# Arginine and nitric oxide (NO) metabolism during bone healing and non-union development - early prognostic markers

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Primary Objective: To study the arginine-NO metabolism during fracture healing and dysfunctional fracture healingSecondary Objective(s): To investigate if differences or decreased arginine and NO concentrations in bone healing form a prognostic…

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| **Ethical review** | Approved WMO |
| **Status** | Recruiting |
| **Health condition type** | Fractures |
| **Study type** | Observational invasive |

## Summary

### ID

NL-OMON39646

**Source**
ToetsingOnline

**Brief title**
Arginine and NO early prognostic markers for non-union development

### Condition

- Fractures

**Synonym**
impaired fracture healing, non-union

**Research involving**
Human

### Sponsors and support

**Primary sponsor:** Algemene Heelkunde

**Source(s) of monetary or material Support:** ZonMW

## Intervention

**Keyword:** arginine, fracture healing, Nitric Oxide (NO), Non-union

## Outcome measures

### Primary outcome

Primary endpoint are the arginine, citrulline, ornithine and Nitric Oxide

levels in the plasma during normal and dysfunctional fracture healing the bone

in patients with and without non-union

### Secondary outcome

none

# Study description

### Background summary

The incidence of hospital admission in the Netherlands due to a fracture is 50,000 per year, of which about 28,000 are operated upon. This incidence is rising in the last decades due to increased aging of the population and increased mobility of this group. Increased duration of hospital stay has a major impact on medical and hospital resources and adequate recovery of the patient with a normal fracture healing is, therefore, essential.
A fracture of the bone implies loss of mechanical integrity and continuity of the bone.
This will induce a process of bone healing, which will restore the integrity and continuity, when a number of conditions are met: absence of movements in the fractured bone parts, absence of distraction of the fracture, and adequate blood supply to the fracture site. Internal factors during this healing process are bone growth factors and absence of osteoporosis and external factors are adequate tissue coverage of the bone and adequate nutritional status.
Failure of bone healing, or non-union, occurs as a consequence of a standstill in the healing process. A non-union can be defined as a fracture which has ceased to show any evidence of healing, as indicated by persistent fracture lines, sclerosis at the fracture ends, a gap, and hypertrophic or absent callus. A non-union, which develops despite callus formation, is called hypertrophic non-union, in contrast to atrophic non-union during which no

callus formation is seen. In general terms, unless bone loss is present, a non-union is usually present when consolidation of the fracture has not occurred 6-8 months after the occurrence of the fracture.

Arginine, a semi-essential amino acid, plays an important role in the transport, storage and excretion of nitrogen. Moreover, arginine is an essential factor in the metabolism, since it is a precursor of nitric oxide (NO), polyamines and other molecules.NO is formed from arginine by the expression of the enzyme NO synthase (NOS).Some studies indicate that NO has a biphasic or dual effect on bone healing. High concentrations of NO inhibit bone resorption by suppressing osteoclast formation and the resorbing function of osteoclasts. Low concentrations of NO augment cytokine-induced bone resorption. In line with these data an inhibition of growth and differentiation of osteoblasts by high concentrations of NO was found, where low concentrations regulate normal growth and function of osteoblasts.
Preliminary data from our PILOT experiment (MEC04-021) concerning the influence of arginine, NO or polyamine metabolism on the development of non-union, indicate that a non-union possibly occurs during a disturbed production of NO.
In the PILOT phase, bone callus and plasma amino acid concentrations were evaluated to investigate disturbances in the amino acid profile during the development of a non-union. Patients with either atrophic or hypertrophic non-union had completely different amino acid concentrations of arginine, citrulline and ornithine. The patients with an atrophic non-union showed an overall decrease in the bone callus concentrations of arginine, citrulline and ornithine, compared to control samples, as the hypertrophic non-unions showed the opposite phenomenon with increased concentrations of these amino acids in callus tissue. In our PILOT study (MEC 04-021), the disturbances in the arginine-NO metabolism were determined at one time point, after the development of a non-union, and not immediately after the onset of the fracture. During fracture healing, fluctuations in the arginine-NO metabolism are possible, combined with the changes in the NOS expressions during healing. Therefore, early and frequent determination of the arginine -No metabolism in the different phases of fracture healing are essential to gather insight in the role of arginine-No in the development of a non-union. Furthermore, the different measurement time points of the arginine-NO metabolism, may be used as diagnostic time points, for the start of future therapeutic interventions to prevent the development of a non-union. Prior to this, early detection, during the healing process, of abnormal levels of arginine is a prerequisite for this intervention.
Bone turnover rates play a role in the healing process of fractures. A disturbed bone turnover may be a factor influencing the development of non-unions. Developing a method with which the turnover rates of bone can be measured adequately is therefore of importance.

**Study objective**

Primary Objective:

To study the arginine-NO metabolism during fracture healing and dysfunctional fracture healing

Secondary Objective(s):
To investigate if differences or decreased arginine and NO concentrations in bone healing form a prognostic marker for non-union development

Research questions
1. What are the changesof the arginine and NO metabolism in the onset of fracture healing in humans?
2. Can disturbances in the arginine and NO concentrations be used as prognostic markers for non-union development or for normal fracture healing?
3. Are there fluctuations present in the arginine - NO metabolism during fracture healing, which may be used as therapeutic intervention points in future research?

Hypothesis
Early detection of disturbances in the Arginine and nitric oxide metabolism during fracture healing are a good prognostic marker for non-union development

**Study design**

Prospective observational study.
Duration: 2-year period

This study is conducted in all acute fracture patients, with a fracture of the tibia or femur attending the Department of General Surgery, to investigate the Arginine -NO metabolism during normal fracture healing and possible dysfunctional healing.
It is anticipated that changes in the arginine-NO metabolism occur rapidly after the fracture onset, indicating that patients need to be included as soon as possible.

The experimental design of the study is described below
1. After inclusion, baseline demographic details are registered, including Injury Severity Score.
2. During the surgical procedure the bone pulp during intramedullary reaming will be collected and snap frozen in liquid nitrogen. This reaming and thereby harvesting of bone pulp is part of the normal procedure during fracture osteosynthesis.
3. One blood samples (5 mL venous blood) will be collected to measure amino acid levels in plasma of the patient at the primary surgical procedure and dialy during the stay in the hospital ( 5mL venous blood per day).
4. In patients with a fracture of the femur, a small muscle biopsy will be obtained in the fracture region.

5. For all patients bone healing will be followed closely during standard follow-up. The development of delayed-union/ non-union or re-fracture in the primary group and persistence of the non-union in the non-union group will be noted.
6. Blood samples will be collected during standard follow-up at several time points.
These time points are, approximately:
- 4 weeks after the primary surgical procedure
- 3months after the initial fracture
- 6 months after the initial fracture
7. During secondary surgical procedures:
A. Patient with a non-union fracture of the femur or tibia who will undergo surgical correction, or
B. Patient undergoing removal of the intramedullary device after healing of the fracture of the femur or tibia.

**Study burden and risks**

Only a venapuncture will be done during this study, which will not include extra risk or a high extent of the burden associated with participation.
In patients with a femur fracture, a muscle biopsy will be obtained during the surgical procedure. A small risk of haematoma and bleeding is existent.

## Contacts

**Public**
Selecteer

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**Scientific**
Selecteer

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## Trial sites

### Listed location countries

Netherlands

## Eligibility criteria

**Age**
Adults (18-64 years)
Elderly (65 years and older)

### Inclusion criteria

1. Written informed consent
2. Age > 18 years
3. Patient with a fracture of the femur or tibia at the primary surgical procedure and for which a surgical procedure providing bone debris is performed as therapy.

### Exclusion criteria

1. Patients with another bone fracture in their recent medical history (results in disturbances in the arginine-NO metabolism)
2. Infectious complications, such as infected pseudo-arthrosis (infections causes decreased argininine production, possibly resulting in impaired fracture healing).
3. Use of chronic corticosteroids (suppresses the immunesystem) or nitrovasodilating medication (improves the NO metabolism).
4. Patients with severe metabolic disturbances (liver, and renal insufficiency, diabetes).
5. Patients with metastases, haematological malignancies or chemotherapy (results in disturbances in the arginine-NO metabolism).

## Study design

### Design

**Study type:** Observational invasive

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| Masking: | Open (masking not used) |
| Control: | Uncontrolled |
| Primary purpose: | Diagnostic |

### Recruitment

NL

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| Recruitment status: | Recruiting |
| Start date (anticipated): | 29-03-2010 |
| Enrollment: | 100 |
| Type: | Actual |

## Ethics review

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| Approved WMO Date: | 03-03-2010 |
| Application type: | First submission |
| Review commission: | METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht) |
| Approved WMO Date: | 10-05-2010 |
| Application type: | Amendment |
| Review commission: | METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht) |
| Approved WMO Date: | 22-04-2015 |
| Application type: | Amendment |
| Review commission: | METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht) |

## Study registrations

### Followed up by the following (possibly more current) registration

No registrations found.

### Other (possibly less up-to-date) registrations in this register

No registrations found.

## In other registers

| Register | ID |
| --- | --- |
| ClinicalTrials.gov | NCT01070576 |
| CCMO | NL30839.068.09 |