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ASSOCIATION OF BONE FRACTURE TYPE AND DEGREE OF CALLUS FORMATION WITH LEPTIN CONCENTRATION IN CHILDREN WITH LONG BONE FRACTURES

Zoran Paunovic¹, Ivan Stanojevic², Dzihan Abazovic³, Mia Rakic⁴, Nikola Stankovic¹, Mirjana Djukic⁵, Sanja Milutinovic⁷, Srdjan Starcevic⁶,⁷, Gordana Supic²,⁷, Danilo Vojvodic²,⁷, Milena Jovic⁸, Dusan Maric⁹

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Abstract

**Background / Aim.** Recent studies indicate that adipokines have important role in bone physiology and pathology. Recent data indicate that adipokine leptin functions as a regulator of bone growth at multiple levels, systemically and locally. So far, it has been shown that leptin influence bone volume and bone mineral density in a population with metabolic and/or hormonal abnormality. Data concerning leptin values in non-obese children with fractures are scarce. **Methods.** 93 non-obese children with long bone fractures (LBF), 14 children with short bone fractures (SBF) and 19 healthy children. Leptin concentration was determined in 2 blood samples (0d and 21d) and analyzed according to gender, fracture type, fracture anatomical localization, fracture topography, callus formation and the healing outcome. **Results.** Children with LBF demonstrated significantly increased leptin comparing to control group (both 0d/21d). In control group girls had significantly more leptin than boys. Leptin value was significantly influenced by anatomical localization, since boys and girls with humerus fracture and girls with femur fracture had the highest average leptin concentration in initial sample. Boys with incomplete callus formation had the highest leptin concentration (both 0d/21d) significantly elevated comparing to control boy samples, boys samples with intermediary and well formed callus and also increased comparing to initial samples of girls with incomplete callus. Better callus formation in girls was associated with increment of leptin concentrations in second over initial sample. Girls with partially and satisfactory formed callus had significantly increased leptin concentration in second sample (21d) comparing to boys group. **Conclusion.** Leptin concentration was significantly increased (both samples) in children with LBF comparing to children with SBF and corresponding controls. Leptin concentration was highly influenced by gender. High leptin in boys or low leptin concentration in girls immeditately upon fracture could be used to identify groups of children with incomplete callus formation.

Abstrakt

**Uvod / Cilj.** Nedavne studije pokazuju da adipokini imaju važnu ulogu u fiziologiji i patologiji kostiju. Najnoviji podaci ukazuju da adipokin leptin funkcioniše kao regulator rasta kostiju sistemski i lokalno. Pokazano je da leptin utiče na volumen kostiju i mineralnu
gustinu kostiju u populaciji sa metaboličkom i ili hormonalnom abnormalnošću. Podaci o vrednostima leptina kod negožne dece sa frakturama su oskudni. **Metode.** 93 negožna deteta sa prelomima dugih kostiju (LBF), 14 deteta sa prelomima malim kostiju (SBF) i 19 zdravih deteta. Koncentracija leptina određena je u 2 uzorka krvi (0d i 21d) i analizirana prema polu, tipu frakture, lokalizaciji anatomske frakture, topografiji frakture, formiranju kalusa i ishodu zarastanja. **Rezultati.** Deca sa LBF imala su značajno povećan leptin u poređenju sa kontrolnom grupom (oba 0d / 21d). U kontrolnoj grupi devojčice su imale značajno više leptina od đeca. Na vrednost leptina značajno je uticala anatomska lokalizacija, jer su đeci i devojčice sa prelomom humerusa i devojčice sa prelomom femura imali najveću prosečnu koncentraciju leptina u početnom uzorku. Đeci sa nepotpuno formiranim kalusom imali su najveću koncentraciju leptina (oba 0d / 21d) značajno povišenim u odnosu na kontrolne uzorke đeca, uzorke đeca s intermedijarnim i dobro formiranim kalusom, a takođe povećani u odnosu na početne uzorke djevojčica s nepotpunim kalusom. Bolje formiranje kalusa kod devojčica je povezano sa povećanjem koncentracije leptina u drugom u odnosu na početni uzorak. Devojčice sa delimično i zadovoljavajuće formiranim kalusom imale su značajno povećanu koncentraciju leptina u drugom uzorku (21d) u odnosu na grupu đeca. **Zaključak.** Koncentracija leptina je značajno povećana (oba uzorka) kod dece sa LBF u poređenju sa dekom sa SBF i odgovarajućim kontrolama. Koncentracija leptina je zavisna od pola. Visok leptin kod đeca ili niska koncentracija leptina kod devojčica odmah nakon preloma može se koristiti za identifikaciju grupa dece sa nepotpunim formiranjem kalusa.

