (Ting et al., 2003).

4. In a single case study, a woman took 0.5 g inositol per day in the first trimester of her third pregnancy, after two previous pregnancies were terminated because of NTDs (she took FA in both). The third pregnancy was uneventful, and a normal baby was born (Cavalli and Copp, 2002). Subsequently, four other women with a predisposition to NTDs
(with a past history of affected pregnancies) have taken 0.5 g inositol and all have had babies unaffected by NTDs (Cavalli, personal communication).

5. Inositol therapy appears safe during mouse pregnancy. Detailed pathological analysis of inositol-treated curly tail mice revealed no major fetal defects and no increase in fetal loss (Cogram et al., 2002). Treated pregnant females showed no adverse effects.

6. Regarding the safety of inositol in human pregnancy, the mother who took inositol in a third pregnancy reported no side effects (Cavalli and Copp, 2002). Trials in adults for depressive psychiatric disorders, to improve insulin sensitivity in polycystic ovary syndrome, and to ameliorate psoriasis in patients on lithium therapy have all identified beneficial effects of inositol with no major side effects. In children, inositol has been used to treat autism and to prevent sequelae of respiratory distress syndrome in premature babies. Relatively high inositol doses have been used: up to 20 g per day in adults and 200 mg/kg in children. Inositol is a constituent of living cells, is widespread in many foods, and is an essential nutrient included in the culture medium of many cell lines (for more information see Investigators Brochure). It seems unlikely that exogenous inositol therapy will pose a risk to the mother and/or embryo/fetus.

Our aim is to develop a randomised, double blind clinical trial to compare (i) folic acid plus placebo, or (ii) folic acid plus myo-inositol (inositol), for prevention of NTD recurrence. This will evaluate the effectiveness of inositol in the context of co-administration with folic acid. Hence, we will adopt a simple two-arm trial protocol in which all subjects receive identical folic acid supplementation at the ‘high dose’ level of 5 mg/day, typically used in pregnancies at risk of NTD. Inositol dose level is to be set at 1 g/day. Placebo will be used instead of inositol in the control group. We will recruit into the trial women who are planning a pregnancy and who have a history of one or more NTD pregnancies. Supplementation will begin prior to conception and continue until the 12th week of pregnancy. Pregnancy outcome will be determined by second trimester ultrasound scanning plus follow up of term pregnancies. Compliance will be monitored by the collection of urine samples prior to trial entry, 6 weeks after trial entry and following conception at the first antenatal clinic.

This clinical trial application is for a pilot study to assess the feasibility of conducting a full-term trial. The eventual proposed full-term trial will assess safety and efficacy of inositol in the prevention of neural tube defects. An amended application will be made for the full-term trial.

As part of the pilot study, we will (a) establish a network of collaborators in the UK who can recruit trial subjects with a previous NTD pregnancy; (b) confirm laboratory assays for inositol to monitor compliance and investigate levels in trial subjects; (c) determine feasibility and enable accurate power analysis for a larger scale trial.
1. **General Information**

1.1 **Sponsor Responsibilities**
   Request for Authorisation: Dr. Lyn Chitty
   GCP Conduct: Prof. Andrew Copp and members of the Research & Development office at Institute of Child Health
   Pharmacovigilance: Dr. Nicholas Greene to report to Research & Development office at Institute of Child Health who will report to MHRA.

1.2 **Trial monitor**
   Members of Research and Development at Institute of Child Health

1.3 **Sponsor’s medical expert for the trial**
   Medical expert to Chief Investigator: Dr. Lyn Chitty
   Medical experts to patients: Prof. Andrew Copp, Dr. Lyn Chitty and Dr. Therese Hesketh (For all addresses and telephone see page 1)

1.4 **Qualified physician responsible for all trial-site related medical decisions**
   Prof. Andrew Copp (For address and telephone see page 1)

1.5 **Clinical laboratories and other medical and/or technical departments and/or institutions involved in the trial**
   - Neural Development, Institute of Child Health, 30 Guilford Street, London. WC1N 1EH
   - Biochemistry, Endocrinology and Metabolism, Institute of Child Health, 30 Guilford Street, London. WC1N 1EH
   - Pharmacy, Great Ormond Street Hospital (GOSH Pharmacy), Great Ormond Street, London. WC1N 3JH
   - Pharmacy, University College London Hospitals NHS Foundation Trust, 235 Euston Road, London. N1W 2BU.
   - Paediatric Epidemiology and Biostatistics, Institute of Child Health, 30 Guilford Street, London. WC1N 1EH

1.6 **Person(s) authorised to sign the protocol and the protocol amendments**
   Prof. Andrew Copp, Dr. Lyn Chitty, Dr. Therese Hesketh and Dr. Nicholas Greene

2. **Background Information**

2.1 **Names of the trial treatments**

   **Full Name of IMP:** Myo-Inositol  
   **Full Name of Placebo:** Dextrose Monohydrate  
   **Full Name of treatment given in conjunction with IMP:** Folic Acid

   *Note: This is not an IMP but a concomitant medicine that will be prescribed in conjunction with Inositol and Placebo.*
2.2 Suppliers and manufacturers of the trial treatments

Supplier of Inositol (active ingredient):  
Tsuno Rice Fine Chemicals Co Ltd, 2283 Chonomachi, Ito Gun, Wakayama, Japan  
Manufacturer of Inositol Tablets:  
Pharmacy, University College London Hospitals NHS Foundation Trust, 235 Euston Road, London. N1W 2BU.

