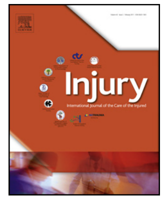
G Model
JINJ-6701; No. of Pages 10
ARTICLE IN PRESS
Injury, Int. J. Care Injured xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury



Obesity does not affect the healing of femur fractures in mice

T. Histing [a,*], A. Andonyan [a], M. Klein [a], C. Scheuer [b], D. Stenger [a], J.H. Holstein [a], N.T. Veith [a], T. Pohlemann [a], M.D. Menger [b]

[a] Department of Trauma, Hand and Reconstructive Surgery, University of Saarland, Homburg/Saar, Germany
[b] Institute for Clinical & Experimental Surgery, University of Saarland, Homburg/Saar, Germany

ARTICLE INFO

Article history:
Accepted 21 April 2016

Keywords:
Obesity
Fracture healing
Bone formation
Bone remodelling
Callus adiposity

ABSTRACT

Obesity is reported to be both protective and deleterious to bone. Lipotoxicity and inflammation might be responsible for bone loss through inhibition of osteoblasts and activation of osteoclasts. However, little is known whether obesity affects the process of fracture healing. Therefore, we studied the effect of high fat diet-induced (HFD) obesity on callus formation and bone remodelling in a closed femur fracture model in mice. Thirty-one mice were fed a diet containing 60 kJ% fat (HFD) for a total of 20 weeks before fracture and during the entire postoperative observation period. Control mice ($n = 31$) received a standard diet containing 10 kJ% fat. Healing was analyzed using micro-CT, biomechanical, histomorphometrical, immunohistochemical, serum and protein biochemical analysis at 2 and 4 weeks after fracture. HFD-fed mice showed a higher body weight and increased serum concentrations of leptin and interleukin-6 compared to controls. Within the callus tissue Western blot analyses revealed a higher expression of transcription factor peroxisome proliferator-activated receptor γ (PPARγ) and a reduced expression of runt-related transcription factor 2 (RUNX2) and bone morphogenetic protein (BMP)-4. However, obesity did not affect the expression of BMP-2 and did not influence the receptor activator of nuclear factor κB (RANK)/RANK ligand/osteoprotegerin (OPG) pathway during fracture healing. Although the bones of HFD-fed animals showed an increased number of adipocytes within the bone marrow, HFD did not increase callus adiposity. In addition, radiological and histomorphometric analysis could also not detect significant differences in bone formation between HFD-fed animals and controls. Accordingly, HFD did not affect bending stiffness after 2 and 4 weeks of healing. These findings indicate that obesity does not affect femur fracture healing in mice.

© 2016 Elsevier Ltd. All rights reserved.

Introduction

The effect of obesity on bone metabolism is still controversially discussed. There are reports indicating that obesity is beneficial to bone due to the increased biomechanical load which stimulates bone formation [1]. Accordingly, bone mineral density was shown to positively correlate with the increased body weight [2]. In contrast, other studies have indicated that obesity may not protect against osteoporosis or osteoporotic fractures, and that "lipotoxicity" and subsequent inflammation might be responsible for bone loss [3,4]. The fat in the bone marrow microenvironment is thought to inhibit osteoblast function and to increase osteoclast activation and differentiation [5]. In addition, bone marrow adipogenesis may be inversely related to osteoblastogenesis, because adipocytes and osteoblasts are derived from a common multipotent mesenchymal stem cell [6]. Several studies have analyzed the effect of high fat diet (HFD)-induced obesity on the mechanical properties of the bone. Cao et al. [7] found that despite the higher weight HFD-fed mice have a lower femoral bone volume, a lower trabecular number and a higher trabecular separation than controls. These findings suggest that obesity increases bone resorption.

Adipose tissue is not just an inert organ, but secretes a variety of biologically active molecules, such as leptin, adiponectin, oestrogen and interleukin (IL)-6 [8]. These molecules may also be involved in bone metabolism. In fact, the pro-inflammatory cytokines IL-6 and tumour necrosis factor (TNF)-alpha are capable of stimulating osteoclastogenesis and, thus, bone resorption [9]. Leptin is the most widely recognized adipocyte-derived hormone. Leptin levels rise with increased body fat and stimulate bone formation through its direct effect on osteoblasts and through central effects, including the growth hormone insulin like growth factor (GH-IGF)-1 axis and the suppression of neuropeptide Y.

* Corresponding author at: Department of Trauma, Hand and Reconstructive Surgery, University of Saarland, D-66421 Homburg/Saar, Germany.
Tel.: +49 6841 16 31502; fax: +49 6841 16 31503.
E-mail address: tina.histing@uks.eu (T. Histing).

http://dx.doi.org/10.1016/j.injury.2016.04.030
0020–1383/© 2016 Elsevier Ltd. All rights reserved.

Stimulation of beta2-adrenergic receptors through hypothalamic leptin receptors, however, increase bone remodelling [10]. These findings suggest that body fat may regulate bone mass and structure through molecular pathways that are independent of load-bearing.

Although there is substantial interest how obesity may influence bone regeneration, there is little information, whether obesity affects the process of fracture healing. In a recent study, Brown et al. [11] indicated a delayed healing of rotationally non-stabilized tibia osteotomies in HFD-fed mice. Because the process of healing is thought to differ between osteotomies and fractures and depends on the osteosynthesis technique, we herein now studied in a murine model the effect of obesity on the healing process of closed femur fractures after intramedullary screw fixation which provides some rotational stability.