**Abstract**

**Background / Aim.** Recent studies indicate that adipokines have important role in bone physiology and pathology. Recent data indicate that adipokine leptin functions as a regulator of bone growth at multiple levels, systemically and locally. So far, it has been shown that leptin influence bone volume and bone mineral density in a population with metabolic and/or hormonal abnormality. Data concerning leptin values in non-obese children with fractures are scarce. **Methods.** 93 non-obese children with long bone fractures (LBF), 14 children with short bone fractures (SBF) and 19 healthy children. Leptin concentration was determined in 2 blood samples (0d and 21d) and analyzed according to gender, fracture type, fracture anatomical localization, fracture topography, callus formation and the healing outcome. **Results.** Children with LBF demonstrated
significantly increased leptin comparing to control group (both 0d/21d). In control group
girls had significantly more leptin than boys. Leptin value was significantly influenced by
anatomical localization, since boys and girls with humerus fracture and girls with femur
fracture had the highest average leptin concentration in initial sample. Boys with
incomplete callus formation had the highest leptin concentration (both 0d/21d) significantly
elevated comparing to control boy samples, boys samples with intermediary and well
formed callus and also increased comparing to initial samples of girls with incomplete
callus. Better callus formation in girls was associated with increment of leptin
concentrations in second over initial sample. Girls with partially and satisfactory formed
callus had significantly increased leptin concentration in second sample (21d) comparing to
boys group. Conclusion. Leptin concentration was significantly increased (both samples)
in children with LBF comparing to children with SBF and corresponding controls. Leptin
concentration was highly influenced by gender. High leptin in boys or low leptin
concentration in girls immediately upon fracture could be used to identify groups of
children with incomplete callus formation.

Introduction

Recovery of fractured bone represent unique biological phenomena in which healing
bone repairs itself through complex interaction with immune cells, blood, bone marow cells
and soft tissue cells. Finally, healing bone again gains previous functional mechanical
stability. According to classic understanding, there are three phases of this process, each
represented with specific mediators. Initial, inflammatory and early remodeling phase,
starting imediately after bone injury is characterized with increased secretion of IL-1, IL-6
and TNF-alpha (1,2). Reparative phase, that starts after few hours upon fracture is
conducted by local and systemic production of numerous growth and differentiation
factors, such as transforming growth factors (TGF), bone morphogenic proteins (BMP),
platelet derived growth factors (PDGF), fibroblast growth factor (FGF) and others (3,4).
Third, remodeling phase that starts with endochondral ossification is mediated with
metalloproteainases and angiogenic factors, like vascular endotelial growth factors (VEGF)
and angiopeotins (5).