Packaging and Labelling of Inositol:  
Pharmacy, Great Ormond Street Hospital (GOSH Pharmacy), Great Ormond Street, London. WC1N 3JH  
Each container of 56 tablets will be labelled with its full identity (ie: not blinded). The labels will be attached to vinyl flag labels, which can be cut off at the time of dispensing by GOSH Pharmacy, so attaining blinding requirements. Labels will be generated from a protected template, using commercial label software, by thermal wax transfer onto permanent self-adhesive labels 89 mm x 36 mm.

Dosage of Inositol:  
1g (0.5g per tablet; two tablets to be taken daily to provide a dose of 1g/day)

Supplier of Placebo (active ingredient):  
Dextrose monohydrate supplied by Tate & Lyle.  
Manufacturer of Placebo Tablets:  
Pharmacy, University College London Hospitals NHS Foundation Trust, 235 Euston Road, London. N1W 2BU.

Packaging and Labelling of Placebo:  
Pharmacy, Great Ormond Street Hospital (GOSH Pharmacy), Great Ormond Street, London. WC1N 3JH  
Dosage of Placebo:  
1g (0.5g per tablet; two tablets to be taken daily to provide a dose of 1g/day)

Supplier of Folic Acid tablets (concomitant medicine):  
Marketing Authorisation number: 0142/5522R  
Holder: Alphapharma Limited (Actavis UK), Whiddon Valley, Barnstaple, EX32 8NS  
Labelling:  
Pharmacy, Great Ormond Street Hospital (GOSH Pharmacy), Great Ormond Street, London. WC1N 3JH  
Dosage of folic acid:  
5mg (5mg per tablet; one tablet to be taken daily to provide a dose of 5mg/day)

Details of Packaging and Labelling:  
A four week supply (28 tablets) of folic acid will be packaged in a bottle clearly labelled as folic acid. A four week supply (56 tablets) of inositol or placebo will be packaged in a bottle labelled as shown in appendix 15. Tablets will be dispensed and sent to patients in twelve weekly batches, therefore they will receive three bottles of folic acid and three bottles of inositol/placebo.
2.3 A description of and justification of the trial treatments

**Route of administration:** Oral

**Dosage:**
Inositol dose level is set at 1g/day. This is consistent with previous clinical trials of inositol in other indications. Placebo will be used instead of inositol in the control groups. All subjects receive identical folic acid supplementation of 5mg/day. This is the dose typically used in pregnancies at risk of recurrent NTD.

**Dosage regimen:** Each subject will take three tablets once per day.

Control group:
1 tablet containing 5mg folic acid and 2 tablets each containing 0.5g placebo.

Study group:
1 tablet containing 5mg folic acid and 2 tablets each containing 0.5g inositol

**Administration of drugs:**
Inositol and Placebo Tablets will be manufactured by UCLH Pharmacy and QP released to GOSH Pharmacy. Following manufacture, all drugs will be held at the GOSH pharmacy and dispensed from there on a named person basis. A medically qualified research team member (AC, LC, TH) will prescribe folic acid and inositol or placebo for a 6 month period for each women recruited to the trial. Randomisation and blinding will occur in the pharmacy, who will dispense the drugs in 12 weekly batches to the women. No drugs will be dispensed until the first urine sample has been received for monitoring. The trial co-ordinator will contact the women monthly by phone to ensure drugs have arrived, no problems have arisen and to determine if conception has occurred.

**Treatment periods:**
Upon receipt of the prescribed drugs by post, medication will begin immediately and will continue whilst the women is trying to conceive. Following conception, medication will cease at the end of the 12th postmenstrual week. It is not possible to state definitively the expected treatment period as this will depend on the time taken to conceive. The minimum period will be one month preconception and three months of pregnancy on medication. The maximum period of treatment within the confines of the pilot study will be twelve months preconception and three months of pregnancy on medication. We will stop prescription of the tablets if the woman fails to conceive within twelve months, as the risks of long term treatment are unknown. We shall inform women when this is due to happen. Recruitment will stop three months before the end of the pilot study and no more tablets will be prescribed after this period. Therefore women who are participating in the trial will no longer receive tablets if they are not pregnant at this point. We shall inform women when this is due to happen.

