



## Clinical trial results:

### Repetitive blood donations and endurance sport performance: does iron supplementation limit the negative effects on hematological parameters?

#### Summary

|                          |                |
|--------------------------|----------------|
| EudraCT number           | 2017-003406-40 |
| Trial protocol           | BE             |
| Global end of trial date | 31 May 2018    |

#### Results information

|                                |               |
|--------------------------------|---------------|
| Result version number          | v1 (current)  |
| This version publication date  | 20 April 2019 |
| First version publication date | 20 April 2019 |

#### Trial information

##### Trial identification

|                       |        |
|-----------------------|--------|
| Sponsor protocol code | RK2017 |
|-----------------------|--------|

##### Additional study identifiers

|                                    |   |
|------------------------------------|---|
| ISRCTN number                      | - |
| ClinicalTrials.gov id (NCT number) | - |
| WHO universal trial number (UTN)   | - |

Notes:

#### Sponsors

|                              |  |
|------------------------------|--|
| Sponsor organisation name    | Université catholique de Louvain   |
| Sponsor organisation address | Place de l'Université 1, Louvain-La-Neuve, Belgium, 1348   |
| Public contact               | Louise Deldicque, Université catholique de Louvain, 0032 10474443, louise.deldicque@uclouvain.be |
| Scientific contact           | Louise Deldicque, Université catholique de Louvain, 0032 10474443, louise.deldicque@uclouvain.be |

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:

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**Results analysis stage**

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|  |                 |
|--|-----------------|
| Analysis stage                                       | Final           |
| Date of interim/final analysis                       | 01 October 2018 |
| Is this the analysis of the primary completion data? | Yes             |
| Primary completion date                              | 31 May 2018     |
| Global end of trial reached?                         | Yes             |
| Global end of trial date                             | 31 May 2018     |
| Was the trial ended prematurely?                     | No              |

Notes:

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**General information about the trial**

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Main objective of the trial:

We propose to measure the impact of 3 blood donations, interspersed by 3 months, on blood parameters and on performance during a maximal incremental test and to see whether iron supplementation can limit or even reverse the negative effects of donations on blood parameters reflecting iron status.

Protection of trial subjects:

\* At the Rode Kruis Vlanderen, during the blood donation, numerous physicians were present in case of emergency. At the Faculty of Motor Science (Louvain-La-Neuve), during the exercise test, the hemoglobin mass measurement and the blood sample collection, there was always a physician present in case of emergency.

\* Plasma ferritin concentrations was checked before starting and during the whole study (1 week before iron supplementation and at day 2, 7, 14, 28 after the beginning of iron supplementation, day 28 being the last day of supplementation. Analyses were repeated at each iron supplementation following blood donation). If a value above 300µg/l ferritin would have been measured, iron supplementation would have been immediately stopped.

\* Exercise testing: a medical screening was performed before the beginning of the experiment to ensure that the participants were able to perform maximal exercise tests. In addition, participants were told that they can stop exercising at any time. A test was terminated if a subject shows any untoward signs and symptoms, including pallor, severe nausea and dizziness.

\* Subjects were asked to report every adverse events to the investigator until 2 months after the end of the trial and therefore the iron supplementation, even in case of premature clinical trial termination.

Every adverse events reported by the subjects was recorded by the Principal investigator and the promotor. The physician evaluated the seriousness and the causality with the product and decided the action to take.

Background therapy: -

Evidence for comparator: -

|   |                   |
|---|-------------------|
| Actual start date of recruitment                          | 18 September 2017 |
| Long term follow-up planned                               | No                |
| Independent data monitoring committee (IDMC) involvement? | No                |

Notes:

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**Population of trial subjects**

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**Subjects enrolled per country**

|                                      |             |
|--------------------------------------|-------------|
| Country: Number of subjects enrolled | Belgium: 44 |
| Worldwide total number of subjects   | 44          |
| EEA total number of subjects         | 44          |

Notes:

| <b>Subjects enrolled per age group</b>    |    |
|---|----|
| 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)                      | 44 |
| From 65 to 84 years                       | 0  |
| 85 years and over                         | 0  |

## Subject disposition

### Recruitment

Recruitment details:

Recruitment between September 2017 and November 2017

### Pre-assignment

Screening details:

Inclusion criteria:

- age between 18-50 years
- BMI between 20-28 kg/m<sup>2</sup>
- hours sport per week between 1-6

Screening of 50 subjects. 6 screening failure

### Period 1

|                              |                                |
|------------------------------|--------------------------------|
| Period 1 title               | Overall trial (overall period) |
| Is this the baseline period? | Yes                            |
| Allocation method            | Randomised - controlled        |
| Blinding used                | Single blind                   |
| Roles blinded                | Subject                        |

