Introduction

It is a known fact that body fat regulates bone metabolism by means of hormonal factors, and furthermore, that the effects of muscle and loading are signaling factors in mechanotransduction [12, 40]. Leptin, a peptide hormone produced predominantly by white fat cells, is one of these hormonal factors [1, 13, 14, 18, 30, 42]. Leptin inhibits appetite; consequently, leptin deficiency induces obesity. With regard to bone metabolism it is not clear whether leptin is a stimulator or an inhibitor of bone growth. Some study authors noted a positive relationship between serum leptin levels and bone mineral density [16, 28], whereas others observed a negative or no relationship [4, 33, 24, 29], which further confounds the interpretation of the effect of leptin on bone mass. Simha et al. concluded that the role played by leptin in bone metabolism in humans may depend on the stage of life [35]. This might explain its positive effect on bone formation in the early stages of development. Leptin is also known to increase both proliferation and differentiation of the chondrocyte population of skeletal growth centers in organ cultures [23, 36]. All these findings strongly support the theory that leptin could stimulate bone growth in the early stages of life.

In a few recent reports the animal model was a useful surrogate to investigate the mechanism of leptin. Mice with deficiency of leptin (obese; ob/ob) or its receptor (diabetic; db/db) are obe-
Both leptin and its receptors were found in murine fetal cartilage and bone template, as well as in the growth plate [21]. Takeda et al. and Karsenty described the leptin-dependent control of bone remodeling via the sympathetic nervous system [18, 19, 36, 37]. The sterility of ob/ob mice should increase bone resorption and indeed, osteoclast numbers and the parameters of bone resorption did increase in ob/ob mice. Nevertheless, leptin-deficient mice have a higher bone mass than wild-type mice and completely correct their high bone mass by intracerebroventricular infusion of leptin at a rate that does not result in any detectable leak of leptin in general circulation [10].

In general, mechanical loading stress on bones causes tissue deformation within the bone and stimulates the bone to adapt and remodel, ultimately improving resistance to osteoporosis. While previous studies have shown that bone parameters improved with weight-bearing exercise in normal subjects, we were interested in the co-influence of biomechanical loading on body weight and bone metabolism in subjects with leptin deficiency. There is so far no agreement regarding the positive effect of leptin in the early stages of life.

The ability of three-dimensional micro-computed tomography to detect changes in a rat model was evaluated for bone mass [2, 9, 22, 27, 32, 34, 38, 39]. It is a method to image, quantify and study trabecular bone and the bone remodeling [32]. After induction of osteoarthritis by drugs in the murine knee, Botter et al. showed that significant changes in subchondral bone architecture and could be detected and quantified in 3D using micro-CT analysis [5]. Ankle micro-CT analyses demonstrated bone destruction in a rat model [20]. The aim of this study was to investigate the differential effects of weight-bearing on joint space in mice without the stimulation of leptin.

Materials and Methods

Animals

C57BL/6J-Lep (ob/ob) female mice were received from the Janvier Laboratory (Le Genest St Isle, France) at the age of five weeks. The animal experiment was approved by the local authorities according to the official guidelines. The effects of dietary restriction in ob/ob and wild-type mice has been described in several studies [7, 15, 25]. The animals were housed one per cage with access to water ad libitum and a standard rodent diet (8640 Harlan Teklad 22/5; Harlan Teklad, Madison, WI, USA) containing 1.13% calcium and 0.94% phosphorus. The animals were maintained under conditions of a twelve-hour light and dark cycle with the light switched on at 6.00 a.m.. Food intake and body weight were recorded daily. There were two experimental groups divided according to the provision of food. Group A included 20 ob/ob mice with access to food ad libitum, the other 20 ob/ob mice in Group B received a limited amount of food (6 gr. each day). All the animals were euthanized by mechanical procedure at the age of twenty weeks.

Micro-CT

A high resolution micro-CT (SkyScan 1072, Aartselaar, Belgium) was used to perform qualitative and quantitative analysis of the hip and knee. All samples were scanned by a commercially available microcomputed tomographic scanner at the Department of Cardiology of the West German Heart Centre at the University of Duisburg-Essen.

Technical specifications of the micro-CT

The micro-CT system is based on a scanner developed for high-resolution imaging [up to 4 μm cubic voxels] of sample sizes up to 2 cm³. The scanner uses a field x-ray tube with an 8 μm spot-size and expected lifetime of > 10,000 hours. The tube is operated at between 20 and 100 kilovoltam, and a current of up to 100 micron amperes. For scanning, the samples are placed between the micro-focus X-ray source and a Charge-Coupled-Device (CCD) detector (matrix size: 1024 x 1024 pixels, field of view: 25 mm²). In order to prevent samples from moving during scanning, the limbs are placed in a tightly fitting rigid plastic tube. In the scanner’s chamber the specimens are placed on a stack of computer-controlled precision stages which are rotated in equiangular steps of 0.9° around an angle of 180°. When the object is placed between the X-ray source and the CCD-detector, the cone-beam of X-rays passes the object and then hits the CCD-detector producing 2D-X-ray images. A personal computer is used to control the scanner and store the CCD image data recorded at each angle of view during the scanning process.

Tomographic image reconstruction

The X-ray projection data of the scanned samples are then submitted to the resident reconstruction program (Cone-beam Reconstruction, SkyScan, Aartselaar, Belgium), which is based on a Feldkamp filtered back projection algorithm [43] resulting in a volume image of up to 10,243 voxels, each cubic voxel being 4–19 μm on one side, depending on how much of the specimen has to be imaged.