2.4 A summary of findings from non-clinical studies that potentially have clinical significance and from clinical trials that are relevant to the trial

**Supporting evidence from mouse models of NTD**
NTDs in the curly tail mutant mouse, which particularly resemble human NTDs, are resistant to FA therapy. FA-resistance is also observed in the NTDs of ephrin-A5 and Zic3 mutant mice (Holmberg et al., 2000; Franke et al., 2003). Among the vitamins, and other related small molecules, only inositol is required for the rodent neural tube to close (Cockroft, 1988; Cockroft et al., 1992). We found that both myo-inositol and D-chiro-inositol prevent the NTDs in the FA-resistant NTD model, curly tail, suggesting inositol
therapy as a possible adjunct therapy to FA in humans (Greene and Copp, 1997; Cogram et al., 2002). Inositol is effective when administered to pregnant female mice both orally and by subcutaneous infusion (Cogram et al., 2002). Direct treatment of curly tail embryos in culture normalises neural tube closure in the low spinal region, preventing spina bifida. The biochemistry of how inositol may be preventing NTD has been published (Greene and Copp, 1997; Cogram et al., 2004).

Other clinical trials involving Inositol (Other indications)
Previous clinical trials involving adults have demonstrated that inositol is effective in the treatment of psoriasis in patients taking lithium, polycystic ovary syndrome, bulimia nervosa, binge eating, panic attacks, depression, Alzheimer’s disease and obsessive compulsive disorder. In addition to these uses, inositol administered to preterm infants reduces the severity of respiratory distress syndrome. No side effects have been reported when the dose administered is less than 6 g per day. For further information see the Investigator’s Brochure.

Other clinical trials involving Folic acid for prevention of NTD
Randomised clinical trials of folic acid for the prevention of NTDs performed in the UK and Hungary showed that folic acid administered early in pregnancy can prevent up to 70% of NTD recurrences with a significant effect also on the first occurrence of NTDs (Wald et al., 1991; Czezial and Dudás, 1992). Folic acid will be provided in this trial as a concomitant medicine as it would be unethical to undertake a study whereby women with a history of a neural tube defect are required not to take folic acid. By prescribing folic acid as a concomitant medicine, the trial will evaluate the effectiveness of inositol in the context of co-administration with folic acid for the prevention of NTD.

2.5 Summary of the known and potential risks and benefits to human subjects
No adverse effects are expected from myo-inositol as this is a naturally occurring substance and none have been described in patients taking it for other indications at a dose less than 6 g per day. The potential for benefit to research participants is a reduction in recurrence of a neural tube defect

2.6 GCP compliance
The trial will be conducted in compliance with the protocol and in compliance with the Sponsor’s latest version of the appropriate Standard Operating Procedures.

2.7 References (from section 2.4 and summary)

Cockroft DL. Changes with gestational age in the nutritional requirements of postimplantation rat embryos in culture. Teratology 1988; 38: 281-90


### 3. Trial objectives and purpose

As a pilot study, the primary outcome measure is to enable a subsequent, appropriately-powered clinical trial of inositol to prevent NTDs, in conjunction with folic acid supplementation. As part of the pilot study, we will (a) establish a network of collaborators in the UK who can recruit trial subjects with a previous NTD pregnancy; (b) develop laboratory assays for inositol to monitor compliance and investigate levels in trial subjects; (c) determine feasibility and enable accurate power analysis for a larger scale trial.

The safety and effectiveness of inositol in the prevention of recurrent NTDs, in conjunction with folic acid, will be evaluated. However it is appreciated that the number of participants expected to be involved in the pilot study would not enable a sufficiently powered analysis of the data to be performed.

As a full-term trial, a positive outcome would demonstrate that inositol is effective in reducing the recurrence of NTDs. This would indicate the immediate need to change clinical practice by introducing inositol alongside folic acid in supplements recommended/prescribed to women planning a pregnancy.
4. **Trial design**

4.1 **Primary endpoint**
The primary endpoint to be measured will be the pregnancy outcome i.e. the occurrence of a neural tube defect. All women recruited to the study will be given a pregnancy outcome sheet to complete and return when the outcome of the pregnancy is known (see Appendix 13). The majority of NTDs (particularly in high risk women) are diagnosed in pregnancy at the time of the routine second trimester anomaly scan. Consent will be obtained at the outset of the study to contact the woman's GP, obstetrician or geneticist for further details of pregnancy outcome if required.

4.2 **A description of the type/design of the trial to be conducted**
The trial will be conducted in a controlled, randomised, double blind manner to compare (i) folic acid (5 mg daily) plus placebo, or (ii) folic acid plus inositol (1 g daily), for the prevention of NTD recurrence.