Blinding implementation details:

\* Blood donation: subjects were isolated from each other and were blinded using a sterile field so that they were not be able to see their arm. Moreover, they listened to music through a headphone, . They were all stitched by a needle but no blood was collected for the control-control group

\* No information was added on the pill to allow subject to recognize iron or glucose supplementation

### Arms

|                              |               |
|------------------------------|---------------|
| Are arms mutually exclusive? | Yes           |
| <b>Arm title</b>             | Placebo group |

Arm description:

- No blood donation
- Glucose supplementation during 4 weeks following each blood donations for the other groups

|  |               |
|--|---------------|
| Arm type                               | Placebo       |
| Investigational medicinal product name | Glucose       |
| Investigational medicinal product code |               |
| Other name                             |               |
| Pharmaceutical forms                   | Capsule, hard |
| Routes of administration               | Oral use      |

Dosage and administration details:

400mg/capsule, 1 capsule per day during 4 weeks following each blood donation

|                  |                          |
|------------------|--------------------------|
| <b>Arm title</b> | Donation - placebo group |
|------------------|--------------------------|

Arm description:

- Blood donation (3 blood donations with three months of interval)
- Glucose supplementation 4 weeks after each blood donations

|  |               |
|--|---------------|
| Arm type                               | Placebo       |
| Investigational medicinal product name | Glucose       |
| Investigational medicinal product code |               |
| Other name                             |               |
| Pharmaceutical forms                   | Capsule, hard |
| Routes of administration               | Oral use      |

Dosage and administration details:

400mg/capsule, 1 capsule per day during 4 weeks following each blood donation

|                  |                            |
|------------------|----------------------------|
| <b>Arm title</b> | Donation - 20mg iron group |
|------------------|----------------------------|

Arm description:

- Blood donation (three blood donations with three months of interval)
- Iron supplementation: 173.72mg of ferrous gluconate per day during 4 weeks following each blood donation

|  |                     |
|--|---------------------|
| Arm type                               | Experimental        |
| Investigational medicinal product name | Losferron 1/4       |
| Investigational medicinal product code |                     |
| Other name                             |                     |
| Pharmaceutical forms                   | Effervescent tablet |
| Routes of administration               | Oral use            |

Dosage and administration details:

1/4 losferron tablet (173.72 mg of ferrous gluconate) per day during 4 weeks following each blood donation.

|                  |                            |
|------------------|----------------------------|
| <b>Arm title</b> | Donation - 80mg iron group |
|------------------|----------------------------|

Arm description:

- Blood donation (three blood donations with three months of interval)
- iron supplementation : 695mg of ferrous gluconate per day during 4 weeks following each blood donation

|  |                     |
|--|---------------------|
| Arm type                               | Experimental        |
| Investigational medicinal product name | Losferron           |
| Investigational medicinal product code |                     |
| Other name                             |                     |
| Pharmaceutical forms                   | Effervescent tablet |
| Routes of administration               | Oral use            |

Dosage and administration details:

1 tablet of losferron per day (695mg of ferrous gluconate) during 4 weeks following each blood donation

| <b>Number of subjects in period 1</b> | Placebo group | Donation - placebo group | Donation - 20mg iron group |
|---------------------------------------|---------------|--------------------------|----------------------------|
| Started                               | 8             | 12                       | 12                         |
| Completed                             | 7             | 11                       | 11                         |
| Not completed                         | 1             | 1                        | 1                          |
| Adverse event, non-fatal              | -             | 1                        | -                          |
| Lost to follow-up                     | 1             | -                        | 1                          |

| <b>Number of subjects in period 1</b> | Donation - 80mg iron group |
|---------------------------------------|----------------------------|
| Started                               | 12                         |
| Completed                             | 11                         |
| Not completed                         | 1                          |
| Adverse event, non-fatal              | -                          |
| Lost to follow-up                     | 1                          |