Data from growing number of studies indicate that adipokines, mediators that are
discovered primarily as fat and energy regulators, are also involved in remodeling and
development processes in bone physiology. Adiponectin, leptin, resistin, visfatin and others are recognized as important regulators of bone metabolism (6). Beyond its effects on fat tissue, leptin modulate immune response and inflammation (7). Arguments demonstrating leptin influence on bone tissue came both from experimental and clinical data. Mesenchymal stem cells and osteoblasts express functional leptin receptors (8,9), and leptin ligation of specific receptor induced cell proliferation (10) comparably intensive as seen in response to IFG-I, major anabolic bone stimulator (11). Furthermore, it has been shown that leptin induce differentiation of mesenchymal cells to osteoblasts, increasing both mRNA and protein levels of osteocalcin, type I collagen, ALP and mineralization (12,13). Experimental leptin administration in vivo resulted in increase of bone mineral density and bone length in geneticaly manipulated ob/ob mice (14), significantly reduced bone fragility in male mice (11), and prevented bone lose in estrogen deficient ovariectomized rats (15). It seems that leptin acts directly on bone, because study where ovariectomized rats were treated with virus vector expressing leptin directly in the hypothalamus showed no changes in bone parameters, comparing to peripheral administration of leptin (16).

Data that were connecting leptin with bone physiology in human came mainly from studies of osteoporotic woman. These results are still controversial, indicating that there is no leptin influence on bone metabolism (17), or demonstrating association of leptin and bone mineral density (18,19). Newer results indicate that leptin valuable marker in osteoporosis (20), and that increase in serum leptin correlated with bone mineral density increase in postmenopausal woman with primary knee osteoarthritis (21).

Studies that determined leptin concentration in children were mainly focused at obese population and they demonstrated direct correlation between serum leptin value with body mass index and overwieght (22,23). Beside data from control children in those studies (24), we did not found any study that investigated adipokines influence on bone recovery after fracture in population of healthy children.
Methods

Participants

All children were admitted, diagnosed and treated at Department of Orthopedics and Joints/Bone Trauma, Institute for Health Protection of Mother and Child, Dr. Vukan Cupic, Belgrade Serbia. This study was approved by Ethics Committee of Institute for Health Protection of Mother and Child of Serbia (No 8/26, 13/10/2015). Parents of all investigated children were informed by the attending medical doctor and gave written consent for participation in a study.

Inclusion criteria

Non-obese children (Body Mass Index - BMI 15.0 – 24.0), boys and girls, aged from 4 to 18 years. Investigated group consisted of children with long bone fractures (LBF, n = 93), children with fingers fractures - short bone fractures (SBF, n = 14) and control group of children (Control, n = 19) (Table 1.). Control group consisted of children admitted to Clinic due to the trauma of extremities but without evidence of bone fracture.

Exclusion criteria

Obese children (BMI over 24.0), children with other injury beyond long bone fracture, children with malignant disease, systemic connective tissue diseases, metabolic diseases / disorders and children with congenital anomalies of the joint/bone were not included in study.

Clinical - radiological parameters

For each patient radiological records were made as a standard two X-ray projections (AP and profile), a total of 5 records at different time intervals (immediately before the orthopedic procedure, immediately after the intervention, control after 7 days, control after 21 days and after removing the cast immobilization). All fractures were classified according to type (open, close fracture), anatomical localisation (humerus, radius, radius + ulna, femur, tibia, tibia + fibula), topography of affected bone (fracture of proximal or distal segment, diaphysis fractures), the severity of bone injury (easy, heavy, complicated), callus formation (based on radiological analysis as <25% - incomplete, <50% - partially, >75% - satisfactory) and the healing outcome (unsatisfactory, incomplete, complete). According to data obtained from parents children were analysed for previous bone fractures, propensity and history of upper respiratory tract infections, allergy, previous nursery or school residence and feeding habits.
Study design

The study was designed as a cross-sectional investigation. From all investigated children first, initial blood sample ("0d"), 2 ml of venous blood from cubital vein was taken inside 1st hour upon admission. Where possible, second blood sample ("21"), 2 ml of venous blood from cubital vein was taken after 21 days of bone fracture. After separation of serum, samples were frozen to – 70 °C until testing.

Leptin determination

Concentration of leptin was determined with commercial flow cytometric test (LEGENDplex 13-plex Human Adipokine Panel) on a flow cytometer Beckman Coulter FC500.