**Brief synopsis of the planned research for the pilot study**

**Recruitment**
Once ethical and MHRA approval is granted we will recruit women who have had one or more NTDs in a number of ways;

- We will circulate a leaflet to all fetal medicine units and all maternity units in the UK to inform them of the trial (Appendix 1) and request that they give information to any women in their care who have had a pregnancy affected by a NTD (Appendix 2). Local consultants will also be encouraged to refer women to the trial team direct.

- All regional genetic units will be contacted and informed of the study (Appendix 1). They will be encouraged to inform parents of the existence of the trial, give them a patient information leaflet (Appendix 2) and either refer direct, if appropriate, or allow the woman to self refer at a later date if she so wishes.

- Paediatric surgeons responsible for the care of neonates with spina bifida will be contacted and informed of the study.
- Information leaflets will be sent to ARC (Antenatal Results and Choices) and ASBAH (Association for Spina Bifida and Hydrocephalus) to send to any woman contacting them because they have had a pregnancy affected by a NTD (Appendix 2).

- ARC will place an advertisement in their regular patient information leaflet that is sent out to all members and will also put a link on their website to the trial website (Appendix 3A and 3B).

**Trial entry**
On receipt of a referral from any source, we will contact the woman by phone and arrange for her to complete a telephone questionnaire to determine eligibility (appendix 4). We will seek permission to approach relevant health professionals to determine any missing information or to ascertain that there are no exclusion criteria.

Once eligibility has been confirmed the trial coordinator will write to the woman offering her entry into the trial and asking her to make contact with the trial centre if required.
(Appendix 5). Initially the trial coordinator will discuss the details of the trial over the phone. If required, the trial coordinator will make arrangements for the mother (and father where necessary) to speak to one of the medical members of the research team (Lyn Chitty – LC, Therese Hesketh – TH or Andrew Copp – AC). After this conversation, if the mother is still interested in participating and is planning a pregnancy, she needs to complete the written consent form (Appendix 6) and the personal information sheet (Appendix 7). These need to be returned to the trial team in the pre-paid addressed envelope along with her first urine sample. Details on how to take the urine sample and sample collection tubes will be provided (Appendix 8).

If at any point there is any problem identified that requires addressing outside the remit of the study, the woman will be referred to her GP, obstetrician or geneticist as appropriate. As recruitment is taking place nationwide it will not be possible to see the woman or her partner for a consultation in person. However, if any participants request that, it will be arranged with one of the medical members of the research team (LC, TH or AC).

No further approaches to the woman or her partner will be made without receipt of the signed consent form (Appendix 6). Once the research team have received the consent form, it will be signed and dated by a member of the research team and at this point the participant will be assigned a unique study number. The participant number will enable all further data capture sheets to be anonymous.

The woman will be provided with a letter to forward to her GP to notify that she is taking part in the trial and that we will be prescribing folic acid 5 mg daily (Appendix 10). Further copies of this letter can be provided for other health professionals involved in the woman’s care.

On receipt of the signed consent form, if the woman is trying to conceive, she will be randomised by GOSH Pharmacy to the treatment or control arm of the trial using a computer generated table of random numbers.

**Administration of drugs**
All subjects receive identical FA supplementation at the ‘high dose’ level of 5 mg, typically used in pregnancies at risk of NTD. Inositol dose level is set at 1 g daily. Placebo will be used instead of inositol in the control group.

All drugs will be held in the pharmacy at GOSH and will be dispensed from there on a named person basis. One of the medically qualified research team (LC, TH or AC) will prescribe folic acid and inositol or placebo for a 6 month period for each woman recruited to the trial. Randomisation will occur in the pharmacy, where they will dispense the drugs in 3 monthly batches to the women. No drugs will be dispensed until the first urine sample has been received for monitoring. The trial coordinator will contact the women by phone to ensure drugs have arrived. As this is a pilot study, regular telephone contact will be made with the women to ensure that no problems have arisen and to determine if conception has occurred. Medication will cease at the end of the 12th postmenstrual week.

**Monitoring and Compliance**
No adverse effects are expected as this is a naturally occurring substance and none have been described in patients taking it for other indications. Nevertheless, participants will...
receive an adverse reaction monitoring leaflet (Appendix 11A) and monitoring forms (Appendix 11B), which will explain the correct procedures for reporting expected and unexpected adverse reactions. Compliance will be monitored by measurement of inositol in urine at various points within the trial period: one sample before starting the trial, one sample after one week of taking the trial treatments and one sample six weeks after conception. Urine collection tubes will be sent to the participants when the time is near.

Collection of outcome data
All women recruited to the study will be given a pregnancy information sheet to complete and return when conception has occurred (Appendix 12). They will also receive a pregnancy outcome sheet to complete and return once the outcome of pregnancy is known (Appendix 13). Consent will be obtained at the outset of the study to contact the woman's GP, obstetrician or geneticist for further details of pregnancy outcome if required. The majority of NTDs (particularly in high risk women) are diagnosed in pregnancy at the time of the routine second trimester anomaly scan.

