Estrogen receptor α- (ERα), but not ERβ-signaling, is crucially involved in mechanostimulation of bone fracture healing by whole-body vibration

Melanie Haffner-Luntzer et.al.
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Abstract

• Mechanostimulation by low-magnitude high frequency vibration (LMHFV) provoke anabolic effects on the intact skeleton

• Experimental studies revealed that, during bone fracture healing, the effect of whole-body vibration is profoundly influenced by the estrogen status.

• LMHFV significantly improved fracture healing in ovariectomized (OVX) mice and was significantly reduced in non-OVX mice

• ERα and ERβ were differentially expressed in the callus depending on the estrogen status
Estrogen receptors

• ERα
  Breast, Uterus, Hypophyse, Hypothalamus
• is considered the most essential receptor for mediating estrogen effects on bone
• Crucial for anabolic effects of mechanical loading
• ERβ
  Bone, Vascular System, Prostate, Ovary, Lungs, Brain
• reverses or inhibits ERα-mediated gene transcription

• both ERs can exert signaling both in the presence and absence of their ligand estrogen
  the activated genes are highly dependent on the receptor subtype
Estrogenes in Osteoporosis

• Significant reduction of fractures

• Not used anymore due to:
  • risk-benefit negative:
    increased breast cancer risk
    higher thrombosis risk
SERM

- **Raloxifen**

- **Bazedoxifene**
  (Biskobing D.M. Update on bazedoxifene: A novel selective estrogen receptor modulator; Palacios S. Efficacy and safety of bazedoxifene, a novel selective estrogen receptor modulator for the prevention and treatment of postmenopausal osteoporosis)

- **IND:** prevention and treatment of osteoporosis in postmenopausal women

- Agonistic effects on bone tissue and lipid metabolism

- Antagonistic effects on breast and uterus
Material & Methods

- At the age of 12 weeks female mice received either a bilateral ovariectomy (OVX) or were sham-operated, and femur osteotomy was performed 4 weeks after OVX/sham operation.
- Estrogen serum levels were determined at day 21 after surgery using an estradiol ELISA.
- The osteotomy was created at the right femur diaphysis using a 0.4 mm gigli wire saw (RISystem, Davos, Switzerland) and stabilized by a semi-rigid external fixator (RISystem).
- Half of all mice received LMHFV. All mice were sacrificed 21 days after surgery using an isoflurane overdose.
Table 1
Overview about experimental groups and number of used mice per group.

<table>
<thead>
<tr>
<th>genotype</th>
<th>WT</th>
<th>ERα-KO</th>
<th>ERβ-KO</th>
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<tbody>
<tr>
<td>sham-OVX</td>
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<tr>
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<tr>
<td>sham-vibration</td>
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<tr>
<td>vibration</td>
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LHVVF

- Mice were placed on custom-made vibration platforms for 20 min per day for 5 days per week, starting with the third postoperative day.
- Vibration settings were 0.3 g sinusoidal peak-to-peak acceleration and 45 Hz frequency. The amplitude and frequency were continuously recorded using integrated accelerometers at the platform.
Biomechanical testing and μCT analysis

- Fractured femurs were explanted after euthanasia and subjected to a non-destructive three-point bending test.
- A force of maximal 4 N was applied on top of the cranio-lateral callus side and deflection was measured using a materials testing machine. Flexural rigidity was calculated from the slope of the linear region of the force-deflection curve.
- After biomechanical testing, bones were fixed in 4% paraformaldehyde and scanned in a μCT device to determine the apparent bone mineral density.
Histomorphometry and immunohistochemistry

• Longitudinal sections of 7 μm were stained with Giemsa or Safranin O for histomorphometric tissue quantification.

• The number and surface of osteoblasts were determined using Toluidine blue staining.

• The number and surface of osteoclasts were determined using tartrate-resistant alkaline phosphatase (TRAP) staining.

• ERα and ERβ expression were detected using a primary antibody against ERα and ERβ, and secondary antibodies against rabbit IgG were used for immunohistochemistry.
Expression of ERs in the fracture callus

• ERβ-KO mice displayed a striking increase in ERα expression in almost all cells of the fracture callus
• WT mice displayed only low amount of the receptor, mostly on osteoclasts.
• only non-vibrated OVX mice additionally displayed some ERβ expression in osteoblasts
Body weight change
Estrogen serum level

A

WT

estrogen serum level in pg/ml

OVX
Vibration

-     -     +     +

G

ERβ⁻⁻

estrogen serum level in pg/ml

OVX
Vibration

-     -     +     +

D

ERα⁻⁻

estrogen serum level in pg/ml

OVX
Vibration

-     -     +     +

#
Uterus weight

- WT
- ERα−/
- ERβ−/
Bone healing WT

A

B

C

D

BV/TV in %

TV in mm³

bridging

flexural rigidity in N/mm²

OVX Vibration

- - - + +

- - - + +

- - - + +

- - - + +

- - - + +

- - - + +

- - - + +

- - - + +

- - - + +

- - - + +
Bone healing ERα
Bone healing ERβ

A

B

C

D

BV/TV in %

TV in mm³

bridging

flexural rigidity in N/mm²

OVX Vibration

- - + + +

- - + + +

- - + + +

- - + + +
Osteoblasts

[Graphs showing different conditions and their effects on osteoblast activity.]
Osteoclasts
Histology (1)
Histology (2)
Histology (3)

B

<table>
<thead>
<tr>
<th>OVX</th>
<th>Vibration</th>
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WT

ERβ-KO

Ob
Discussion

• the results suggest a critical role of ERα-, but not of ERβ-signaling in the effects of mechanostimulation of bone fracture healing.

• to date, there are no other studies that investigated the influence of ERα on bone healing, and only one study that investigated fracture healing in ERβ- KO mice.

• BUT healing was unaffected at a later stage they conclude that under normal healing conditions in young, healthy mice, both ERs are not crucial for fracture healing.

• These results are against our expectations, since both ERs were known to significantly influence the endochondral ossification process during long bone growth, which is recapitulated during fracture healing.