Statistical analysis

Parameters of descriptive statistics were used to estimate central tendency of data (mean, median), and to analyze group variability (standard deviation, standard error, range, 95% interval of confidentiality). Analysis between more than two groups, groups according to fracture type (oblique, transverse, spiral), anatomical localization of fracture (humerus, radius, radius + ulna, femur, tibia, tibia + fibula) and between groups according to degree of callus formation (<25%, <50%, >75% of formed callus) was performed using oneway analysis of variance (ANOVA), with Bonferroni posttesting. Mann – Whitney test was used for all other comparison between two independent groups. The sensitivity and specificity of leptin determination was analyzed with receiver-operator characteristic (ROC) curves constructed on the basis of values detected in control boys and girls groups. All statistical analyses were done using the statistical package GraphPad Prism 5.01 (GraphPad Prism Software Inc. California, USA).

Results

Leptin concentration in children with long bone fractures vs children with small bone fractures and control healthy children

Average leptin concentration in samples of all investigated children with long bone fractures was insignificantly increased comparing to control group, in both time intervals (Table 2.). Same data were demonstrated in groups of boys and girls, both groups had increased leptin value comparing to their adequate peer. Both groups showed slight leptin increase in second sample. Boys with long bone fractures had insignificantly more leptin
comparing to boys with fractures of short bones (SBF). Leptin was significantly increased in samples of girls from control group comparing to boys from control group (Table 3.).

**Leptin concentration in children with long bone fractures according to the type of fracture**

Analysis of all investigated children showed no difference between groups with oblique, transverse or spiral long bone fracture (Table 2.). There was a slight increase of leptin concentration in samples after 3 weeks, irrespective of fracture type. Girls with transverse and spiral type of fracture had increased leptin concentration comparing to boys, while boys with oblique fractures demonstrated increased average concentration of leptin after 3 weeks.

**Anatomical localization of bone fracture and leptin concentration in children with long bone fractures**

Different localization of long bone fracture was associated with various leptin concentration in investigated children (Table 2.). Initially, the highest average leptin concentration was detected in samples of girls with femur fracture, and boys and girls with humerus fracture. After 21 days from fracture, the highest average leptin concentration was detected in children with fractures of radius and ulna, both in girls and boys. Analysis of boys samples only demonstrated less variation in leptin concentration, both between different anatomical localization or between samples and controls. Contrary, girls samples showed significant variation, between those that suffered femur and humerus fracture opposing to girls with radius fracture in initial samples (Table 3.).

**Callus formation and leptin concentration in children with long bone fractures**

Stratification of children according to a level of callus formation demonstrated increased average leptin concentration in all investigated samples comparing to the controls (Table 2.). Children with incompletely formed callus had the highest leptin value, both in initial and second sample, insignificantly elevated comparing to all to others and controls.

More detailed diversification of examinants in groups of boys and girls with long bone fractures according to quality of formed callus showed significant differences, both inside and between investigated groups (Table 3.). Boys with incomplete callus formation (<25%) had the highest leptin concentration both at the time of trauma (initial sample) and after 3 weeks. Average concentration of leptin in their initial samples was significantly elevated comparing to control boy samples, samples of boys with intermediary and well formed
callus and also increased comparing to initial samples of girls with incomplete callus (Table 2.) (Figure 1.). Average concentration of leptin after 3 weeks of boys with incomplete callus formation was still significantly increased comparing to second sample of boys with intermediary formed callus and comparing to control values in boys samples. According to callus quality, later incomplete callus formation was associated with the highest leptin values in boys samples and with the lowest leptin concentration in girls samples, at the time of bone trauma. Leptin in inital samples of boys and girls with intermediary and well formed callus did not differ significantly.

After 3 weeks, leptin concentration in boys with incompletely formed callus decreased significantly, but still was higher than the level measured in other groups. Intermediary and well formed callus was associated with insigificant leptin change. In girls samples after 3 weeks incomplete callus formation was followed with further decreament of leptin concentration. Contrary, better callus formation in girls was associated with increament of leptin concentrations in second over initial samples.

Girls with partially (<50%) and satisfactory (>75%) formed callus had significantly increased leptin concentration in second sample (21d) comparing to boys group.