See Appendix 15 for a schematic diagram of the trial design.

4.3 A description of the measures taken to minimise/avoid bias
Randomisation: On receipt of the signed consent form, women will be randomised to the treatment or control arm of the trial using a computer generated table of random numbers (Stata V9 software). Randomisation will occur in the pharmacy prior to dispensing the drugs, independently of the researchers.

Blinding: The trial will be a double blind, so that both the participant and the GP/obstetrician/geneticist will be blind to the participant's treatment group. In addition to this, the principal investigators (AC, LC, TH) and the trial co-ordinator (KB) will be blind to the allocation of the recruited participants to each trial treatment.

The pharmacy will keep a record of the treatment group of each participant as determined by the randomisation procedure. Sub-Investigator Dr. Nicholas Greene (NG) will have access to this throughout the trial. This will enable any adverse events to be correlated to medication whilst the trial is progressing. Intermediate statistical analysis of pregnancy outcome for both the control and study group will also be assessed by NG to determine the improbable need for early completion of the pilot study. NG will have no contact with trial participants and he will be under obligation not to disclose the randomisation code to any other member of the trial team.

4.4 The expected duration of the subject participation and a description of the sequence and duration of all trial periods, including follow-up, if any.
It is not possible to state definitively the expected total duration of participation in the study for each woman. The minimum period will be one month preconception and 3 months of pregnancy on medication and then the participant will be required to inform us of the pregnancy outcome after delivery. The involvement for some women will be longer and will depend on the interval from trying to conceive to the known outcome of pregnancy.

The maximum period of treatment within the confines of the pilot study will be twelve months preconception, three months of pregnancy on medication. We will stop
prescription of the tablets if the woman fails to conceive within twelve months, as the risks of long term treatment are unknown. We shall inform women when this is due to happen.

Recruitment will stop three months before the end of the pilot study and no more tablets will be prescribed after this period. Therefore women who are participating in the trial will no longer receive tablets if they are not pregnant at this point. We shall inform women when this is due to happen.

4.5 A description of the “stopping rules” or “discontinuation criteria” for individual subjects, parts of the trial and entire trial

Women would be required to stop participation in the trial if the following criteria were met whilst taking the prescribed drugs;

- Diagnosed with epilepsy and were therefore required to take anti-epileptics.
- Failed to conceive within one year of starting the trial.
- They no longer wished to conceive.

4.6 Accountability procedures for the investigational product(s), including the placebo

Once a woman has conceived we will prescribe enough drugs to last until the twelfth week post conception. At that time, if the women has continued to participate as required, there should be no drugs remaining. However if a participant withdraws from the trial early, and still has drugs remaining, we will contact the participant and advise her to either return the unused drugs to us, or destroy them by flushing them down the toilet as soon as possible.

4.7 Maintenance of trial treatment randomisation codes and procedures for breaking codes

Only the pharmacy and one of the principal investigators (NG) will have access to the randomisation code. NG will be aware of all adverse events and pregnancy outcomes to enable monitoring throughout the trial of any correlations between trial treatment group and adverse events/pregnancy outcome.

The circumstances under which the randomisation code may need to be broken are;

- A significant number of unexpected adverse reactions in either the study or the control group.
- A significant increase in the rate of NTD in either the study or the control group.

If such a circumstance were to arise, it would be quickly identified by regular analysis of the trial data by NG throughout the duration of the trial. If a problem is identified by NG, the randomisation code will be broken to ensure all teams member are aware of the problem. Following consultation with the medical experts (AC, LC, TH), it will be decided if it is necessary to stop the trial.
5. **Selection and withdrawal of subjects**

5.1 **Description of the population to be studied**
The participants to be studied will be healthy volunteers, with a history of one or more NTD pregnancies and who wish to embark upon a further pregnancy.

5.2 **Anonymity**
All subjects will be anonymous to comply with the Data Protection Act 1998.

5.3 **Subject inclusion criteria**
Women with a history of one or more NTD pregnancies (spina bifida, anencephaly or encephalocoele) who wish to embark upon a further pregnancy. The trial is designed to reduce recurrence of NTDs and thus this is the sole inclusion criteria.