Materials and methods

Animals

For the present study a total of sixty-two 4-week-old male C57BL/6J mice were purchased from Janvier Labs (Saint-Berthevin Cedex, France). They were kept at a 12-h light and dark cycle. Room temperature was 22 °C and humidity was 55 ± 10%. The animals received water and standard chow ad libitum for 4 weeks. Then, the 8-week-old animals were randomized into two groups. Thirty-one mice were fed a diet containing 60 kJ% fat (ssniff GmbH, Soest, Germany) for a 20-week period before surgery as well as during the 4-week period after surgery. Control mice ($n = 31$) received for a 20-week period a standard diet containing 10 kJ% fat (ssniff GmbH, Soest, Germany), which was also continued during the 4 weeks after surgery. Body weight (BW) of the animals was measured weekly. Before surgery, the mice were kept in groups of four mice in standard cages. After surgery, the mice were singly housed until the end of the experiment. All animal procedures were performed according to the National Institute of Health guidelines for the use of experimental animals and were approved by the German legislation on the protection of animals.

Surgical procedure

Mice were anesthetized by an intraperitoneal injection of xylazine (15 mg/kg BW) and ketamine (75 mg/kg BW). Under aseptic conditions a 4 mm medial parapatellar incision was performed at the right knee to dislocate the patella laterally. After drilling a hole (0.5 mm in diameter) into the intercondylar notch, an injection needle with a diameter of 0.4 mm was drilled into the intramedullary canal. Subsequently, a tungsten guide wire (0.2 mm in diameter) was inserted through the needle into the intramedullary canal. After removal of the needle, the femur was fractured by a 3-point bending device and an intramedullary medical grade stainless steel screw (17.2 mm length, 0.5 mm in diameter) was implanted over the guide wire to stabilize the fracture [12]. The screw consisted of a distal cone-shaped head (diameter 0.8 mm) and a proximal thread (M 0.5 mm, length 4 mm) (Research Implants Systems (RIS), Davos, Switzerland). After fracture fixation, the wound was closed using 6-0 synthetic sutures. Fracture and implant position were confirmed by radiography (MX-20, Faxitron X-ray Corporation, Wheeling, IL, USA). All fractures were simple, transverse midshaft fractures according to the AO classification type A3 fracture. In none of the animals a comminuted or incomplete fracture was observed. For analgesia the mice received tramadol-hydrochloride (Grünenthal, Aachen, Germany) in the drinking water (2.5 mg/100 ml) from day 1 before surgery until day 3 after surgery.

Serum analysis

At 2 and 4 weeks after fracture, 1 ml blood was collected from the vena cava and serum was frozen at −80° C after centrifugation. Therefore, mice were anesthetized by an intraperitoneal injection of xylazine (15 mg/kg BW) and ketamine (75 mg/kg BW). After resection of the femur for further analysis, the blood was collected from the vena cava inferior through a laparotomy using an injection needle. After blood collection the mice were killed by cervical dislocation. Leptin, a hormone secreted by adipocytes, was measured using a sandwich enzyme immunoassay (Mouse Leptin ELISA, BioVendor GmbH, Heidelberg, Germany). Interleukin-6 (IL-6), a pro-inflammatory cytokine, was measured using a solid-phase ELISA (R&D Systems, Wiesbaden, Germany). IL-6 was measured because obesity is characterized by chronic inflammation, which is indicated by increased levels of circulating factors such as C-reactive protein (CRP), tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) [13]. It is well documented that IL-6 production by adipose tissue is enhanced in obesity [14,15]. Of interest, 15–30% of the circulating IL-6 levels derive from adipose tissue in the absence of an acute inflammation [16].

Biomechanical analysis

For biomechanical analysis, the resected femora were freed from soft tissue. After removing the implants, callus stiffness was measured with a non-destructive bending test using a 3-point bending device (Mini-Zwick Z 2.5, Zwick GmbH, Ulm, Germany) [17]. Due to the different states of healing (2 weeks vs. 4 weeks), the loads which had to be applied varied markedly between the individual animals. Loading was stopped individually in every case when the actual load-displacement curve deviated more than 1% from linearity [18]. To guarantee standardized measuring conditions, femora were mounted always with the ventral aspect upwards. A working gauge length of 6 mm was used. Applying a gradually increasing bending force with 1 mm/min, the bending stiffness (N/mm) was calculated from the linear elastic part of the load-displacement diagram. Control that the load was not destructive was performed macroscopically and microscopically (histology). To account for differences in bone stiffness of the individual animals, the unfractured left femora were also analyzed, serving as an internal control. All values of the fractured femora are given in percent of the corresponding unfractured femora.

Radiological analysis

At the end of the 2- and 4-week observation period, fracture healing was analyzed using a high-resolution micro-CT imaging system (Skyscan 1172, Kontich, Belgium). The femora were placed in a 2 ml Eppendorf tube filled with 70% of methanol. Then, the femora were aligned orthogonally to the X-ray beam. The complete callus area was scanned at a resolution of 4 μm, the X-ray tube was operated at 50 keV and 200 μA. The integration time was set to 500 ms, the pixel matrix was set to 1336 × 2000. By this, the following parameters were analyzed: tissue volume (TV, mm^3), bone volume (BV, mm^3), the ratio of bone volume to tissue volume (BV/TV, %), trabecular thickness (mm), trabecular number (1/mm) and trabecular separation (mm).

Histomorphometrical analysis

For histology, bones were fixed in IHC zinc fixative (BD Pharmingen, Heidelberg, Germany) for 24 h, decalcified in 10% EDTA solution for 2 weeks and then embedded in paraffin. Longitudinal sections of 5 μm thickness were stained according to the trichrome method. At a magnification of 12.5× (Olympus BX60

Download English Version:

https://daneshyari.com/en/article/6082726

Download Persian Version:

https://daneshyari.com/article/6082726

Daneshyari.com