Finally, leptin concentration was significantly increased in girls control samples comparing to boys control samples. Leptin was increased in all samples of boys with long bone fractures, both at the time of trauma and 3 weeks after over control values. Girls with incomplete callus formation had lower leptin comparing to their control values, while better callus formation was associated with increased leptin concentration.

**Leptin ROC curve analysis in children with long bone fractures**

Roc curve analysis comparing the diagnostic power of serum leptin concentration in children with long bone fractures demonstrated further difference between boys and girls (Figure 2.). More uniform distribution of leptin levels in boys resulted with better area under curve (AUC=0.777) comparing to girls group (AUC=0.600). Cut off leptin concentration for 100% specifity in boys was above 2.49 ng/ml, comparing to 3.00 ng/ml in girls. Leptin concentration above cut off in boys group was significantly associated with fractures localized esclusively in arm, and in those with incomplete callus formation. Interestingly, analysis showed that high leptin concentration were detected in majority of
Discussion

Leptin is a representative adipokine produced by differentiated adipocytes, crucial in regulation of energy balance and fat storage. Newer data demonstrated that leptin has almost the same relevance in numerous physiological processes, such as regulation of both female and male reproduction, hematopoiesis and angiogenesis, glucoregulation, wound healing, inflammation and osteogenesis (25). Furthermore, leptin has a major role in chronic inflammatory mechanism characteristic for obesity and atherosclerosis (26), sepsis (27), chronic viral infection (28) and cancer (29). Leptin exert its action by binding specific membrane receptor (Ob-R), expressed on different cell types, including immune cells (30). On the other side, microbial products as LPS, together with inflammatory cytokines stimulate intense leptin production, indicating complex mutuality between this hormone and inflammatory process. As well as immune cells, mesenchymal stem cells and osteoblasts express both types of leptin receptors on their membrane (8,9). Leptin functions as a regulator of bone growth at multiple levels, both systemically and locally. After penetrating blood brain barrier and binding to specific receptors, leptin indirectly regulate bone metabolism through sympathetic nervous system (31, 32, 33). There are still controverses about leptin and bone mineral density, at least regarding conditions without bone fractures. Several studies pointed out that there is no significant correlation between leptin levels and bone density (38,39,4,35,36,37,38,39,40). It must be emphasized that most frequently studied population was among post menopausal women, although from different ethnicities. Contrary to these data, Rhie et al reported positive correlation of serum leptin level with bone density (measured as spine and femoral bone mineral density) in group of pre pubertal obese girls comparing to age adjusted group, indicating positive role of leptin in bone metabolism (41).

In experimental model where rats were subjected both to trauma of brain and femoral fracture, two studies demonstrated leptin significance in process of healing bone (42,43). Callus formation was significantly associated both with increased serum leptin values and with abundant presence of leptin in various cells in fracture site, osteoblasts, fibroblasts and
mesenchymal cells. Serum concentration of leptin and local expression of leptin documented by immunohistochemical analysis peaked at 4 weeks after trauma.

There are also clinical data supporting leptin importance in bone homeostasis. Women with hypothalamic amenorrhea demonstrate low serum leptin concentration together with decreased estrogen, growth hormones and thyroid hormones. These hormonal disorder is associated with insufficient bone mass density, which is often a cause of bone fractures despite age (44,45).

Interestingly, supportive therapy with synthetic leptin preparate improves not only hormonal abnormalities but significantly amends bone density (44,45,46,47).

Increased serum leptin concentration immediately after bone trauma could be explained from several aspects. Bone fracture is accompanied with hypermetabolic response, characterized with mobilization of free fatty acids, which cause leptin rise by neuroendocrine mechanism (48,49,50,33). Early inflammatory response to acute bone trauma is mediated with inflammatory cytokine production, that also results in stimulation of further leptin secretion (51,52,). Another important source of postraumatic leptin rise is bone marrow, its adipose part (“medulla flava”), which abundantly release leptin at the edges of fractured bone (53). Beside these, complex neuroendocrine response after bone trauma represented in release of multiple cytokines and hormones, influences the production and levels of leptin.