5.4 **Subject exclusion criteria**
- Women who are unable to give informed consent for any reason (language difficulties, low IQ). We will not be recruiting women who fall into this category, as we do not have funding to translate the information leaflets or employ interpreters for the telephone interviews. If the pilot trial is successful we will take appropriate measures to include these groups in the definitive trial.
- Maternal age outside 18 - 40 years of age. An upper limit of forty years has been stipulated because there is increased risk of a pregnancy being affected by chromosomal abnormality which may result in a NTD.
- Pregnancies resulting from assisted conception (e.g. IVF).
- Cases where the previous affected child had other abnormalities suggestive of another underlying genetic aetiology or fetal valproate syndrome, or where the chromosomes were abnormal. In these cases the underlying pathology is different and we would not expect folic acid or inositol to influence recurrence risk.
- Women who are epileptic and/or taking anti-epileptic medications including valproate. It is recognised that some anti-epileptic drugs increase the predisposition to neural tube defects and, as the mechanism for this is unknown, it may be unresponsive to inositol. For the purposes of this pilot study including these cases may skew the data and thus will need to be excluded.
- Women who do not have a GP or obstetrician in the UK. We need to be able to monitor the pregnancy, confirm there are no contraindications and seek outcome data. We do not have the facilities within this pilot trial to seek this information for cases outside the UK.

5.5 **Method of subject recruitment**
To identify potential participants, we will use the methods described in section 4.2.

5.6 **Subject withdrawal criteria and procedures**
We would remove a participant from the trial if there was a lack of communication and/or correspondence, or if they moved abroad.
6. Treatment of subjects

6.1 The treatment to be administered and follow-up period

Treatment to be administered

Upon receipt of the prescribed drugs by post, medication will begin immediately and will continue whilst the woman is trying to conceive. Following conception, medication will cease at the end of the 12th postmenstrual week. Participants will be required to take three tablets each day (one folic acid tablet and two inositol/placebo tablets) at a suitable time for them. Patients will be advised on how to take the medication in the welcome letter (Appendix 9).

Follow-up period

Pregnancy outcome will be determined by second trimester ultrasound scanning, plus follow up of all term pregnancies.

6.2 Medication/treatments permitted and not permitted before and/or during trial

- Anti-epileptic medications will not be permitted before and up to the 12th week of pregnancy. It is recognised that some anti-epileptic drugs increase the predisposition to NTDs and, as the mechanism for this is unknown, it may be unresponsive to inositol. For the purposes of this pilot study including these cases may skew the data and thus will need to be excluded.

- Additional folic acid supplements will not be permitted before and up to the 12th week of pregnancy. All trial participants will receive identical folic acid supplements of 5 mg/day. This is the dose typically used in pregnancies at risk of recurrent NTD. Taking greater than 5 mg/day of folic acid may lead to unknown complications. This includes multi-vitamin preparations and “Pregnacare” tablets which contain folic acid.

- Additional inositol supplements will not be permitted before and up to the 12th week, including multi-vitamin preparations which include inositol.

6.3 Procedures of monitoring subject compliance

Compliance will be monitored by the measurement of inositol levels in urine. Once eligibility for the trial has been confirmed, women will be sent a sterile pot for urine collection, together with appropriate pre-addressed and pre-paid packaging. They will also receive an information leaflet detailing how and when the samples should be taken (see Appendix 8). This first set of samples will enable us to measure inositol levels before medication is prescribed. A further urine sample will be required after taking the medication for six weeks. The study co-ordinator will send out the packs for urine collection at this time. If the participant conceives before the six weeks is up, this sample will still be collected to monitor compliance despite pregnancy. A third sample will be requested during pregnancy whilst taking the medication, preferably around the 10th - 11th week of pregnancy.
6.4 A description of the plan for the provision of any additional care of the subjects once their participation in the trial has ended from what is normally expected according to the subject’s medical condition

No provision will be made for additional care of the subjects upon completion of trial participation. Their pregnancy care will be provided as per local healthcare practise.

7. Assessment of efficacy

7.1 Specification of the efficacy parameters
Efficacy will be assessed by the prevention of neural tube defects in the study group.

7.2 Methods and timing for assessing, recording and analysing of efficacy parameters
Ultrasound scans are capable of detecting NTDs during pregnancy. The majority of NTDs will first be detected following the 20 week total anomaly scan, although it is possible that some cases of anencephaly will be detected earlier, at the 12th or 16th week scan. Routine post-mortem analysis reports of terminated or miscarried pregnancies will also enable us to record any NTDs. All full term pregnancies will be followed up until birth.

8. Assessment of safety

8.1 Specification of safety parameters
Safety parameters for inositol will need to be established during pregnancy. Safety assessment will monitor incidence of the following:

- Premature births
- NTDs
- Other birth defects
- Low birth weight
- Pregnancy complications
- Maternal symptoms

8.2 Expected adverse reactions
No adverse effects to Inositol are expected as this is a naturally occurring substance and none have been described in patients taking it for other indications. However pregnancy itself can induce a number of adverse events/reactions (unrelated to IMP). This is a list of such events/reactions;

Expected non-serious adverse events following conception;

- Menstrual cycle stops
- Light bleeding approximately 8-10 days after ovulation
- Nausea and vomiting following conception (“morning sickness”)
- Swollen and tender breasts
- Need to urinate more frequently
- Tiredness
- Dizziness and/or fainting
- Mild constipation, indigestion and heartburn
- Haemorrhoids
- Stretch marks
- Bleeding gums
- Headaches
- Mood swings
- Weight gain
- Nose bleeds
- Mild swelling of hands, ankles and face (oedema)
- Itchy skin and/or change in skin pigment
- Mild backache
- Food aversions

