# Clinical trial results:
# Feasibility study on the effects of L-citrulline on uteroplacental and cardiovascular function in hypertensive pregnant women.

## Summary

| | |
|---|---|
| EudraCT number | 2015-005792-25 |
| Trial protocol | GB |
| Global end of trial date | 06 March 2019 |

### Results information

| | |
|---|---|
| Result version number | v1 (current) |
| This version publication date | 18 March 2020 |
| First version publication date | 18 March 2020 |
| Summary attachment (see zip file) | Cherry results (CHERRY Final Analysis Report v3.0 20190920.pdf) |

## Trial information

### Trial identification

| | |
|---|---|
| Sponsor protocol code | R04341 |

### Additional study identifiers

| | |
|---|---|
| ISRCTN number | ISRCTN12695929 |
| ClinicalTrials.gov id (NCT number) | - |
| WHO universal trial number (UTN) | - |
| Other trial identifiers | REC number: 16/NW/0557 |

Notes:

### Sponsors

| | |
|---|---|
| Sponsor organisation name | Manchester University NHS Foundation Trust |
| Sponsor organisation address | Oxford Road, Manchester, United Kingdom, M13 9WL |
| Public contact | Dr Lynne Webster, Manchester University NHS Foundation Trust, research.sponsor@mft.nhs.uk |
| Scientific contact | Dr Lynne Webster, Manchester University NHS Foundation Trust, research.sponsor@mft.nhs.uk |

Notes:

### Paediatric regulatory details

| | |
|---|---|
| Is trial part of an agreed paediatric investigation plan (PIP) | No |
| Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial? | No |
| Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial? | No |

Notes:

## Results analysis stage

| | |
|---|---|
| Analysis stage | Final |
| Date of interim/final analysis | 07 September 2018 |
| Is this the analysis of the primary completion data? | Yes |
| Primary completion date | 07 September 2018 |
| Global end of trial reached? | Yes |
| Global end of trial date | 06 March 2019 |
| Was the trial ended prematurely? | No |

Notes:

## General information about the trial

Main objective of the trial:

Process outcome:
Recruitment rate (number of women eligible, recruited and completing study per month per centre)

Clinical outcome:
Reduction in diastolic blood pressure following L-citrulline supplementation compared with placebo (baseline compared 8 weeks post treatment)

Protection of trial subjects:

Trial monitoring was carried out to ensure that the rights and well-being of human participants were protected during the course of a clinical trial. A detailed risk assessment was performed for CHERRY to determine the level and type of monitoring required for specific hazards. Monitoring activities were carried out via central monitoring, this included safety and consent monitoring and site visits were conducted when required. A trial steering committee and Idependent data safety & monitoring committee were convened and met regulalry throughout the trial to provide idependent oversight of the trial.

Background therapy: -

Evidence for comparator: -

| | |
|---|---|
| Actual start date of recruitment | 04 July 2017 |
| Long term follow-up planned | No |
| Independent data monitoring committee (IDMC) involvement? | Yes |

Notes:

## Population of trial subjects

### Subjects enrolled per country

| | |
|---|---|
| Country: Number of subjects enrolled | United Kingdom: 36 |
| Worldwide total number of subjects | 36 |
| EEA total number of subjects | 36 |

Notes:

### Subjects enrolled per age group

| | |
|---|---|
| In utero | 0 |
| Preterm newborn - gestational age < 37 wk | 0 |
| Newborns (0-27 days) | 0 |
| Infants and toddlers (28 days-23 months) | 0 |
| Children (2-11 years) | 0 |

| | |
|---|---|
| Adolescents (12-17 years) | 0 |
| Adults (18-64 years) | 36 |
| From 65 to 84 years | 0 |
| 85 years and over | 0 |

## Subject disposition

### Recruitment

Recruitment details:

Randomisation start (first recruting site opened): 04/07/2017. Randomisation end (last recruting site closed): 31/01/2018. Recruitment planned at 2 sites in UK. Only 1 site opened.

### Pre-assignment

Screening details:

42 patients screened, 41 eligible, 36 consented, 36 randomised.

### Pre-assignment period milestones

| | |
|---|---|
| Number of subjects started | 42[1] |
| Intermediate milestone: Number of subjects | Screened: 42 |
| Intermediate milestone: Number of subjects | Eligible: 41 |
| Intermediate milestone: Number of subjects | Consented: 36 |
| Intermediate milestone: Number of subjects | Randomised: 36 |
| Number of subjects completed | 36 |

### Pre-assignment subject non-completion reasons

| | |
|---|---|
| Reason: Number of subjects | Did not provide consent: 5 |
| Reason: Number of subjects | Ineligible: 1 |

Notes:

[1] - The number of subjects reported to have started the pre-assignment period are not the same as the worldwide number enrolled in the trial. It is expected that these numbers will be the same.
Justification: The number of patients who started the pre-assignment period (screened = 42) is larger than the number who enrolled in the trial (randomised = 36).