## Baseline characteristics

### Reporting groups

|  |                            |
|--|----------------------------|
| Reporting group title  | Placebo group              |
| Reporting group description:   |                            |
| <ul style="list-style-type: none"> <li>- No blood donation</li> <li>- Glucose supplementation during 4 weeks following each blood donations for the other groups</li> </ul>  |                            |
| Reporting group title  | Donation - placebo group   |
| Reporting group description:   |                            |
| <ul style="list-style-type: none"> <li>- Blood donation (3 blood donations with three months of interval)</li> <li>- Glucose supplementation 4 weeks after each blood donations</li> </ul>   |                            |
| Reporting group title  | Donation - 20mg iron group |
| Reporting group description:   |                            |
| <ul style="list-style-type: none"> <li>- Blood donation (three blood donations with three months of interval)</li> <li>- Iron supplementation: 173.72mg of ferrous gluconate per day during 4 weeks following each blood donation</li> </ul> |                            |
| Reporting group title  | Donation - 80mg iron group |
| Reporting group description:   |                            |
| <ul style="list-style-type: none"> <li>- Blood donation (three blood donations with three months of interval)</li> <li>- iron supplementation : 695mg of ferrous gluconate per day during 4 weeks following each blood donation</li> </ul>   |                            |

| Reporting group values                             | Placebo group | Donation - placebo group | Donation - 20mg iron group |
|--|---------------|--------------------------|----------------------------|
| Number of subjects                                 | 8             | 12                       | 12                         |
| Age categorical                                    |               |                          |                            |
| 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)                               | 8             | 12                       | 12                         |
| From 65-84 years                                   | 0             | 0                        | 0                          |
| 85 years and over                                  | 0             | 0                        | 0                          |
| Age continuous                                     |               |                          |                            |
| Units: years                                       |               |                          |                            |
| arithmetic mean                                    | 24.3          | 21.5                     | 22.5                       |
| full range (min-max)                               | 18 to 32      | 18 to 33                 | 19 to 33                   |
| Gender categorical                                 |               |                          |                            |
| Units: Subjects                                    |               |                          |                            |
| Female   | 0             | 0                        | 0                          |
| Male   | 8             | 12                       | 12                         |

| Reporting group values | Donation - 80mg iron group | Total |  |
|------------------------|----------------------------|-------|--|
| Number of subjects     | 12                         | 44    |  |

|   |          |    |  |
|---|----------|----|--|
| Age categorical<br>Units: Subjects                    |          |    |  |
| In utero  | 0        | 0  |  |
| Preterm newborn infants<br>(gestational age < 37 wks) | 0        | 0  |  |
| Newborns (0-27 days)                                  | 0        | 0  |  |
| Infants and toddlers (28 days-23<br>months)           | 0        | 0  |  |
| Children (2-11 years)                                 | 0        | 0  |  |
| Adolescents (12-17 years)                             | 0        | 0  |  |
| Adults (18-64 years)                                  | 12       | 44 |  |
| From 65-84 years                                      | 0        | 0  |  |
| 85 years and over                                     | 0        | 0  |  |
| Age continuous<br>Units: years                        |          |    |  |
| arithmetic mean                                       | 24       |    |  |
| full range (min-max)                                  | 19 to 31 | -  |  |
| Gender categorical<br>Units: Subjects                 |          |    |  |
| Female  | 0        | 0  |  |
| Male  | 12       | 44 |  |

## End points

### End points reporting groups

|   |                            |
|---|----------------------------|
| Reporting group title   | Placebo group              |
| Reporting group description:  |                            |
| <ul style="list-style-type: none"><li>- No blood donation</li><li>- Glucose supplementation during 4 weeks following each blood donations for the other groups</li></ul>  |                            |
| Reporting group title   | Donation - placebo group   |
| Reporting group description:  |                            |
| <ul style="list-style-type: none"><li>- Blood donation (3 blood donations with three months of interval)</li><li>- Glucose supplementation 4 weeks after each blood donations</li></ul>   |                            |
| Reporting group title   | Donation - 20mg iron group |
| Reporting group description:  |                            |
| <ul style="list-style-type: none"><li>- Blood donation (three blood donations with three months of interval)</li><li>- Iron supplementation: 173.72mg of ferrous gluconate per day during 4 weeks following each blood donation</li></ul> |                            |
| Reporting group title   | Donation - 80mg iron group |
| Reporting group description:  |                            |
| <ul style="list-style-type: none"><li>- Blood donation (three blood donations with three months of interval)</li><li>- iron supplementation : 695mg of ferrous gluconate per day during 4 weeks following each blood donation</li></ul>   |                            |

### Primary: peak oxygen consumption

|   |                         |
|---|-------------------------|
| End point title   | peak oxygen consumption |
| End point description:  |                         |
| Measurement of this endpoint at several time points:  |                         |
| <ul style="list-style-type: none"><li>- 1 week before first blood donation</li><li>- 2 days after first blood donation</li><li>- 1 week after first blood donation</li><li>- 2 weeks after first blood donation</li><li>- 4 weeks after first blood donation</li><li>- 1 week before second blood donation</li><li>- 2 days after second blood donation</li><li>- 1 week after second blood donation</li><li>- 2 weeks after second blood donation</li><li>- 4 weeks after second blood donation</li><li>- 1 week before third blood donation</li><li>- 2 days after third blood donation</li><li>- 1 week after third blood donation</li><li>- 2 weeks after third blood donation</li><li>- 4 weeks after third blood donation</li></ul> |                         |
| End point type  | Primary                 |
| End point timeframe:  |                         |
| 4 weeks after the third blood donation  |                         |

| End point values                 | Placebo group   | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|-----------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5               | 11                       | 11                         | 11                         |
| Units: mL/min/kg                 |                 |                          |                            |                            |
| arithmetic mean (standard error) | 56.4 (± 4.3)    | 48.6 (± 1.7)             | 47.4 (± 3.5)               | 53.5 (± 2.4)               |

## Statistical analyses

|   |  |
|---|--|
| <b>Statistical analysis title</b>   | mixed ANOVA model  |
| Statistical analysis description:<br>A mixed ANOVA model (SAS Statistical Software) was used with the subjects as a random variable and groups (placebo, donation alone, donation + 20mg iron and donation + 80mg iron) and condition (time) as fixed independent variables. Contrast analyses were performed to compare means, applying a Sidak correction. Statistical significance was set at $p < 0.05$ |  |
| Comparison groups   | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis   | 38   |
| Analysis specification  | Pre-specified  |
| Analysis type   | other  |
| P-value   | $< 0.05$   |
| Method  | Mixed models analysis  |
| Parameter estimate  | Mean difference (net)  |
| Variability estimate  | Standard error of the mean   |

## Secondary: hemoglobin

|  |            |
|--|------------|
| End point title  | hemoglobin |
| End point description:<br>Measurement of this endpoint at several time points: <ul style="list-style-type: none"><li>- 1 week before first blood donation</li><li>- 2 days after first blood donation</li><li>- 1 week after first blood donation</li><li>- 2 weeks after first blood donation</li><li>- 4 weeks after first blood donation</li><li>- 1 week before second blood donation</li><li>- 2 days after second blood donation</li><li>- 1 week after second blood donation</li><li>- 2 weeks after second blood donation</li><li>- 4 weeks after second blood donation</li><li>- 1 week before third blood donation</li><li>- 2 days after third blood donation</li><li>- 1 week after third blood donation</li><li>- 2 weeks after third blood donation</li><li>- 4 weeks after third blood donation</li></ul> |            |
| End point type   | Secondary  |
| End point timeframe:<br>4 weeks after third blood donation   |            |

| End point values                 | Placebo group   | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|-----------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5               | 11                       | 11                         | 11                         |
| Units: g/dL                      |                 |                          |                            |                            |
| arithmetic mean (standard error) | 14.6 (± 0.4)    | 14.5 (± 0.3)             | 14.4 (± 0.3)               | 14.1 (± 0.2)               |

## Statistical analyses

|   |  |
|---|--|
| Statistical analysis title              | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 38   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

## Secondary: red blood cells

|   |                 |
|---|-----------------|
| End point title   | red blood cells |
| End point description:  |                 |
| Measurement of this endpoint at several time points:  |                 |
| <ul style="list-style-type: none"> <li>- 1 week before first blood donation</li> <li>- 2 days after first blood donation</li> <li>- 1 week after first blood donation</li> <li>- 2 weeks after first blood donation</li> <li>- 4 weeks after first blood donation</li> <li>- 1 week before second blood donation</li> <li>- 2 days after second blood donation</li> <li>- 1 week after second blood donation</li> <li>- 2 weeks after second blood donation</li> <li>- 4 weeks after second blood donation</li> <li>- 1 week before third blood donation</li> <li>- 2 days after third blood donation</li> <li>- 1 week after third blood donation</li> <li>- 2 weeks after third blood donation</li> <li>- 4 weeks after third blood donation</li> </ul> |                 |
| End point type  | Secondary       |
| End point timeframe:  |                 |
| 4 weeks after third blood donation  |                 |

| End point values                 | Placebo group    | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|------------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group  | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5                | 11                       | 11                         | 11                         |
| Units: 10E12/L                   |                  |                          |                            |                            |
| arithmetic mean (standard error) | 4.6 ( $\pm$ 0.1) | 4.7 ( $\pm$ 0.1)         | 4.6 ( $\pm$ 0.1)           | 4.6 ( $\pm$ 0.1)           |

## Statistical analyses

|   |  |
|---|--|
| Statistical analysis title              | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 38   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

## Secondary: Iron

|   |           |
|---|-----------|
| End point title   | Iron      |
| End point description:  |           |
| Measurement of this endpoint at several time points:  |           |
| <ul style="list-style-type: none"> <li>- 1 week before first blood donation</li> <li>- 2 days after first blood donation</li> <li>- 1 week after first blood donation</li> <li>- 2 weeks after first blood donation</li> <li>- 4 weeks after first blood donation</li> <li>- 1 week before second blood donation</li> <li>- 2 days after second blood donation</li> <li>- 1 week after second blood donation</li> <li>- 2 weeks after second blood donation</li> <li>- 4 weeks after second blood donation</li> <li>- 1 week before third blood donation</li> <li>- 2 days after third blood donation</li> <li>- 1 week after third blood donation</li> <li>- 2 weeks after third blood donation</li> <li>- 4 weeks after third blood donation</li> </ul> |           |
| End point type  | Secondary |
| End point timeframe:  |           |
| 4 weeks after third blood donation  |           |

| End point values                 | Placebo group   | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|-----------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5               | 11                       | 11                         | 11                         |
| Units: µg/dL                     |                 |                          |                            |                            |
| arithmetic mean (standard error) | 102 (± 15)      | 85 (± 13)                | 97 (± 7)                   | 116 (± 12)                 |

## Statistical analyses

|   |  |
|---|--|
| Statistical analysis title              | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 38   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

## Secondary: Transferrin

|   |             |
|---|-------------|
| End point title   | Transferrin |
| End point description:  |             |
| Measurement of this endpoint at several time points:  |             |
| <ul style="list-style-type: none"> <li>- 1 week before first blood donation</li> <li>- 2 days after first blood donation</li> <li>- 1 week after first blood donation</li> <li>- 2 weeks after first blood donation</li> <li>- 4 weeks after first blood donation</li> <li>- 1 week before second blood donation</li> <li>- 2 days after second blood donation</li> <li>- 1 week after second blood donation</li> <li>- 2 weeks after second blood donation</li> <li>- 4 weeks after second blood donation</li> <li>- 1 week before third blood donation</li> <li>- 2 days after third blood donation</li> <li>- 1 week after third blood donation</li> <li>- 2 weeks after third blood donation</li> <li>- 4 weeks after third blood donation</li> </ul> |             |
| End point type  | Secondary   |
| End point timeframe:  |             |
| 4 weeks after third blood donation  |             |

| End point values                 | Placebo group     | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|-------------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group   | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5                 | 11                       | 11                         | 11                         |
| Units: g/L                       |                   |                          |                            |                            |
| arithmetic mean (standard error) | 2.5 ( $\pm$ 0.03) | 2.79 ( $\pm$ 0.09)       | 2.72 ( $\pm$ 0.09)         | 2.61 ( $\pm$ 0.09)         |

## Statistical analyses

|   |  |
|---|--|
| Statistical analysis title              | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 38   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

## Secondary: Ferritin

|   |           |
|---|-----------|
| End point title   | Ferritin  |
| End point description:  |           |
| Measurement of this endpoint at several time points:  |           |
| <ul style="list-style-type: none"> <li>- 1 week before first blood donation</li> <li>- 2 days after first blood donation</li> <li>- 1 week after first blood donation</li> <li>- 2 weeks after first blood donation</li> <li>- 4 weeks after first blood donation</li> <li>- 1 week before second blood donation</li> <li>- 2 days after second blood donation</li> <li>- 1 week after second blood donation</li> <li>- 2 weeks after second blood donation</li> <li>- 4 weeks after second blood donation</li> <li>- 1 week before third blood donation</li> <li>- 2 days after third blood donation</li> <li>- 1 week after third blood donation</li> <li>- 2 weeks after third blood donation</li> <li>- 4 weeks after third blood donation</li> </ul> |           |
| End point type  | Secondary |
| End point timeframe:  |           |
| 4 weeks after third blood donation  |           |

| End point values                 | Placebo group   | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|-----------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5               | 11                       | 11                         | 11                         |
| Units: µg/L                      |                 |                          |                            |                            |
| arithmetic mean (standard error) | 64 (± 8)        | 28 (± 9)                 | 31 (± 4)                   | 36 (± 6)                   |

## Statistical analyses

|   |  |
|---|--|
| Statistical analysis title              | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 38   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

## Secondary: Hepcidin

|   |           |
|---|-----------|
| End point title   | Hepcidin  |
| End point description:  |           |
| Measurement of this endpoint at several time points:  |           |
| <ul style="list-style-type: none"> <li>- 1 week before first blood donation</li> <li>- 2 days after first blood donation</li> <li>- 1 week after first blood donation</li> <li>- 2 weeks after first blood donation</li> <li>- 4 weeks after first blood donation</li> <li>- 1 week before second blood donation</li> <li>- 2 days after second blood donation</li> <li>- 1 week after second blood donation</li> <li>- 2 weeks after second blood donation</li> <li>- 4 weeks after second blood donation</li> <li>- 1 week before third blood donation</li> <li>- 2 days after third blood donation</li> <li>- 1 week after third blood donation</li> <li>- 2 weeks after third blood donation</li> <li>- 4 weeks after third blood donation</li> </ul> |           |
| End point type  | Secondary |
| End point timeframe:  |           |
| 4 weeks after third blood donation  |           |

| End point values                 | Placebo group       | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|---------------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group     | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5                   | 11                       | 11                         | 11                         |
| Units: pg/mL                     |                     |                          |                            |                            |
| arithmetic mean (standard error) | 18698 ( $\pm$ 6770) | 3824 ( $\pm$ 1095)       | 7859 ( $\pm$ 2827)         | 7851 ( $\pm$ 2875)         |

## Statistical analyses

|   |  |
|---|--|
| Statistical analysis title              | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 38   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

## Secondary: Erythropoietin

|   |                |
|---|----------------|
| End point title   | Erythropoietin |
| End point description:  |                |
| Measurement of this endpoint at several time points:  |                |
| <ul style="list-style-type: none"> <li>- 1 week before first blood donation</li> <li>- 2 days after first blood donation</li> <li>- 1 week after first blood donation</li> <li>- 2 weeks after first blood donation</li> <li>- 4 weeks after first blood donation</li> <li>- 1 week before second blood donation</li> <li>- 2 days after second blood donation</li> <li>- 1 week after second blood donation</li> <li>- 2 weeks after second blood donation</li> <li>- 4 weeks after second blood donation</li> <li>- 1 week before third blood donation</li> <li>- 2 days after third blood donation</li> <li>- 1 week after third blood donation</li> <li>- 2 weeks after third blood donation</li> <li>- 4 weeks after third blood donation</li> </ul> |                |
| End point type  | Secondary      |
| End point timeframe:  |                |
| 4 weeks after third blood donation  |                |

| End point values                 | Placebo group   | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|-----------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5               | 11                       | 11                         | 11                         |
| Units: mIU/mL                    |                 |                          |                            |                            |
| arithmetic mean (standard error) | 8 ( $\pm$ 1.6)  | 10.8 ( $\pm$ 1.4)        | 7.4 ( $\pm$ 0.6)           | 9.9 ( $\pm$ 1.0)           |

## Statistical analyses

|   |  |
|---|--|
| Statistical analysis title              | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 38   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

## Secondary: Soluble transferrin receptor

|   |                              |
|---|------------------------------|
| End point title   | Soluble transferrin receptor |
| End point description:  |                              |
| Measurement of this endpoint at several time points:  |                              |
| <ul style="list-style-type: none"> <li>- 1 week before first blood donation</li> <li>- 2 days after first blood donation</li> <li>- 1 week after first blood donation</li> <li>- 2 weeks after first blood donation</li> <li>- 4 weeks after first blood donation</li> <li>- 1 week before second blood donation</li> <li>- 2 days after second blood donation</li> <li>- 1 week after second blood donation</li> <li>- 2 weeks after second blood donation</li> <li>- 4 weeks after second blood donation</li> <li>- 1 week before third blood donation</li> <li>- 2 days after third blood donation</li> <li>- 1 week after third blood donation</li> <li>- 2 weeks after third blood donation</li> <li>- 4 weeks after third blood donation</li> </ul> |                              |
| End point type  | Secondary                    |
| End point timeframe:  |                              |
| 4 weeks after third blood donation  |                              |

| End point values                 | Placebo group   | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|-----------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5               | 11                       | 11                         | 11                         |
| Units: mg/L                      |                 |                          |                            |                            |
| arithmetic mean (standard error) | 2.15 (± 0.24)   | 2.72 (± 0.19)            | 2.24 (± 0.18)              | 2.18 (± 0.19)              |

## Statistical analyses

|   |  |
|---|--|
| Statistical analysis title              | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 38   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

## Secondary: Hemoglobin mass

|   |                 |
|---|-----------------|
| End point title   | Hemoglobin mass |
| End point description:  |                 |
| Measurement of this endpoint at several time points:  |                 |
| <ul style="list-style-type: none"> <li>- 1 week before first blood donation</li> <li>- the day of the first blood donation</li> <li>- 2 days after first blood donation</li> <li>- 1 week after first blood donation</li> <li>- 2 weeks after first blood donation</li> <li>- 4 weeks after first blood donation</li> <li>- 1 week before second blood donation</li> <li>- the day of the second blood donation</li> <li>- 2 days after second blood donation</li> <li>- 1 week after second blood donation</li> <li>- 2 weeks after second blood donation</li> <li>- 4 weeks after second blood donation</li> <li>- 1 week before third blood donation</li> <li>- the day of the third blood donation</li> <li>- 2 days after third blood donation</li> <li>- 1 week after third blood donation</li> <li>- 2 weeks after third blood donation</li> <li>- 4 weeks after third blood donation</li> </ul> |                 |
| End point type  | Secondary       |
| End point timeframe:  |                 |
| 4 weeks after third blood donation  |                 |

| End point values                 | Placebo group       | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|---------------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group     | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 3                   | 5                        | 6                          | 5                          |
| Units: g/kg                      |                     |                          |                            |                            |
| arithmetic mean (standard error) | 13.59 ( $\pm$ 1.16) | 12.22 ( $\pm$ 0.91)      | 12.36 ( $\pm$ 0.55)        | 12.75 ( $\pm$ 0.36)        |

## Statistical analyses

|   |  |
|---|--|
| Statistical analysis title              | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 19   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

## Secondary: Lactate max

|   |             |
|---|-------------|
| End point title   | Lactate max |
| End point description:  |             |
| Measurement of this endpoint at several time points:  |             |
| <ul style="list-style-type: none"> <li>- 1 week before first blood donation</li> <li>- 2 days after first blood donation</li> <li>- 1 week after first blood donation</li> <li>- 2 weeks after first blood donation</li> <li>- 4 weeks after first blood donation</li> <li>- 1 week before second blood donation</li> <li>- 2 days after second blood donation</li> <li>- 1 week after second blood donation</li> <li>- 2 weeks after second blood donation</li> <li>- 4 weeks after second blood donation</li> <li>- 1 week before third blood donation</li> <li>- 2 days after third blood donation</li> <li>- 1 week after third blood donation</li> <li>- 2 weeks after third blood donation</li> <li>- 4 weeks after third blood donation</li> </ul> |             |
| End point type  | Secondary   |
| End point timeframe:  |             |
| 4 weeks after third blood donation  |             |

| End point values                 | Placebo group   | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|-----------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5               | 11                       | 11                         | 11                         |
| Units: mM                        |                 |                          |                            |                            |
| arithmetic mean (standard error) | 8.8 (± 1.5)     | 7.4 (± 1.1)              | 11.1 (± 1.1)               | 9.1 (± 0.9)                |

### Statistical analyses

|   |  |
|---|--|
| Statistical analysis title              | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 38   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

### Secondary: Heart rate max

|   |                |
|---|----------------|
| End point title   | Heart rate max |
| End point description:  |                |
| Measurement of this endpoint at several time points:  |                |
| <ul style="list-style-type: none"> <li>- 1 week before first blood donation</li> <li>- 2 days after first blood donation</li> <li>- 1 week after first blood donation</li> <li>- 2 weeks after first blood donation</li> <li>- 4 weeks after first blood donation</li> <li>- 1 week before second blood donation</li> <li>- 2 days after second blood donation</li> <li>- 1 week after second blood donation</li> <li>- 2 weeks after second blood donation</li> <li>- 4 weeks after second blood donation</li> <li>- 1 week before third blood donation</li> <li>- 2 days after third blood donation</li> <li>- 1 week after third blood donation</li> <li>- 2 weeks after third blood donation</li> <li>- 4 weeks after third blood donation</li> </ul> |                |
| End point type  | Secondary      |
| End point timeframe:  |                |
| 4 weeks after third blood donation  |                |

| <b>End point values</b>          | Placebo group   | Donation - placebo group | Donation - 20mg iron group | Donation - 80mg iron group |
|----------------------------------|-----------------|--------------------------|----------------------------|----------------------------|
| Subject group type               | Reporting group | Reporting group          | Reporting group            | Reporting group            |
| Number of subjects analysed      | 5               | 11                       | 11                         | 11                         |
| Units: bpm                       |                 |                          |                            |                            |
| arithmetic mean (standard error) | 180 ( $\pm$ 4)  | 184 ( $\pm$ 3)           | 191 ( $\pm$ 3)             | 180 ( $\pm$ 3)             |

### Statistical analyses

|   |  |
|---|--|
| <b>Statistical analysis title</b>       | mixed ANOVA model  |
| Comparison groups                       | Placebo group v Donation - placebo group v Donation - 20mg iron group v Donation - 80mg iron group |
| Number of subjects included in analysis | 38   |
| Analysis specification                  | Pre-specified  |
| Analysis type                           | other  |
| P-value                                 | < 0.05   |
| Method                                  | Mixed models analysis  |
| Parameter estimate                      | Mean difference (net)  |

## Adverse events

### Adverse events information

Timeframe for reporting adverse events:

- 1 week before blood donation
- blood donation
- 2 days after blood donation
- 1 week after blood donation
- 2 weeks after blood donation
- 4 weeks after blood donation

Repeated at every 3 blood donations, interval 3 months

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

### Dictionary used

|                 |        |
|-----------------|--------|
| Dictionary name | MedDRA |
|-----------------|--------|

|                    |   |
|--------------------|---|
| Dictionary version | 1 |
|--------------------|---|

### Reporting groups

|                       |                            |
|-----------------------|----------------------------|
| Reporting group title | Donation - 80mg iron group |
|-----------------------|----------------------------|

Reporting group description: -

|                       |                          |
|-----------------------|--------------------------|
| Reporting group title | Donation - placebo group |
|-----------------------|--------------------------|

Reporting group description: -

|                       |               |
|-----------------------|---------------|
| Reporting group title | Placebo group |
|-----------------------|---------------|

Reporting group description: -

|                       |                            |
|-----------------------|----------------------------|
| Reporting group title | Donation - 20mg iron group |
|-----------------------|----------------------------|

Reporting group description: -

| Serious adverse events                            | Donation - 80mg iron group | Donation - placebo group | Placebo group |
|---|----------------------------|--------------------------|---------------|
| Total subjects affected by serious adverse events |                            |                          |               |
| subjects affected / exposed                       | 0 / 11 (0.00%)             | 0 / 11 (0.00%)           | 0 / 7 (0.00%) |
| number of deaths (all causes)                     | 0                          | 0                        | 0             |
| number of deaths resulting from adverse events    | 0                          | 0                        | 0             |

| Serious adverse events                            | Donation - 20mg iron group |  |  |
|---|----------------------------|--|--|
| Total subjects affected by serious adverse events |                            |  |  |
| subjects affected / exposed                       | 0 / 11 (0.00%)             |  |  |
| number of deaths (all causes)                     | 0                          |  |  |
| number of deaths resulting from adverse events    | 0                          |  |  |

Frequency threshold for reporting non-serious adverse events: 5 %

| <b>Non-serious adverse events</b>                     | Donation - 80mg iron group | Donation - placebo group | Placebo group  |
|---|----------------------------|--------------------------|----------------|
| Total subjects affected by non-serious adverse events |                            |                          |                |
| subjects affected / exposed                           | 1 / 11 (9.09%)             | 2 / 11 (18.18%)          | 1 / 7 (14.29%) |
| Gastrointestinal disorders                            |                            |                          |                |
| Diarrhoea   |                            |                          |                |
| subjects affected / exposed                           | 1 / 11 (9.09%)             | 1 / 11 (9.09%)           | 1 / 7 (14.29%) |
| occurrences (all)                                     | 14                         | 1                        | 1              |
| Constipation  |                            |                          |                |
| subjects affected / exposed                           | 0 / 11 (0.00%)             | 0 / 11 (0.00%)           | 0 / 7 (0.00%)  |
| occurrences (all)                                     | 0                          | 0                        | 0              |
| Discomfort  |                            |                          |                |
| subjects affected / exposed                           | 0 / 11 (0.00%)             | 0 / 11 (0.00%)           | 1 / 7 (14.29%) |
| occurrences (all)                                     | 0                          | 0                        | 1              |

| <b>Non-serious adverse events</b>                     | Donation - 20mg iron group |  |  |
|---|----------------------------|--|--|
| Total subjects affected by non-serious adverse events |                            |  |  |
| subjects affected / exposed                           | 2 / 11 (18.18%)            |  |  |
| Gastrointestinal disorders                            |                            |  |  |
| Diarrhoea   |                            |  |  |
| subjects affected / exposed                           | 0 / 11 (0.00%)             |  |  |
| occurrences (all)                                     | 0                          |  |  |
| Constipation  |                            |  |  |
| subjects affected / exposed                           | 2 / 11 (18.18%)            |  |  |
| occurrences (all)                                     | 3                          |  |  |
| Discomfort  |                            |  |  |
| subjects affected / exposed                           | 0 / 11 (0.00%)             |  |  |
| occurrences (all)                                     | 0                          |  |  |

## **More information**

### **Substantial protocol amendments (globally)**

Were there any global substantial amendments to the protocol? No

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### **Interruptions (globally)**

Were there any global interruptions to the trial? No

### **Limitations and caveats**

None reported