**Expected serious adverse events following conception;**
- Miscarriages
- Ectopic Pregnancy
- NTDs (in this population of subjects)

### 8.3 Recording and reporting of all adverse events
All adverse events and reactions (expected, unexpected, serious) will be recorded and reported (to the REC, MHRA, and Sponsor) according to the Standard operating Procedures outlined in the sponsors document: GOSH ICH/05/S05/00 (latest version). All expected SAE will be reported to the sponsor within 30 days using the sponsor’s forms. All SUSARs will be reported to the sponsor as soon as possible.

### 8.4 The methods and timing for assessing, recording and analysing safety parameters
Participants will be sent an adverse reaction monitoring leaflet and forms with their dispensed drugs (see Appendix 10 and 11). This will contain information about expected adverse reactions that may occur during the participation of the trial and will explain what action must be taken by the woman when an expected and unexpected adverse reaction occurs. Following completion of the forms, we will request that participants return these to us in a prepaid envelope for analysis.

Collated data from every thirty forms returned will be analysed by the pharmaco-vigilance investigator (NG). If there is a significant increase in the rate of a listed expected adverse event then he will be able to correlate it with trial treatment if needed. NG is the only sub-investigator who will be allowed access to the randomisation code and only under these circumstances. Following this, it can then be reported as stated in local Standard Operating Procedures. In addition to adverse reaction monitoring forms, the trial co-ordinator will have regular telephone contact with each participant to help ensure correct reporting of any adverse reactions.

### 8.5 Procedures for eliciting reports of, and for recording, inter-current illnesses
All inter-current illnesses reported on the Adverse Reaction Monitoring Form (appendix 11B) or following telephone contact will be transcribed on to case report forms. Unless
listed in section 4.5 as a discontinuation criterion, inter-current illnesses would not prevent a woman from completing her participation in the trial.

8.6 The type and duration of the follow-up of subjects after adverse events
We propose to follow-up all participants until the outcome of pregnancy is known and the pregnancy outcome sheet has been completed.

8.7 Declaration of ‘End of Trial’
The trial will be complete 1 year after the last assessment/visit of the last recruited patient.

9. Statistics

9.1 Description of the statistical methods to be employed, including timing of any interim analyses.
Statistical analysis of efficacy will be by the Chi-square contingency test of two proportions (affected vs. unaffected pregnancies in each group) after approximately 100 ‘at risk’ pregnancies have been monitored. However, it is expected that the pilot study will not be adequately powered to give a statistically significant result in this analysis. As this is a pilot study, the aim will be to quantify the success of our recruitment process, our success in assessing compliance and the results of the urine analyses.

It is not possible to track the extent of non-responders to the initial recruitment but we will be able to track the extent of non-consent following an offer of entry. We will also be able to extrapolate from our results which population groups were more successfully recruited. The responders will be assessed for age, frequency of previous affected pregnancy and method of recruitment. Results will highlight which population groups have not responded to recruitment or have been inadequately targeted during recruitment. This will then enable us to amend recruitment procedures as required for the full term trial.

The success of compliance monitoring will be determined by the ability to measure an increase in the level of inositol in participants in the treatment arm and not in participants who were in the control arm. This will involve a comparison of levels/trajectories between individuals in the two arms. Each participant will provide three samples. The first urine sample will give an indication of inositol level prior to entry into the trial. The second two urine samples taken whilst participating in the trial can then be compared to the initial baseline concentration. It can then be assessed which of these two samples is more indicative of patient compliance. All of these data will be critical for determining how and when to measure compliance in the full term trial.

9.2 Number of subjects planned to be enrolled

For a full-term trial;
Recurrence after one case of NTD is generally estimated at 1 in 25 (approximately 4% risk). Although 70% of NTD recurrences were prevented in the MRC trial (Wald et al., 1991), we predict a smaller effect of FA in the present trial. This is because low dose (0.4 mg) FA supplementation is now widespread, presumably preventing a number of FA
sensitive cases. Today, NTD cases are likely to be either from unsupplemented pregnancies (potentially FA sensitive) or else FA-resistant. Hence, our study population (previous NTD case) will be enriched for FA-resistant pregnancies. We predict, therefore, that FA alone may prevent only 35% of recurrent NTDs (half the effect of the MRC trial), giving an expected 2.6% recurrence in the FA-only group. To detect a further 45% reduction in frequency in the group treated with FA + inositol (to a 0.8% recurrence risk) requires a sample size of 878 in each study arm (for $p = 0.05$; power = 0.8). Hence, we will be aiming for similar study numbers as in the original MRC trial (Wald et al., 1991).