### Period 1

| | |
|---|---|
| Period 1 title | Baseline & Analysis (overall period) |
| Is this the baseline period? | Yes |
| Allocation method | Randomised - controlled |
| Blinding used | Double blind |
| Roles blinded | Investigator, Data analyst, Subject |

### Arms

| | |
|---|---|
| Are arms mutually exclusive? | Yes |
| **Arm title** | Arm A |

Arm description:

L-citrulline

| | |
|---|---|
| Arm type | Experimental |
| Investigational medicinal product name | L-citrulline |
| Investigational medicinal product code | |
| Other name | |
| Pharmaceutical forms | Oral solution |
| Routes of administration | Oral use |

Dosage and administration details:

Dietary supplements of L-Citrulline (3g)/ placebo were taken twice daily from randomisation, 14 +/-2 weeks gestational age unitl 22+/-2 weeks gestational age (maximum 10 weeks).
Each 30ml of L Citrulline solution contains:
L Citrulline 3g

Orange syrup 4.5mL
Sodium methylhydroxybenzoate 24mg
Sodium propylhydroxybenzoate 6mg
Dilute hydrochloric acid 10% 0.069mL
Purified water to 30ml

| **Arm title** | Arm B |
|---|---|

Arm description:

Placebo

| | |
|---|---|
| Arm type | Placebo |
| Investigational medicinal product name | Placebo |
| Investigational medicinal product code | |
| Other name | |
| Pharmaceutical forms | Oral solution |
| Routes of administration | Oral use |

Dosage and administration details:

Dietary supplements of L-Citrulline (3g)/ placebo were taken twice daily from randomisation, 14 +/-2 weeks gestational age unitl 22+/-2 weeks gestational age (maximum 10 weeks).
Each 30ml of placebo solution contains:
Orange syrup 4.5mL
Sodium methylhydroxybenzoate 24mg
Sodium propylhydroxybenzoate 6mg
Dilute hydrochloric acid 10% 0.069mL
Purified water to 30ml

| **Number of subjects in period 1** | Arm A | Arm B |
|---|---|---|
| Started | 24 | 12 |
| Completed | 24 | 12 |

## Baseline characteristics

| Reporting groups | |
|---|---|
| Reporting group title | Arm A |
| Reporting group description: L-citrulline | |
| Reporting group title | Arm B |
| Reporting group description: Placebo | |

| Reporting group values | Arm A | Arm B | Total |
|---|---|---|---|
| Number of subjects | 24 | 12 | 36 |
| Age categorical | | | |
| Age | | | |
| Units: Subjects | | | |
| In utero | 0 | 0 | 0 |
| Preterm newborn infants (gestational age < 37 wks) | 0 | 0 | 0 |
| Newborns (0-27 days) | 0 | 0 | 0 |
| Infants and toddlers (28 days-23 months) | 0 | 0 | 0 |
| Children (2-11 years) | 0 | 0 | 0 |
| Adolescents (12-17 years) | 0 | 0 | 0 |
| Adults (18-64 years) | 24 | 12 | 36 |
| From 65-84 years | 0 | 0 | 0 |
| 85 years and over | 0 | 0 | 0 |
| Age continuous | | | |
| Units: years | | | |
| arithmetic mean | 33.73 | 34.26 | |
| standard deviation | ± 4.09 | ± 4.42 | - |
| Gender categorical | | | |
| Units: Subjects | | | |
| Female | 24 | 12 | 36 |
| Male | 0 | 0 | 0 |
| Antihypertensive medication | | | |
| Any antihypertensive medication taken if last 12 months | | | |
| Units: Subjects | | | |
| Yes | 15 | 6 | 21 |
| No | 9 | 6 | 15 |
| Cardiac disease | | | |
| Units: Subjects | | | |
| Yes | 0 | 0 | 0 |
| No | 23 | 12 | 35 |
| Data unobtainable | 1 | 0 | 1 |
| Diagnosis | | | |
| Units: Subjects | | | |
| Primary | 19 | 11 | 30 |
| Secondary | 4 | 1 | 5 |
| Data unobtainable | 1 | 0 | 1 |

| | | | |
|---|---|---|---|
| Ethnicity<br>Units: Subjects | | | |
| Black African | 6 | 2 | 8 |
| Black Caribbean | 1 | 2 | 3 |
| East/ Central Asian | 2 | 0 | 2 |
| South Asian | 2 | 0 | 2 |
| White | 12 | 8 | 20 |
| Other | 1 | 0 | 1 |
| Viable pregnancies | | | |
| Number of past viable pregnancies | | | |
| Units: Subjects | | | |
| Zero | 6 | 0 | 6 |
| One | 8 | 8 | 16 |
| Two | 3 | 1 | 4 |
| Three | 6 | 1 | 7 |
| Four | 0 | 0 | 0 |
| Five | 1 | 1 | 2 |
| Six | 0 | 0 | 0 |
| Seven | 0 | 1 | 1 |
| Presence of Proteinuria<br>Units: Subjects | | | |
| Yes | 2 | 0 | 2 |
| No | 22 | 12 | 34 |
| BMI<br>Units: Kg/ m^2 | | | |
| arithmetic mean | 31.13 | 33.36 | |
| standard deviation | ± 7.59 | ± 10.16 | - |
| Diastolic Blood pressure<br>Units: mmHg | | | |
| arithmetic mean | 86.96 | 93.47 | |
| standard deviation | ± 8.08 | ± 10.21 | - |
| Gestational age<br>Units: days | | | |
| arithmetic mean | 94.08 | 95.42 | |
| standard deviation | ± 8.14 | ± 5.25 | - |
| Systolic blood pressure<br>Units: mmHg | | | |
| arithmetic mean | 131.4 | 138.08 | |
| standard deviation | ± 11.65 | ± 15.47 | - |
| Time since diagnosis<br>Units: Years | | | |
| median | 3.35 | 4.71 | |
| inter-quartile range (Q1-Q3) | 0.98 to 10.23 | 1.46 to 9.71 | - |

## End points

### End points reporting groups

| | |
|---|---|
| Reporting group title | Arm A |
| Reporting group description: L-citrulline | |
| Reporting group title | Arm B |
| Reporting group description: Placebo | |

### Primary: Diastolic blood pressure

| | |
|---|---|
| End point title | Diastolic blood pressure |

End point description:

Change in diastolic BP (average of 3 readings) between randomisation (visit 1) and the 8 week clinic visit (visit 3). Within-patient change in diastolic BP from randomisation will be calculated by subtracting each patient's average diastolic BP measurement at randomisation (visit 1) from their average diastolic BP measurement at 8 weeks (visit 3). Standardised within-patient change in diastolic BP will be calculated by dividing each patient's change in diastolic BP from baseline by the number of days between visit 3 and visit 1.
Within-patient change and standardised within-patient change from visit 1 to visit 3 of dBP was presented using mean and 95% confidence interval for each treatment group separately. (See supplementary material for confidence intervals).

| | |
|---|---|
| End point type | Primary |

End point timeframe:

Change in diastolic BP between randomisation (visit 1) and the 8 week clinic visit (visit 3).

| End point values | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 24 | 12 | | |
| Units: mmHg | | | | |
| arithmetic mean (standard deviation) | | | | |
| dBP differences | -1.82 (± 9.56) | -5 (± 12.21) | | |
| dBP standardised differences | -0.03 (± 0.16) | -0.08 (± 0.2) | | |

### Statistical analyses

| Statistical analysis title | Exploratory regression dBP |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| | |
|---|---|
| Comparison groups | Arm A v Arm B |

| | |
|---|---|
| Number of subjects included in analysis | 36 |
| Analysis specification | Pre-specified |
| Analysis type | other[1] |
| P-value | = 0.857 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | -0.64 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -7.77 |
| upper limit | 6.5 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 3.5 |

Notes:

[1] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size

## Primary: Recrutiment rates

| | |
|---|---|
| End point title | Recrutiment rates[2] |

End point description:

The primary process outcomes is overall recruitment rate, based on the screening logs. Recruitment rate = total number recruited / total eligible women entered on screening log. Recruitment rate (averaged over the entire recruitment period) was presented with 95% CI, along with the number of women eligible and recruited (see supplementary material for confidence interval).

| | |
|---|---|
| End point type | Primary |

End point timeframe:

Screening occured prior to randomisation.

Notes:

[2] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No between group comparisons were conducted for this outcome.

| End point values | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 24[3] | 12[4] | | |
| Units: persons | | | | |
| Eligible | 41 | 41 | | |
| Randomised | 24 | 12 | | |

Notes:

[3] - The number eligible (above) can only be provided overall for both arms (41).

[4] - The number eligible (above) can only be provided overall for both arms (41).

## Statistical analyses

No statistical analyses for this end point

## Primary: Acceptability of intervention

| | |
|---|---|
| End point title | Acceptability of intervention[5] |

End point description:

Acceptability of the intervention was assessed based on feedback from questions 1-3 of a patient questionnaire:

Q1: The number (and percentage) of women who selected each response (i.e. who found taking the treatment easy, neither difficult/easy or difficult) will be summarised for each treatment group separately.
Q2: The number (and percentage) of women who described the taste of the treatment as delicious/pleasant (as opposed to neither unpleasant/awful) will be summarised for each treatment group separately.
Q3: The number (and percentage) of women who selected each response (i.e. missed doses every day, 1-2 times per week, 1-2 per month or hardly ever) will be summarised for each treatment group separately.

| End point type | Primary |
|---|---|

End point timeframe:

After completion of intervention.

Notes:

[5] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No between group comparisons were conducted for this outcome.

| **End point values** | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 22[6] | 10[7] | | |
| Units: persons | | | | |
| Q1: Easy | 18 | 8 | | |
| Q1: Neither difficult or easy | 2 | 2 | | |
| Q1: Difficult | 2 | 0 | | |
| Q2: Delicious | 1 | 0 | | |
| Q2: Pleasent | 12 | 4 | | |
| Q2: Unpleasant | 8 | 6 | | |
| Q2: Awful | 1 | 0 | | |
| Q3: Every day | 0 | 1 | | |
| Q3: Once/ twice per week | 8 | 2 | | |
| Q3: Once/ twice per month | 4 | 2 | | |
| Q3: Hardly ever | 10 | 5 | | |

Notes:

[6] - n missing = 2

[7] - n missing = 2

## Statistical analyses

No statistical analyses for this end point

## Secondary: Ambulatory BP monitor

| End point title | Ambulatory BP monitor |
|---|---|

End point description:

Within-patient change in average day and night time systolic and diastolic ABPM measurements from randomisation will be calculated by subtracting each patient’s average visit 1 systolic ABPM measurement from their average visit 3 systolic ABPM measurement, for day and night time averages separately. Standardised within-patient change in average day and night time systolic and diastolic ABPM from baseline will be calculated by dividing each patient’s change in average day and night time systolic ABPM by the number of days between visit 3 and visit 1, for day and night time averages separately.
Within-patient change and standardised within-patient change from visit 1 to visit 3 of ABPM measurements was presented using mean and 95% confidence interval for each treatment group separately. (See supplementary material for confidence intervals).

| End point type | Secondary |
|---|---|

End point timeframe:

Change in average day and night time ambulatory BP monitor (ABPM) measurements (systolic and diastolic) between randomisation (visit 1) and the 8 week clinic visit (visit 3).

| **End point values** | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 20[8] | 9[9] | | |
| Units: mmHg | | | | |
| arithmetic mean (standard deviation) | | | | |
| day time sBP differences | -0.35 (± 7.18) | -2.78 (± 6.82) | | |
| day time sBP standardised differences | -0.003 (± 0.12) | -0.04 (± 0.12) | | |
| night time sBP differences | 0.18 (± 8.92) | 3.29 (± 8.6) | | |
| night time sBP standardised differences | 0.01 (± 0.16) | 0.06 (± 0.15) | | |
| day time dBP differences | -2.05 (± 7.25) | -3.11 (± 5.97) | | |
| day time dBP standardised differences | -0.03 (± 0.13) | -0.05 (± 0.1) | | |
| night time dBP differences | 0.41 (± 7.67) | 2 (± 5.63) | | |
| night time dBP standardised differences | 0.01 (± 0.13) | 0.04 (± 0.09) | | |

Notes:

[8] - Day time: n=20, n missing= 4. Night time: n=17, n missing=7

[9] - Day time: n=9, n missing= 3. Night time: n=7, n missing=5

## Statistical analyses

| **Statistical analysis title** | Exploratory regression day time sBP (ABPM) |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| Comparison groups | Arm A v Arm B |
|---|---|
| Number of subjects included in analysis | 29 |
| Analysis specification | Pre-specified |
| Analysis type | other[10] |
| P-value | = 0.354 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | 2.71 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -3.2 |
| upper limit | 8.63 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 2.87 |

Notes:

[10] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.

| **Statistical analysis title** | Exploratory regression night time sBP (ABPM) |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| Comparison groups | Arm A v Arm B |
|---|---|
| Number of subjects included in analysis | 29 |
| Analysis specification | Pre-specified |
| Analysis type | other[11] |
| P-value | = 0.4128 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | -3.33 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -11.62 |
| upper limit | 4.97 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 3.98 |

Notes:

[11] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.
Note: for ABPM night time exploratory regression analysis the group numbers are n=17 for L-citrulline and n=7 for placebo (overall n=24).

| **Statistical analysis title** | Exploratory regression day time dBP (ABPM) |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| Comparison groups | Arm A v Arm B |
|---|---|
| Number of subjects included in analysis | 29 |
| Analysis specification | Pre-specified |
| Analysis type | other[12] |
| P-value | = 0.9779 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | -0.08 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -6.04 |
| upper limit | 5.88 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 2.89 |

Notes:

[12] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.

| **Statistical analysis title** | Exploratory regression night time dBP (ABPM) |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| | |
|---|---|
| Comparison groups | Arm A v Arm B |
| Number of subjects included in analysis | 29 |
| Analysis specification | Pre-specified |
| Analysis type | other[13] |
| P-value | = 0.4103 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | -2.52 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -8.78 |
| upper limit | 3.74 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 3 |

Notes:

[13] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.
Note: for ABPM night time exploratory regression analysis the group numbers are n=17 for L-citrulline and n=7 for placebo (overall n=24).

## Secondary: Cardiovascular compliance

| End point title | Cardiovascular compliance |
|---|---|

End point description:

Within-patient change in central BP, PWV and normalised augmentation index was calculated by subtracting each patient's randomisation measurements from their 8 week measurements. Standardised within-patient change in measurement from baseline was calculated by dividing each patient's change in measurement from randomisation by the number of days between visit 3 and visit 1.

Normalised augmentation index aortic values are calculated as follows:
Augmentation Index Aortic – 0.431*(75-Heart rate)

Standardised within-patient change from visit 1 to visit 3 of each vascular compliance measurement was presented using mean and 95% confidence interval for each treatment group separately. (See supplementary material for confidence intervals).

| End point type | Secondary |
|---|---|

End point timeframe:

Change in vascular compliance measurements (central BP, pulse wave velocity (PWV) and normalised augmentation index) measured at randomisation (visit 1) and the 8 week clinic visit (visit 3).

| End point values | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 14[14] | 8[15] | | |
| Units: (see below) | | | | |
| arithmetic mean (standard deviation) | | | | |
| central BP differences (mmHg) | -4.86 (± 19.42) | -6.28 (± 29.03) | | |
| central BP standardised differences (mmHg) | -0.08 (± 0.32) | -0.1 (± 0.5) | | |
| PWV differences (m/s) | -0.36 (± 0.94) | 0.04 (± 1.51) | | |
| PWV standardised differences (m/s) | -0.01 (± 0.02) | 0.0006 (± 0.03) | | |
| Augmentation index differences (%) | -4.95 (± 10.14) | -1.73 (± 41.82) | | |
| Augmentation index standardised differences (%) | -0.08 (± 0.17) | -0.01 (± 0.74) | | |

Notes:

[14] - n missing = 10

[15] - n missing = 4

## Statistical analyses

| Statistical analysis title | Exploratory regression central BP |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| | |
|---|---|
| Comparison groups | Arm A v Arm B |
| Number of subjects included in analysis | 22 |
| Analysis specification | Pre-specified |
| Analysis type | other[16] |
| P-value | = 0.8498 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | 1.69 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -16.82 |
| upper limit | 20.21 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 8.81 |

Notes:

[16] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.

| Statistical analysis title | Exploratory regression PWV |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| | |
|---|---|
| Comparison groups | Arm A v Arm B |
| Number of subjects included in analysis | 22 |
| Analysis specification | Pre-specified |
| Analysis type | other[17] |
| P-value | = 0.4429 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | -0.42 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -1.56 |
| upper limit | 0.71 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 0.54 |

Notes:

[17] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size

| **Statistical analysis title** | Exploratory regression normalised AIO |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| | |
|---|---|
| Comparison groups | Arm A v Arm B |
| Number of subjects included in analysis | 22 |
| Analysis specification | Pre-specified |
| Analysis type | other[18] |
| P-value | = 0.8788 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | -1.88 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -27.46 |
| upper limit | 23.7 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 12.18 |

Notes:

[18] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.

## Secondary: Vascular compliance

| End point title | Vascular compliance |
|---|---|

End point description:

Within-patient change in vascular compliance measure was calculated by subtracting each patient's visit 1 vascular compliance measurement from their visit 3 vascular compliance measurement. Standardised within-patient change in vascular compliance measurements was calculated by dividing each patient's change in vascular compliance measurement by the number of days between visit 3 and visit 1.

Within-patient change and standardised within-patient change from visit 1 to visit 3 of ADMA concentration and Arginine concentrations was presented using mean and 95% confidence interval for each treatment group separately. (See supplementary material for confidence intervals).

| End point type | Secondary |
|---|---|

End point timeframe:

Change in vascular compliance measurements (cardiac output (CO), cardiac index (CI), stroke volume index (SVI) and total peripheral resistance index (TPRI) between randomisation (visit 1) and the 8 week clinic visit (visit 3).

| **End point values** | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 23[19] | 12[20] | | |
| Units: (see below) | | | | |
| arithmetic mean (standard deviation) | | | | |
| CO (L/minute) differences | -0.54 (± 1.75) | -1.18 (± 1.22) | | |
| CO (L/minute) standardised differences | -0.01 (± 0.03) | -0.02 (± 0.02) | | |
| CI (L/min/m^2) differences | -0.28 (± 0.88) | -0.57 (± 0.63) | | |
| CI (L/min/m^2) standardised differences | -0.01 (± 0.01) | -0.01 (± 0.01) | | |
| SVI (ml/m^2) differences | -4.43 (± 10.69) | -7.82 (± 8.3) | | |
| SVI (ml/m^2) standardised differences | -0.08 (± 0.17) | -0.13 (± 0.14) | | |
| TPRI (mmHg ml^-1 min^-1 kg^-1) differences | 140.35 (± 1508.87) | 574.92 (± 963.77) | | |
| TPRI (mmHg ml^-1 min^-1 kg^-1) standardised diffs | 2.89 (± 23.58) | 9.25 (± 16.61) | | |

Notes:

[19] - n missing = 1

[20] - For CO, CI and TRPI: n=12, n missing = 0
For SVI: n=11, n missing = 1

## Statistical analyses

| **Statistical analysis title** | Exploratory regression CO |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| Comparison groups | Arm A v Arm B |
|---|---|
| Number of subjects included in analysis | 35 |
| Analysis specification | Pre-specified |
| Analysis type | other[21] |
| P-value | = 0.8731 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | 0.06 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -0.72 |
| upper limit | 0.84 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 0.38 |

Notes:

[21] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.

| **Statistical analysis title** | Exploratory regression CI |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| | |
|---|---|
| Comparison groups | Arm A v Arm B |
| Number of subjects included in analysis | 35 |
| Analysis specification | Pre-specified |
| Analysis type | other[22] |
| P-value | = 0.8808 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | 0.03 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -0.34 |
| upper limit | 0.4 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 0.18 |

Notes:

[22] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.

| **Statistical analysis title** | Exploratory regression SVI |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| | |
|---|---|
| Comparison groups | Arm A v Arm B |
| Number of subjects included in analysis | 35 |
| Analysis specification | Pre-specified |
| Analysis type | other[23] |
| P-value | = 0.4211 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | 1.93 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -2.9 |
| upper limit | 6.75 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 2.36 |

Notes:

[23] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.
Note: for SVI exploratory regression analysis the group numbers are n=23 for L-citrulline and n=11 for

placebo (overall n=34).

| **Statistical analysis title** | Exploratory regression TRPI |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| | |
|---|---|
| Comparison groups | Arm A v Arm B |
| Number of subjects included in analysis | 35 |
| Analysis specification | Pre-specified |
| Analysis type | other[24] |
| P-value | = 0.6493 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | -135.64 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -738.18 |
| upper limit | 466.89 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 295.43 |

Notes:

[24] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.

## Secondary: Uteroplacental measurements (continuous)

| End point title | Uteroplacental measurements (continuous) |
|---|---|

End point description:

Within-patient change in RI and PI was calculated by subtracting each patient's visit 1 RI or PI measurement from their visit 3 RI or PI measurement. Standardised within-patient change in RI and PI measurements was calculated by dividing each patient's change in RI or PI measurement by the number of days between visit 3 and visit 1.

Within-patient change and standardised within-patient change from visit 1 to visit 3 of RI and PI was presented using mean and 95% confidence interval for each treatment group separately. (See supplementary material for confidence intervals).

| End point type | Secondary |
|---|---|

End point timeframe:

Change in uteroplacental blood flow measurements (uterine artery resistance index (RI) and pulsatility index (PI)) from randomisation (visit 1) to 8 weeks (visit 3).

| **End point values** | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 23[25] | 12[26] | | |
| Units: Ratio | | | | |
| arithmetic mean (standard deviation) | | | | |
| RI differences | -0.1 (± 0.11) | -0.08 (± 0.09) | | |
| RI standardised differences | -0.002 (± 0.002) | -0.001 (± 0.002) | | |

| | | | | |
|---|---|---|---|---|
| PI differences | -0.43 (± 0.43) | -0.37 (± 0.34) | | |
| PI standardised differences | -0.01 (± 0.01) | -0.01 (± 0.01) | | |

Notes:

[25] - n missing = 1

[26] - n missing = 0

## Statistical analyses

| **Statistical analysis title** | Exploratory regression RI |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| | |
|---|---|
| Comparison groups | Arm A v Arm B |
| Number of subjects included in analysis | 35 |
| Analysis specification | Pre-specified |
| Analysis type | other[27] |
| P-value | = 0.7345 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | 0.01 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -0.05 |
| upper limit | 0.08 |
| Variability estimate | Standard error of the mean |
| Dispersion value | 0.03 |

Notes:

[27] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.

| **Statistical analysis title** | Exploratory regression PI |
|---|---|

Statistical analysis description:

Exploratory regression analyses was conducted using ANCOVA for each continous outcomes to directly account for the duration in days between visit 1 and visit 3 when assessing the change in outcome from visit 1 to visit 3. In particular, the visit 3 outcome was regressed against visit 1 outcome, treatment group and duration (in days) between visit 1 and visit 3.

| | |
|---|---|
| Comparison groups | Arm A v Arm B |
| Number of subjects included in analysis | 35 |
| Analysis specification | Pre-specified |
| Analysis type | other[28] |
| P-value | = 0.8704 |
| Method | ANCOVA |
| Parameter estimate | Mean difference (final values) |
| Point estimate | -0.02 |
| Confidence interval | |
| level | 95 % |
| sides | 2-sided |
| lower limit | -0.24 |
| upper limit | 0.21 |

| | |
|---|---|
| Variability estimate | Standard error of the mean |
| Dispersion value | 0.11 |

Notes:

[28] - Exploratory regression analyses. These analyses are viewed as entirely hypothesis generating, rather than confirmatory analyses, in light of the small sample size.

## Secondary: Uteroplacental measurments (discrete)

| | |
|---|---|
| End point title | Uteroplacental measurments (discrete) |

End point description:

Presence/absence of notching. Bilateral notching is defined as "L Notch" = Yes and "R Notch" = Yes, and change in bilateral notching is defined as patients with bilateral notching at visit 1 no longer having bilateral notching at visit 3.

| | |
|---|---|
| End point type | Secondary |

End point timeframe:

Change in uteroplacental blood flow measurements ( presence of bilateral notching) from randomisation (visit 1) to 8 weeks (visit 3).

| End point values | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 24 | 12 | | |
| Units: persons | | | | |
| Visit 1 notchings present | 10 | 2 | | |
| Visit 3 notchings present | 5 | 0 | | |
| Change in notchings | 5 | 2 | | |

### Statistical analyses

No statistical analyses for this end point

## Secondary: Plasma ADMA and Arginie

| | |
|---|---|
| End point title | Plasma ADMA and Arginie |

End point description:

Within-patient change in plasma ADMA concentration and Arginine concentration was calculated by subtracting each patient's visit 1 concentrations from their visit 3 concentrations. Standardised within-patient change in concentration was calculated by dividing each patient's change in concentration by the number of days between visit 3 and visit 1.

Within-patient change and standardised within-patient change from visit 1 to visit 3 of ADMA concentration and Arginine concentrations was presented using mean and 95% confidence interval for each treatment group separately. (See supplementary material for confidence intervals).

| | |
|---|---|
| End point type | Secondary |

End point timeframe:

Change in plasma ADMA and arginine concentrations between randomisation (visit 1) and the 8 week clinic visit (visit 3)

| End point values | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 21[29] | 11[30] | | |
| Units: µmol/L | | | | |
| arithmetic mean (standard deviation) | | | | |
| Plasma ADMA differences | 0.01 (± 0.05) | -0.01 (± 0.05) | | |
| Plasma ADMA standardised differences | 0.0002 (± 0.0008) | -0.0002 (± 0.0008) | | |
| Arginine differences | 6.86 (± 36.34) | -2.55 (± 13.34) | | |
| Arginine standardised differences | 0.14 (± 0.62) | -0.04 (± 0.23) | | |

Notes:

[29] - Plasma ADMA: n=19, n missing=5
Arginine: n=21, n missing = 3
[30] - Plasma ADMA: n=8, n missing=4
Arginine: n=11, n missing = 1

### Statistical analyses

No statistical analyses for this end point

## Secondary: Antihypertensive therapy

| End point title | Antihypertensive therapy |
|---|---|

End point description:

Antihypertensive therapy (AHT) information was used to determine whether patients were taking antihypertensive therapy at visit 1 or visit 3, for at least one of "Methyldopa", "Labetalol", "Beta blocking agent" or "Calcium channel antagonist". The number of patients who are taking antihypertensive therapy at visit 1 is presented for each treatment group separately. The number of patients who are taking antihypertensive therapy at visit 3 will be presented for each treatment group separately. The number of patients who change from taking (any) antihypertensive therapy from visit 1 to visit 3 is presented for each treatment group separately

| End point type | Secondary |
|---|---|

End point timeframe:

Change in antihypertensive therapy from visit 1 to visit 3.

| End point values | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 24[31] | 12[32] | | |
| Units: persons | | | | |
| Visit 1 AHT | 16 | 6 | | |
| Visit 3 AHT | 13 | 6 | | |

Notes:

[31] - AHT: Visit 1 only n = 3, visit 1 & 3 n = 13 (overall n = 16)

[32] - AHT: Visit 1 only n = 0, visit 1 & 3 n = 6 (overall n = 6)

### Statistical analyses

No statistical analyses for this end point

## Post-hoc: Pregnancy outcomes (continuous)

| End point title | Pregnancy outcomes (continuous) |
|---|---|

End point description:

Post- hoc summaries for the status and outcomes after giving birth. Continous outcomes presented with means and standard deviation. Categorical outcomes present using frequencies.

| End point type | Post-hoc |
|---|---|

End point timeframe:

Status and outcomes of pregnancy after giving birth.

| **End point values** | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 24 | 12 | | |
| Units: (see below) | | | | |
| arithmetic mean (standard deviation) | | | | |
| Gestational age (days) | 264.0 (± 12.2) | 259.8 (± 12.1) | | |
| Birthweight (grams) | 2846.8 (± 622.1) | 3123.6 (± 707.8) | | |

## Statistical analyses

No statistical analyses for this end point

## Post-hoc: Pregnancy outcomes (discrete)

| End point title | Pregnancy outcomes (discrete) |
|---|---|

End point description:

Post- hoc summaries for the status and outcomes after giving birth. Continous outcomes presented with means and standard deviation. Categorical outcomes present using frequencies.

| End point type | Post-hoc |
|---|---|

End point timeframe:

Status and outcomes of pregnancy after giving birth.

| **End point values** | Arm A | Arm B | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 24 | 12 | | |
| Units: persons | | | | |
| Live born: Yes | 24 | 12 | | |
| Gender: Female | 14 | 5 | | |
| Gender: Male | 10 | 7 | | |
| Magnesium sulfate required: Yes | 1 | 0 | | |
| Steroids required: Yes | 5 | 2 | | |
| Preeclampsia: Yes | 5 | 3 | | |
| Chronic hypertension: Yes | 24 | 12 | | |
| SGA by population centile: Yes | 7 | 3 | | |
| FGR: Yes | 7 | 3 | | |
| Pregestational diabetes: Yes | 3 | 3 | | |
| Gestational diabetes: Yes | 4 | 0 | | |

| | | | | |
|---|---|---|---|---|
| Perinatal outcome: Alive | 24 | 12 | | |
| Perinatal survival: Yes | 24 | 12 | | |

## Statistical analyses

No statistical analyses for this end point

## Adverse events

### Adverse events information[1]

Timeframe for reporting adverse events:

The appearance or worsening of any undesirable sign, symptom, or medical condition occurring after the study has commenced, even if not considered to be related to the study.

See uploaded results for details of non-serious adverse events.

Adverse event reporting additional description:

Medical conditions/diseases present before starting the study will only be considered as adverse events if they worsen after the start of the study. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

| | |
|---|---|
| Assessment type | Systematic |

### Dictionary used

| | |
|---|---|
| Dictionary name | MedDRA |
| Dictionary version | 19 |

### Reporting groups

| | |
|---|---|
| Reporting group title | L-citrulline safety set |

Reporting group description:

Any participant who recieved at least 1 dose of L-citrulline.

| | |
|---|---|
| Reporting group title | Placebo safety set |

Reporting group description:

Any participant who recieved at least one dose of placebo.

Notes:

[1] - There are no non-serious adverse events recorded for these results. It is expected that there will be at least one non-serious adverse event reported.
Justification: Non-serious adverse events not coded using MedDRA so these are included in the main study report which is attached.

| Serious adverse events | L-citrulline safety set | Placebo safety set | |
|---|---|---|---|
| Total subjects affected by serious adverse events | | | |
| subjects affected / exposed | 0 / 24 (0.00%) | 1 / 12 (8.33%) | |
| number of deaths (all causes) | 0 | 0 | |
| number of deaths resulting from adverse events | 0 | 0 | |
| Pregnancy, puerperium and perinatal conditions | | | |
| Foetal disorder | Additional description: Fetal ventriculomegaly diagnosed on fetal MRI on 8th May 2018. Participant delivered on 26th May 2018. Baby well at birth and will have neonatal follow-up. | | |
| subjects affected / exposed | 0 / 24 (0.00%) | 1 / 12 (8.33%) | |
| occurrences causally related to treatment / all | 0 / 0 | 0 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |

Frequency threshold for reporting non-serious adverse events: 0 %

| **Non-serious adverse events** | L-citrulline safety set | Placebo safety set | |
|---|---|---|---|
| Total subjects affected by non-serious adverse events<br>subjects affected / exposed | 0 / 24 (0.00%) | 0 / 12 (0.00%) | |

## More information

## Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

| Date | Amendment |
|---|---|
| 19 March 2017 | Amendment 2:<br>Submitted to meet MHRA conditions on initial approval<br>Approved by MHRA – 19/03/2017<br>Approved by REC – 27/03/2017<br>Approved by HRA – 23/03/2017 (initial approval)<br>IB document update -As per MHRA recommendations to clarify stability data testing.<br>Contact card - This was omitted from the original application in error.<br>Change to participant questionnaire - Removal of question 1 |
| 23 March 2017 | Amendment 1:<br>Inclusion of IRAS ID on patient information sheets.<br>Approved by HRA – 23/03/2017 (initial approval)<br>Approved by MHRA – N/A<br>Approved by REC – N/A |
| 03 July 2017 | Amendment 3:<br>Submitted to amend the protocol<br>Approved by MHRA – 18/08/2017<br>Approved by REC – 03/07/2017<br>Approved by HRA - 31/08/2017<br>Protocol - ABPM will now be issued at study visit 1 and 3<br>Patient information sheets ABPM will now be issued at study visit 1 and 3<br>Change of PI at St Thomas' Hospital |
| 24 August 2017 | Amendment 4:<br>Submitted to amend PISCs<br>Approved by HRA and REC - 24/08/2017<br>Approved by MHRA – N/A<br>Amendment to Patient Information sheets to clarify what scans are optional research scans and inclusion of further data protection information |
| 17 November 2017 | Amendment 5:<br>Submitted to clarify that other sites within MFT can identify patients for CHERRY and amend the Sponsor name throughout the protocol<br>Approved by MHRA – N/A<br>Approved by REC – 17/11/2017<br>Approved by HRA – 23/11/2017 |

Notes:

## Interruptions (globally)

Were there any global interruptions to the trial? No

## Limitations and caveats

None reported