Cancellous bone assessment by micro-CT

The regions of interest (ROIs) from the proximal femur and tibia were selected for structural analysis of the cancellous bone. A cubic region of 0.5x0.5x0.5mm³ in the metaphysis of the tibia was adjacent to (1mm away from) the growth plate and femoral head. Trabecular thickness (Th), trabecular number (Tb.N) and trabecular (Th, Tb.N) were based directly on the 3-D data using medial axis transformation and distance transformation.

Joint space measurement by micro-CT

The center of the head of the hip was detected by “data viewer”. The measurements were made in micrometers. The smallest joint space was measured from the longitudinal, sagittal and transversal slices of the center. The joint space of the knee was measured from the transverse and sagittal slices between the medial condyle and the tibial plateau.

Statistical analysis

The data were analyzed and assessed using SPSS software (version 15.0; SPSS Institute Inc, Chicago, USA). Descriptive statistics of all variables were determined including the mean and standard deviation of each group. The difference of all parameters between the two groups was assessed using the Student’s t-Test because all parameters were normally distributed [which was tested using the Kolmogorov Smirnov test]. Pearson’s correlation coefficient was used to assess the relationship between all the trabecular bone parameters of the femur and tibia. A value of p < 0.05 was considered to be statistically significant.

Results

No death or health deterioration occurred during this study. The body weight at each time point in the two groups is shown in Figure 1. Animals with an ad libitum diet (Group A) were found to increase body weight significantly at the age of six weeks in comparison with the lean mice (Group B). From this time point on,
the difference increased constantly. At the age of twenty weeks, the obese mice were almost twice as heavy as the lean mice.

In both Group A and Group B the mice with a high body weight had a lower bone mineral density (BMD) (Figure 2) and a lower trabecular number (Tb.N) in the proximal femur and tibia (Table 1, Figure 3).

The animals with an ad libitum diet (Group A) were found to have a significantly increased joint space in the hip in the coronal, sagittal and transverse planes in comparison with the lean mice (Group B) (Table 2).

The increase in the joint space in the knee in the coronal plane, for the transverse diameter of the tibial plateau and for the subchondral sclerosis in the ad libitum diet Group A was also statistically significant.

Only one significant correlation was found between bone mineral density (measured by micro-CT) and the cross-diameter of the tibial plateau (Figure 3).

Correlation was found between the trabecular number (Tb.N) of the knee and femur (measured by micro-CT) and body weight (Fig. 4).

Correlation was also found between the trabecular number (Tb.N) of the femur (measured by micro-CT) and the diameter of the femoral head in the sagittal, coronary and transverse planes (Fig. 5).

**Discussion**

The aim of the present study was to examine changes in the joint space induced by a response to biomechanical loading in leptin-deficient mice. Correlation of body weight and bone mineral density shows that in both groups bone mineral density decreased as body weight increased. However, this did not induce any significant changes in the joint space in either of the groups. Only one significant correlation was found between bone mineral density and the cross-diameter of the tibial plateau.

A great deal of research has confirmed that greater biomechanical loading due to high body weight contributes to the increased bone dimensions observed not only in our animal mo-

**Table 1: Statistically significant differences between the ad libitum diet Group A and controlled diet Group B for bone mineral density (BMD), body weight and trabecular number (Tb.N) in the ROI of the proximal femur (p < 0.05) and the statistical trend of the trabecular number (Tb.N) in the ROI of the proximal tibia (p = 0.038).**

<table>
<thead>
<tr>
<th>Group</th>
<th>BMD mean</th>
<th>SD</th>
<th>Weight (grams) mean</th>
<th>SD</th>
<th>TbN femur mean</th>
<th>SD</th>
<th>TbN tibia mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>2.57</td>
<td>0.147</td>
<td>52.53</td>
<td>6.36</td>
<td>6.42</td>
<td>0.45</td>
<td>5.63</td>
<td>0.34</td>
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<td>(ad libitum diet)</td>
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<tr>
<td>Group B</td>
<td>2.68</td>
<td>0.138</td>
<td>35.65</td>
<td>3.50</td>
<td>6.76</td>
<td>0.55</td>
<td>5.90</td>
<td>0.52</td>
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<tr>
<td>(controlled diet)</td>
<td></td>
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<tr>
<td>P value</td>
<td>&lt; 0.05</td>
<td>&lt; 0.001</td>
<td></td>
<td>0.038</td>
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<td>0.058</td>
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</table>
Abstract

Leptin was regulate bone development. However, there is so far no agreement concerning the effect of leptin in the early stages of life. The aim of this study was to investigate the differential effects of weight-bearing on joint space in leptin-deficiency mice. Materials, Methods and Results: C57BL/6J-Lep ob/ob female mice with an ad-libitum-diet (Group A) were found to increase body weight significantly at the age of six weeks in comparison with lean mice (Group B). At the age of twenty weeks the obese mice were almost twice as heavy as the lean mice. A high resolution micro-CT (SkyScan 1072) was used to perform qualitative and quantitative analysis of the hip and knee. Significant statistical differences are shown between the two groups for body weight and bone mineral density, for the trabecular number (Tb.N) for the proximal femur, a increased joint space in the hip in the coronary, sagittal and transverse planes for Group A, a increase in the joint space in the hip and knee. Significant correlation was found between bone mineral density and the cross diameter of the tibial plateau, between Tb.N. of knee/fe-
Hansjoerg Heep: Joint Space Gap of Leptin-Def. ob/ob Mice in Response to Loading

Conclusion
In both groups decreased bone mineral density as body weight increased. However, this did not induce any significant changes in the joint space in either of the groups in micro-CT.

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