Several studies reported contradictory finding of improved healing in patients with long bone fracture and concomitant trauma of brain, both at experimental and/or clinical level (54,55,56,57,58,59,60). Although far from clear understanding data from these studies indicate that both serum and cerebrospinal fluid of patients with concomitant brain trauma and long bone fractures have stimulatory effects on bone healing. It is assumed that this osteoinductive factors are secreted and / or released from injured brain. Based on these results, leptin is one among this factors.

Interesting data came from study of Wang et al. They investigated association between serum leptin concentration, bone density and healing of long bone fractures in two group of men, with fractures combined with spinal cord injury and group only with fractures (61). After 4 and 8 week after trauma, patients with spinal cord injury had significantly less formed callus, significantly reduced bone density and significantly increased leptin concentration comparing to fracture only group. Although our investigated population
consisted of children with no other trauma (it was inclusion criteria), some children demonstrated association of very high leptin concentration with unsatisfactory bone recovery. Namely, group of boys with insufficiently formed callus had the highest leptin concentration, both initially after bone fracture and in the samples after 3 weeks. Gender conditionality of high leptin concentrations seems to be important, because the leptin concentration was significantly decreased in girls with insufficient callus formation, in both their samples.

Studies from late 2000 demonstrated strong association leptin and gender, presented with increased serum leptin concentration in girls comparing to boys of same age (62,63,64,65,66). This gender based leptin domination was explained with higher rate of leptin secretion from adipose tissue in girls, genetically determined higher subcutaneous / visceral fat ratio and higher estrogen levels throughout puberty. Our data demonstrated clear difference in leptin concentration between boys and girls, as well as in control group of healthy children comparing to long bone fracture group. Girls generally have higher leptin serum concentration than boys, but this is not an absolute rule, at least in our study. Average leptin concentration was higher in boys with oblique type of fracture (21d), boys that had fracture of humerus (21d), radius (0d, 21d), combined radius and ulna (0d) and most strikingly in boys with incomplete callus formation (0d, 21d).

Theoretically, fracture of larger bones should be associated with more intensive systemic reaction and larger inner surface of fractured bone should induce increased liberation of leptin from frontiers of lesion (53). This is in compliance with our finding that boys and girls with humerus fracture and girls with femur fracture had the highest average leptin concentration in initial sample. Association of high serum leptin with incomplete callus formation is even more complex to explain and leaves space for speculation. In initial sample, association of high serum leptin with unsatisfactory bone recovery in boys could indicate its compensatory increase as a result of ongoing inflammatory process in incompletely formed callus. Contrary, leptin could be considered as a adipokine necessary for bone recovery in a group of girls with insufficiently formed callus could and extremely low leptin. Another possibility is that these boys and girls with incompletely formed callus had leptin abnormality before resulting fracture. This issue should be investigated in the population of children with poor recovery after fractures of long bones. Determination of leptin concentration after 21 day from fracture demonstrated far more variation. At that
time, beyond of processes in fractured bone, leptin concentration could be influenced by numerous factors, such as immobilization quality, discipline of the patient, sleep, feeding, or infection.

**Conclusion**

Children with long bone fractures demonstrated significant increase of leptin concentration in samples taken immediately upon bone trauma and three weeks after comparing to children with short bone fractures and corresponding controls. Leptin concentration is highly influenced by gender. High leptin in boys or low leptin concentration in girls immediately upon fracture could be used to identify groups of children with incomplete callus formation.

**Acknowledgment**

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Table 1

A. Demographic characteristics of investigated children. B. Number (%) of patients according to fracture properties.

A.  

<table>
<thead>
<tr>
<th></th>
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<th>girls</th>
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<td><strong>patients</strong></td>
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<tr>
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<td>9.3 ± 3.4</td>
<td>9.3 ± 3.7</td>
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<td>LBF</td>
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<tr>
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<td>16.6 ± 1.9</td>
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<tr>
<td><strong>patients</strong></td>
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<tr>
<td>age years</td>
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<td>SBF</td>
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<tr>
<td>weight kg (n = 14)</td>
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<td><strong>Controls</strong></td>
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<td>age years (n = 19)</td>
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</tbody>
</table>

B.  

<table>
<thead>
<tr>
<th>LBF patients</th>
<th>all</th>
<th>boys</th>
<th>girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>humerus</td>
<td>25 (27%)</td>
<td>16 (30%)</td>
<td>9 (23%)</td>
</tr>
<tr>
<td>anatomical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>radius</td>
<td>7 (7%)</td>
<td>4 (8%)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>localization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>radius + ulna</td>
<td>33 (35%)</td>
<td>18 (34%)</td>
<td>15 (37%)</td>
</tr>
<tr>
<td>of femur</td>
<td>10 (11%)</td>
<td>5 (9%)</td>
<td>5 (13%)</td>
</tr>
<tr>
<td>fracture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tibia</td>
<td>11 (12%)</td>
<td>6 (11%)</td>
<td>5 (13%)</td>
</tr>
<tr>
<td>tibia + fibula</td>
<td>7 (7%)</td>
<td>4 (8%)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>total</td>
<td>93 (100%)</td>
<td>53 (100%)</td>
<td>40 (100%)</td>
</tr>
<tr>
<td>type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oblique</td>
<td>21 (23%)</td>
<td>14 (27%)</td>
<td>7 (18%)</td>
</tr>
<tr>
<td>of transverse</td>
<td>58 (62%)</td>
<td>33 (62%)</td>
<td>25 (62%)</td>
</tr>
<tr>
<td>fracture</td>
<td>spiral</td>
<td>14 (15%)</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>total</td>
<td>93 (100%)</td>
<td>53 (100%)</td>
<td>40 (100%)</td>
</tr>
<tr>
<td>callus</td>
<td>&lt; 25%</td>
<td>17 (18%)</td>
<td>9 (17%)</td>
</tr>
<tr>
<td>formation</td>
<td>&lt; 50%</td>
<td>43 (46%)</td>
<td>24 (45%)</td>
</tr>
<tr>
<td>&gt; 75%</td>
<td>33 (36%)</td>
<td>20 (38%)</td>
<td>13 (32%)</td>
</tr>
<tr>
<td>total</td>
<td>93 (100%)</td>
<td>53 (100%)</td>
<td>40 (100%)</td>
</tr>
</tbody>
</table>

LBF – long bone fractures, SBF – small bone fractures

Table 2

Average leptin concentration in samples of children with long bone fractures and control children (x±SD, ng/ml). A. Initial sample. B. Sample taken at 21th day of fracture.

A.

<table>
<thead>
<tr>
<th></th>
<th>sample</th>
<th>all</th>
<th>boys</th>
<th>girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBF</td>
<td>0 day</td>
<td>2.46 ± 1.29</td>
<td>2.27 ± 1.22</td>
<td>2.59 ± 1.16</td>
</tr>
<tr>
<td>long bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oblique</td>
<td>0 day</td>
<td>2.85 ± 0.17</td>
<td>2.73 ± 0.19</td>
<td>3.01 ± 0.58</td>
</tr>
<tr>
<td>transverse</td>
<td>0 day</td>
<td>2.31 ± 0.11</td>
<td>2.24 ± 0.13</td>
<td>2.46 ± 0.84</td>
</tr>
<tr>
<td>spiral</td>
<td>0 day</td>
<td>2.14 ± 0.71</td>
<td>2.12 ± 0.76</td>
<td>2.17 ± 0.80</td>
</tr>
<tr>
<td>humerus</td>
<td>0 day</td>
<td>2.72 ± 1.61</td>
<td>2.72 ± 1.74</td>
<td>2.73 ± 0.71</td>
</tr>
<tr>
<td>radius</td>
<td>0 day</td>
<td>1.61 ± 1.12</td>
<td>2.10 ± 0.97</td>
<td>1.12 ± 0.14</td>
</tr>
<tr>
<td>radius + ulna</td>
<td>0 day</td>
<td>2.27 ± 1.10</td>
<td>2.34 ± 1.46</td>
<td>2.19 ± 0.75</td>
</tr>
<tr>
<td>femur</td>
<td>0 day</td>
<td>2.76 ± 1.41</td>
<td>1.98 ± 0.72</td>
<td>3.54 ± 1.78</td>
</tr>
<tr>
<td>tibia + fibula</td>
<td>0 day</td>
<td>1.70 ± 0.34</td>
<td>1.88 ± 0.51</td>
<td>1.52 ± 0.15</td>
</tr>
<tr>
<td>callus</td>
<td>&lt;25%</td>
<td>0 day</td>
<td>3.23 ± 2.05</td>
<td>4.47 ± 2.05</td>
</tr>
<tr>
<td></td>
<td>&lt;50%</td>
<td>0 day</td>
<td>2.42 ± 1.53</td>
<td>2.27 ± 1.29</td>
</tr>
<tr>
<td></td>
<td>&gt;75%</td>
<td>0 day</td>
<td>2.31 ± 0.75</td>
<td>2.20 ± 0.77</td>
</tr>
<tr>
<td>SBF</td>
<td>0 day</td>
<td>2.16 ± 0.41</td>
<td>1.91 ± 0.50</td>
<td>2.47 ± 0.29</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>2.02 ± 0.22</td>
<td>1.67 ± 0.49</td>
<td>2.38 ± 0.41</td>
</tr>
</tbody>
</table>

B.
<table>
<thead>
<tr>
<th>marker</th>
<th>group</th>
<th>sample</th>
<th>callus vs</th>
<th>group</th>
<th>sample</th>
<th>callus vs</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>boys</td>
<td>0d</td>
<td>&lt;25%</td>
<td>boys</td>
<td>0d</td>
<td>&lt;50%</td>
<td>**</td>
</tr>
<tr>
<td>d</td>
<td>boys</td>
<td>0d</td>
<td>&lt;25%</td>
<td>boys</td>
<td>0d</td>
<td>&gt;75%</td>
<td>**</td>
</tr>
<tr>
<td>e</td>
<td>boys</td>
<td>0d</td>
<td>&lt;25%</td>
<td>boys</td>
<td>Control</td>
<td>/</td>
<td>***</td>
</tr>
<tr>
<td>f</td>
<td>boys</td>
<td>0d</td>
<td>&lt;25%</td>
<td>girls</td>
<td>0d</td>
<td>&lt;25%</td>
<td>*</td>
</tr>
<tr>
<td>g</td>
<td>boys</td>
<td>21d</td>
<td>&lt;25%</td>
<td>boys</td>
<td>21d</td>
<td>&lt;50%</td>
<td>**</td>
</tr>
<tr>
<td>h</td>
<td>boys</td>
<td>21d</td>
<td>&lt;25%</td>
<td>boys</td>
<td>Control</td>
<td>/</td>
<td>**</td>
</tr>
<tr>
<td>i</td>
<td>girls</td>
<td>21d</td>
<td>&lt;25%</td>
<td>girls</td>
<td>21d</td>
<td>&gt;75%</td>
<td>*</td>
</tr>
<tr>
<td>j</td>
<td>boys</td>
<td>21d</td>
<td>&lt;50%</td>
<td>girls</td>
<td>21d</td>
<td>&lt;50%</td>
<td>*</td>
</tr>
</tbody>
</table>

LBF – Small Bone Fracture. SBF – Small Bone Fracture.

Superscript letters mark pairs of groups that differ significantly (Mann Whitney test, p< 0.05)

Table 3

Statistical analysis of difference in leptin concentration according to level of callus formation inside groups of boys and girls, and between groups of boys and girls.
| k | boys  | 21d  | >75% | vs | girls | 21d  | >75% | * |

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