Approximately 1,200 cases of NTD are expected annually in the UK (1 per 1000 among 1.2 million births). Recurrent NTDs probably comprise 5-10% of the total, amounting to 60-120 NTD recurrences per year in the UK. At a recurrence risk of 4%, this equates to 1500-3000 pregnancies per year of women who are ‘at risk’ of NTD, having had a previously affected pregnancy. To achieve a sample size of 878 per trial arm would require 5-8 years of recruitment (given that not all women will choose to participate).

For a pilot study to assess feasibility;
We would expect to recruit 50 ‘at risk’ pregnancies during 1.5 years of the pilot study (25 in the control group, 25 in the study group).

9.3 The level of significance to be used
p-values of 0.05 or less will be considered to be significant. All estimates will be presented with 95% confidence intervals.

9.4 Criteria for the termination of the trial
Not applicable for a pilot study.

9.5 Procedure for accounting for missing, unused and spurious data
One of the aims of the pilot study is to determine feasibility, hence data that go missing are important for designing a future trial. Recording missing data will be important because it will highlight problems in the process than can be corrected. Therefore a record will be kept of any missing data and the reasons why the data are missing will be documented. There should not be any unused or spurious data collected, because it is important to the current aims of the pilot study that all data are included to assess study feasibility.

9.6 Procedures for reporting any deviations from the original statistical plan
A major aim of the pilot study is to identify any problems with the clinical trial system. To do this, we plan to monitor closely how each participant is coping by contacting them on a monthly basis to discuss any problems or answer any queries. It is hoped that this will highlight any problems in the system and these can then be resolved in the full-term trial. We will welcome feedback from participants, or from health professionals involved, on the overall conduct of the pilot study.

9.7 The selection of subjects to be included in the analyses
It is hoped that with the recruitment procedures chosen, women with a history of NTDs from all areas of the UK and from all age ranges and backgrounds will be targeted. However as part of the analysis of the final data from the pilot study (see section 9.1) it will be possible to determine if there are any under-represented populations. We can also determine the extent of drop out following the telephone questionnaire.
10. **Direct access to source data/documents**
Any information given to the study team, and the results of the blood and urine testing, will be kept completely confidential. Only the researchers listed will have access to the data collected in the course of this study. Representatives of the hospital’s Research Ethics Committee and inspectors from the MHRA may require access to the data for monitoring and auditing purposes. The use of some types of personal information is safeguarded by the Data Protection Act 1998 (DPA). The DPA places an obligation on those who record or use personal information, but also gives rights to people about whom information is held. Any questions about data protection will be directed to the Data Protection officer via the switchboard on 020 7405 9200 extension 5217. The results of our project will be published as papers in medical journals. No data will be published that allows for individuals to be identified in any way.

11. **Quality control and quality assurance**
The trial will be conducted according to local Standard Operating Procedures.

12. **Ethics**
Trial will be conducted after approval of REC and will be conducted according to the principles that have their origin in the Declaration of Helsinki (1996 version).

13. **Data handling and record keeping**
Source documents (Appendices 4, 7, 11, 12 and 13) and CRF (Appendix 6) will be used to capture data. This will be kept in a locked secure filing cabinet at the Institute of Child Health. When data are entered onto a computer, this will only be accessible by password. All applicants will have access to the data whilst AC will have custodial responsibility.

14. **Financial and insurance matters**
Trial is supported by a MRC grant (Registration Reference No.TP58). The project will be covered by No Fault Compensation Policy held by the Institute of Child Health, University College London. Further details of this policy can be obtained from the R&D Office (see sponsor details).

15. **Publication policy**
The results of the study will be reported and disseminated as follows;
- Peer reviewed scientific journals
- Internal report, plus possible article on Institute web pages (publicly accessible)
- Conference presentation(s)
- Written feedback to patient support groups
We will produce a summary sheet of any findings from the pilot study which will be sent out to all participants, health professionals and patient support groups involved in the pilot trial. The data from the pilot study will be available for the proposed full-term trial and will be displayed on a future website to be set-up as part of the full-term trial.

16. **Supplements**
Appendix 1 Information for Health Professionals
2 Participant Information Sheet
3A Recruitment Poster
3B Outline of Website
4 Telephone questionnaire
5 Offer to participate in clinical trial
6 Consent Form
7 Personal Information Sheet
8 Sample Collection Information Sheet
9 Welcome letter
10 Letter informing Health Professionals
11A Adverse Reaction Monitoring leaflet
11B Adverse Reaction Monitoring sheet
12 Pregnancy Information Sheet
13 Pregnancy Outcome Sheet
14 Inositol / Placebo Bottle Label
15 Flow Chart of Trial Procedures
16 Recruitment Pack Covering Letter

17. Signature Page

Chief Investigator: Prof. Andrew Copp

Date:

Signature: