Leptin reduces in vitro cementoblast mineralization and survival as well as induces PGE2 release by ERK1/2 commitment

G. Ruiz-Heiland1 · J. W. Yong1 · J. von Bremen1 · S. Ruf1

Received: 13 January 2020 / Accepted: 5 August 2020 / Published online: 20 August 2020
© The Author(s) 2020

Abstract
Objectives Juvenile obesity is a complex clinical condition that is present more and more frequently in the daily orthodontic practice. Over-weighted patients have an impaired bone metabolism, due in part to their increased levels of circulating adipokines. Particularly, leptin has been reported to play a key role in bone physiology. Leptin is ubiquitously present in the body, including blood, saliva, and crevicular fluid. If, and to what extent, it could influence the reaction of cementoblasts during orthodontic-induced forces is yet unknown.

Material and methods OCCM-30 cementoblasts were cultivated under compressive forces using different concentrations of leptin. The expression of ObR, Runx-2, Osteocalcin, Rank-L, Sost, Caspase 3, 8, and 9 were analyzed by RT-PCR. Western blots were employed for protein analysis. The ERK1/2 antagonist FR180204 (Calbiochem) was used and cPLA2 activation, PGE2, and cytochrome C release were further evaluated.

Results In vitro, when compressive forces are applied, leptin promotes ERK1/2 phosphorylation, as well as upregulates PGE2 and caspase 3 and caspase 9 on OCCM cells. Blockade of ERK1/2 impairs leptin-induced PGE2 secretion and reduced caspase 3 and caspase 9 expression.

Conclusions Leptin influences the physiological effect of compressive forces on cementoblasts, exerting in vitro a pro-inflammatory and pro-apoptotic effect.

Clinical relevance Our findings indicate that leptin exacerbates the physiological effect of compressive forces on cementoblasts promoting the release of PGE2 and increases the rate of cell apoptosis, and thus, increased levels of leptin may influence the inflammatory response during orthodontically induced tooth movement.

Keywords Cementoblasts · ERK1/2 · Leptin

Introduction
In all age groups, the prevalence of obesity has been increasing worldwide over the last decades. It is well known that obesity is an important risk factor for many chronic diseases (WHO) [1]. Due to the excess of adipose tissue, the number and volume of adipocytes are increased. Adipocytes are a highly active endocrine organ which produces and releases a variety of adipokines, in particular, increased amounts of proinflammatory adipokines, such as leptin, and decreased amounts of anti-inflammatory adipokines, such as adiponectin. This imbalance between pro- and anti-inflammatory adipokines results in a subclinical chronic systemic inflammation status of obese patients [2–4].

Being the first adipokine discovered, leptin has attracted special research interest [5]. In general medicine, higher leptin levels have been associated with several chronic diseases, such as type 2 diabetes, cardiovascular diseases, cancer, and arthritis (WHO) [1]. In dentistry, there has been a special focus on the effects of leptin on the periodontal structures. There is some evidence that higher leptin levels do not only support the initiation and progression of periodontitis but are also associated with compromised healing after periodontal therapy [6]. Thus, nowadays, obesity is considered a major risk factor for periodontal disease [7]. Considering the fact that orthodontic tooth movement requires healthy periodontal structures, it must be questioned whether or not orthodontic therapy of obese individuals possibly bears more risks than treatment of
normal weight patients due to the mild chronic systemic inflammation status.

Furthermore, it has been shown that leptin directly regulates osteoclast formation through a reduced production of RANK and RANK-ligand as well as an increase of osteoprotegerin, which results in reduced osteoclastogenesis [8, 9]. This impaired bone metabolism could also influence orthodontic tooth movement. During orthodontic therapy, the root of the tooth is translated within the alveolar socket resulting in a compressed periodontal ligament (PDL) on the one and stretched periodontal fibers on the opposing side. In a healthy surrounding, this results in bone resorption on the compressed and bone apposition on the stretched side. If, however, higher leptin levels cause more inflammation and less periodontal healing in addition to a compromised bone resorption due to less active osteoclasts, the rate of tooth movement and/or possible side effects of orthodontic tooth movement, such as root resorption, might be affected.

In the biphasic theory of orthodontic tooth movement, the osteoclast activity becomes of even higher importance [10]. Here, it is assumed that orthodontic tooth movement occurs in two phases: first, the osteoclast-driven catabolic phase where bone resorption takes place; and second, the anabolic phase in which bone formation takes place [10]. The first phase, initiated by cytokines, is required for the second phase to begin, and the second phase is regulated by osteoclast-osteoblast interactions. This implies that both phases are dependent on the presence of active osteoclasts and could thus be affected by increased leptin levels.

In addition to an altered reaction of bone and periodontal fibers due to higher leptin levels in obese patients, the cementoblast reaction needs to be considered. So far, this part of the periodontium has received little attention in leptin research. Although it is well accepted that the cementoblasts lining the root surface play an important role in preventing root resorption during orthodontic tooth movement, the causes and predisposing factors for orthodontically induced root resorption are not fully understood [11]. Several studies have confirmed the destructive effect of leptin on periodontal fibroblasts [6, 12–14]. Here, it has been shown that higher leptin levels are associated with an upregulation of somatostatin receptor 2, indicating a potential mechanism how adipokines could contribute to periodontal destruction through their proinflammatory characteristics [14]. Furthermore, the same research group elucidated how different regenerative periodontal ligament cell functions were negatively influenced by the presence of leptin [12]. Supplementation of the available research on fibroblasts, the present study now aimed to analyze the effect of leptin on cementoblasts in the presence or absence of compressive forces as induced during orthodontic tooth movement.

Material and methods

Cell culture

The mouse cementoblast cell line OCCM-30 was kindly provided from Prof. M. Somerman (NIH, NIDCR, Bethesda, Maryland). Cells were obtained from the root surface of the first mandibular molars of osteocalcin-transgenic (OC-Tag) mice. These mice contain the simian virus 40 (SV40) large T-antigen under the control of the osteocalcin promoter, and thus, only cells that express OC also express Tag in accordance with an immortalized cell line [15].

Cell passages 3–5 were cultivated until confluence using α-MEM (11095-080, Gibco) supplemented with 10% FCS (10270-106, Gibco) and 1% penicillin/streptomycin (15140-122, Gibco). To promote calcification, 50 μg/ml ascorbic acid (6288.1, Roth) and 10 mM β-glycerophosphate (#35675, Calbiochem) were added to cell media using different concentrations of mouse leptin (CYT-351, Prospec). Cementoblasts were cultivated under compressive forces as described by Proff et al. [16]. For this purpose, 33-mm diameter glass cylinders with pulled surfaces were fabricated by Reichmann Feinoptik, Brokdorf, Germany. Cylinders of different volumes were fabricated in order to reach pressure forces of 1.2, 2.4, and 4.8 g/cm², respectively.

The cells were seeded into 6-well plates at a density of 3 × 10⁴ cells/well until confluence and covered with the glass cylinders afterwards. Cells were cultivated under compression over different time periods.

Calcified matrix assessment

Cells, plated on 6 wells/plates, were washed with phosphate buffered saline solution, fixed with 70% ethanol, and further stained during 5 min with Alizarin-Red (A5533, Sigma-Aldrich), pH 4.2. Calcified matrix formation was assessed by inverted phase contrast microscopy (Leica) using the LASV4.8 software (Leica). Calcified nodules were surrounded; thereafter, the covered area was measured as previously described [17]. A total of eight different microscopic areas per well and a minimum of 6 wells per group were evaluated.

Alkaline phosphatase enzymatic activity

Alkaline phosphatase enzymatic activity was measured according to an established protocol. Briefly, cells were collected in 0.2% Triton-X and homogenized using an ice-cold Dounce grinder. The protein concentration was measured using Pierce™ BCA protein Assay Kit (23225, Thermo Scientific). Alkaline phosphatase enzymatic activity was measured using p-Nitrophenylphosphat solution (P7998, Sigma) pH 10.5 as substrate and 4-nitrophenol solution (N7660,
Each experiment was repeated at least three times. 

**Quantitative mRNA expression assay**

RNA was isolated employing RNeasy Mini Kit (Cat. N° 74104, Qiagen) and treated with RNAse-Free DNase Set (Cat. N° 79254, Qiagen). One-microgram RNA was transcribed into cDNA using iScript™ CDNA Synthesis Kit (Cat. N° 170-8891, BioRad) according to the manufacturer protocol.

TaqMan real-time polymerase assays were performed utilizing TaqMan Universal PCR Master Mix (4304437, Applied Biosystem) and the following TaqMan Gene Expression assays: Bcl-2 (Mm00477631_m1, Applied Biosystems); Leptin Receptor (Mm00440181_m1, Applied Biosystems); Caspase-9 (Mm00516563_m1, Applied Biosystems); Rank-L (Mm00441906_m1, Applied Biosystems); Runx-2 (Mm00501584_m, Applied Biosystems); Caspase-8 (Mm00802247_m1, Applied Biosystems); Cox-2 (Mm03294838_g1); Pges (Mm00452105_m1); Sost (Mm00470479_m1). Target gene expressions were normalized to the expression of Runx-2 (Mm00452105_m1), Sost (Mm00470479_m1). 

**Western blot**

Cells were collected in RIPA buffer Pierce™ (89901, Thermo Scientific) supplemented with 3% phosphatase and protease inhibitors (78442, Thermo Scientific). Protein concentration was measured using Pierce™ BCA Protein Assay Kit (23225, Thermo Scientific) on a Nanorop 2000 Spectrophotometer (Thermo Scientific) on a Nanodrop 2000 Spectrophotometer. Ten-microgram protein aliquots were loaded on a 12% SDS-PAGE gel, and western blot was loaded on a 12% SDS-PAGE gel, and western blot was probed with monoclonal antibodies and visualized using chemiluminescence. Western blot images were analyzed with the ImageJ software.

Membranes were blocked with 5% non-fat milk (T145.1, Sigma) diluted in 0.2% Triton-X as a standard. Plates were read at 405 nm (xMark™, Microplate Absorbance Spectrophotometer, 1681150 BioRad). A total of 6 wells per group were evaluated.

**Cytochrome C release test**

Cells were washed in cold PBS, collected in 100-μl Cytosol Extraction Buffer containing DTT and protease inhibitors (ab65311, Abcam) and immediately homogenized using Branson Sonifier 150 on ice. Protein concentration was measured employing Pierce™ BCA protein Assay Kit (23225, Thermo Scientific) on a Nanodrop 2000 Spectrophotometer (Thermo Scientific). Ten-microgram protein aliquots were loaded on a 12% SDS-PAGE gel, and western blot was probed with antibodies to the following proteins: leptin receptors (ObR) (ab65311, Abcam); ERK1/2 (MBS8241746, BIOZOL), dilution 1:1000; phospho-ERK1/2 (44-680G, Thermo-Fisher) dilution 1:1000; JNK (MBS840351, BIOZOL) dilution 1:500; phospho-JNK (07-175, Thermo-Fisher) dilution 1:500; P38 MAPK (9212, Cell Signaling Technology) dilution 1:1000; phosPho-P38 MAPK alpha (MA5-15182, Thermo-Fisher) dilution 1:500; STAT1 (AHP2527, Bio-Rad) dilution 1:1000; phospho-STAT1 Tyr701 (05-1064, Thermo-Fisher) dilution 1:1000; phospho-STAT1 S727 (ab109461, Abcam) dilution 1:1000; STAT3 (PA1-86605, Thermo-Fisher) dilution 1:1000; phospho-STAT3 Ser727 (OPA1-03007, Thermo-Fisher) dilution 1:500; Cytochrome C (ab65311, Abcam) dilution 1:1000; cPLA2α (orb100010, BIOZOL) dilution 1:1000; cPLA2β (ab198898, Abcam) dilution 1:1000; SHP2 (PA5-27312, Thermo Fisher) dilution 1:1000; COX 2 (ab62331, Thermo-Fisher) dilution 1:1000. As loading control, β-ACTIN (ab8227, Abcam) dilution 1:2000 was employed. The secondary antibodies: Polyclonal Goat Anti-Rabbit (P0448, Dako); Rabbit Anti-Goat (P0160, Dako); and Polyclonal Goat Anti-Mouse (P0447, Dako) Immunoglobulins/HRP at a dilution 1:2000, were used. The membranes were developed utilizing Amersham ECL Western Blotting Detection Reagents (9838243, GE Healthcare) and detected with Amersham Hyperfilm ECL (28906836, GE Healthcare) on OPTIMAX X-Ray Film Processor (11701-9806-3716, PROTEC GmbH).
performed using the following manufacturer instructions. After membrane develop, the films were scanned (MC332, OKI), and images were analyzed using the Image J 1.42q (National Institutes of Health, USA). Briefly, images were converted to grayscale 8 bit. Around the first lane, a rectangle was drawn using the rectangular selection tool. This first line, corresponding to the control group, served as a reference. Using the function Analyze>Gels>Plots Lanes, the intensity of every other lane in the image was analyzed. Results were expressed as percentage of the total size of all of highlighted peaks. Values were pasted into Excel for further analysis.

**Elisa test**

Prostaglandin E2 (PGE2) release was measured on cell supernatants using a highly sensitive PGE2 ELISA Kit (ADI-900-001, Enzo-Lifesciences). Plates were read at 405 nm and results were analyzed with xMark™ Microplate Absorbance Spectrophotometer (1681150, Bio-Rad) software.

**Statistical analysis**

The results were analyzed using ANOVA or Mann-Whitney U tests. A p value ≤ 0.05 was considered significant. For data distribution, the Shapiro-Wilk test and the Kolmogorov-Smirnov test were used. In addition, QQ plots were performed. Statistical analysis was carried out using the GraphPad Prism 8 software.

**Results**

**Leptin receptors are expressed on cementoblasts and leptin reduces cell mineralization**

First, we performed western blot analysis of OCCM cells to determine if and which isoforms of Ob receptors are present in the cells. The results showed that the long isoform α as well as the short isoform β of leptin receptors is expressed at 130 KDa and 100 KDa, respectively (Fig. 1a). Furthermore, we aimed to elucidate if compression has any influence on the expression of leptin receptors at mRNA level. Therefore, we cultivated cells under compressive forces of 1.2 g/cm², 2.4 g/cm², and 4.8 g/cm² and collected the cells for RNA isolation after 30, 60, 120, 240, and 480 min: Compression did not significantly alter ObR expression at mRNA level at any of the timepoints evaluated (Fig. 1c and d).

**Leptin upregulates COX2 expression as well as STAT1, STAT3, and ERK1/2 phosphorylation irrespective of compression**

Taking into consideration previous data on signals activated by leptin, we stimulated OCCM cementoblasts with leptin and performed western blot analysis at different timepoints (Fig. 2). Cells stimulated with a saturation dose of leptin (500 ng/ml) show activation of COX2, 10 min after adipokine addition. In the same manner, cells show upregulation of STAT3 phosphorylation whereas STAT1 phosphorylation occurs after 5 min. A decrease on phosphorylation levels occurs 2 h after stimulation.

A strong phosphorylation of ERK1/2 occurs again 10 min after leptin addition whereas no upregulation in the levels of P38 or JNK was observed.

**Enhanced expression of Rank-L, Cox2, Pges, and Caspases mRNA expressions by compressive forces after leptin stimulation**

To further elucidate the findings that leptin impairs mineralization, we performed mRNA expression analysis of cells cultivated with and without compression respectively in the presence or absence of leptin, and we analyzed the expression of key molecules involved in cementoblast proliferation, mineralization, and resorption as well as apoptosis (Fig. 3). Cells under compression express increased levels of Rank-L irrespective of leptin whereas no significant differences in Runx-2 levels were observed among groups. The levels of Sclerostin mRNA did not vary after compression or leptin stimulation whereas the stimulation with leptin drastically increased the expression of Cox-2 and Pge, levels increased to 2.46 ± 0.16-folds. It should be noted that there was hardly any difference between cells stimulated with leptin under compression forces or without compression (p > 0.05) both for Cox-2 and Pge.

Interestingly, we observed upregulation of Caspase-3 and Caspase-9 levels by leptin whereas the levels of Caspase-8 where not altered after stimulation. On the contrary, the mRNA expression Bel-2 remained almost identical among groups.

**Rescue from inflammatory and apoptotic molecules induced by leptin after ERK1/2 blockade**

In order to analyze if ERK1/2 plays a central role in cell physiology in response to leptin, we performed a pharmacological inhibition of ERK1/2 using PR180204 (Calbiochem-
Merck) and analyzed the cytosolic activity of PLA2 during 2 h: The addition of leptin into cell culture media immediately upregulates cPLA2 activity. After 15 min, the groups stimulated with leptin showed a cPLA2 activity of 6.5 ± 2.65 nmol/min/ml. When compression was applied, the cPLA2 levels increased to 8.2 ± 1.15 nmol/min/ml. After

Fig. 1 Leptin receptors are present in OCCM-30 mouse cementoblasts. a The short isoform α as well as the long isoform β of leptin receptors were detected by WB at 100 kDa and 130 KDa, respectively (Fig. 1a). The application of compressive forces of 1.2 g/cm², 2.4 g/cm², and 4.8 g/cm² did not significantly affect Ob receptor expression at mRNA level (Fig. 1b). Leptin added to cell culture reduced mineralized matrix production (**p < 0.001) (n = 6). Pictures show alizarin red staining of OCCM cells stimulated during 21 days (Fig. 1c). Leptin reduced alkaline phosphatase enzymatic activity in a dose-dependent manner (Fig. 1d). Each experiment was repeated three times. Bars indicate mean ± SD and stars indicate statistical significance.
120 min, these levels continue to increase up to 60.87 ± 6.51 and 72.2 ± 3.83 nmol/min/ml, respectively. The blockade of ERK1/2 significantly reduces cPLA2 activity levels to 16.54 ± 7 nmol/min/ml (\( p = 0.029 \)) and 22.5 ± 6.71 nmol/min/ml (\( p = 0.011 \)). Meanwhile, the cPLA2 levels were undetectable in the control group at almost all timepoints evaluated, reaching a peak after 120 min of 5.78 ± 4.8 nmol/min/ml (Fig. 4a). A total of 2 samples per timepoint (n = 2) with 3 measurement per sample were performed resulting in 6 observations per timepoint. Data distribution was analyzed using the Kolmogorov-Smirnov test and visually by normal QQ plots. The experiment was repeated three times based on different samples.

Because it is known that phospholipase A2 drives the regulation of PGE2, we hypothesized that cementoblasts can release PGE2 in response to leptin. As expected, 6 h after leptin addition to cell culture, the PGE2 levels on cell supernatants started to increase to 130.63 ± 2.65 pg/ml and reached 1294 ± 68.51 pg/ml after 24 h. The inhibition of ERK1/2 partially reduced PGE2 release to 906 ± 70 pg/ml (\( p = 0.030 \)) whereas the level in the control group was 463.31 ± 7.48 pg/ml. When compression was applied, the PGE2 level reached 1521.55 ± 93.83 pg/ml after 24 h and 1025.3 ± 56.71 pg/ml with the addition of the ERK1/2 inhibitor (\( p = 0.023 \)). Meanwhile, cells compressed for 24 h under forces of 2.4 g/cm² released PGE2 reaching a peak of 1341.83 ± 73.01 pg/ml and similar levels after ERK1/2 blockade (1318.8 ± 10.47 pg/ml), suggesting that compression forces can induce PGE2 release on OCCM cells independently of ERK1/2 (Fig. 4b). A total of 2 samples per timepoint (n = 2) with 3 measurement per sample were performed resulting in a total of 6 observations per timepoint. Data distribution was analyzed using the Kolmogorov-Smirnov test and visually by normal QQ plots. The experiment was repeated three times based on different samples.

To determine the influence of leptin as inductor of apoptosis on cementoblasts, we analyzed the release of cytochrome C from mitochondria into cytosol by immunoblotting. Four hours after leptin stimulation, OCCM cells show a strong upregulation of cytochrome C release in cells stimulated with leptin and in the group of cells stimulated with leptin and cultivated under compressive forces of 2.4 g/cm². On cells cultivated under compression in the absence of leptin, the release of cytochrome C was remarkably low. The blockade of ERK1/2 impaired its release (Fig. 5a and b).

**Discussion**

In the present study, we have shown that cementoblasts can be direct targets of leptin because they express ObR functional receptors. In vitro, leptin enhances the inflammatory response to compressive forces, promoting the upregulation of PGE2, COX2, and RANK-L. We also observed that this adipokine induces cementoblast apoptosis via cPLA2 and cytochrome C release. Leptin strongly activates ERK1/2 and neutralization of ERK1/2 impairs the release of PGE2 as well as cell apoptosis.

To our knowledge, this is the first study to demonstrate that leptin can directly interact with cementoblasts and influence...
Clinical studies have described that the levels of leptin present in crevicular fluid drastically vary during orthodontically induced tooth movement, suggesting that leptin-leptin receptor interactions could play a biological role on this process [18, 19]. Immunohistological analysis performed on primates has shown that leptin receptors are widely expressed in the junctional epithelium and in mineralizing areas of the periodontal ligament [20], as well as it has been demonstrated that the gingiva can directly release soluble leptin receptors into crevicular fluid [21]. Kapur et al. (2010) indicated that leptin receptors are negative modulators of bone mechanosensitivity and that genetic variation in LEPR signaling causes a poor osteogenic response to loading force in mice [22].

Fig. 3 Graphics show the mRNA expression of cementoblasts exposed to compressive forces (2.4 g/cm²), with and without co-stimulation with 50 ng/ml leptin (n = 3). After 30 min, leptin significantly upregulated Cox-2; PGE₃; Caspase-3; and Caspase-9 expression. The application of compression upregulates Rank-L expression irrespective of leptin. Stars indicate statistical significance *p < 0.05 and **p < 0.01. Bars show mean ± SD. Data is normalized to 1.
Various studies indicate that leptin is an important pro-inflammatory mediator in different tissues of the dentoalveolar system: High levels of this adipokine were detected in infected dental pulps and in periapical granulomas [23], and it is well known that increased levels of leptin in saliva and in crevicular fluids, common in individuals with a high white fat mass, elevate
the risk for periodontal disease development [24–26]. In vitro, it has been demonstrated that upregulated levels of leptin promote cytokine release in periodontal ligament cells [24]. Also, the regenerative capacity of cells is impaired in the presence of high levels of leptin due to the downregulation of collagen 1 expression [6].

Regarding the subject that concerns the present study, there is no evidence in vivo that cementocytes play a function in tissue homeostasis, and it has been pointed out that the adjacent periodontal ligament directly influences the physiology of the underlying cement [27, 28]. Nevertheless, in vitro studies indicated that, when cementoblasts are exposed to compressive as well as tensile forces, they can release inflammatory and chemotactic molecules as RANKL and PGE2 [29, 30]. In the present study, we observed that the mRNA expression of Rank-L, Cox-2, and Pges as well as the expression of Caspase-3 and Caspase-9 was strongly upregulated by leptin and that this effect was exacerbated when compressive forces were applied. This data let us suppose that increased levels of leptin could be a potential risk for cementoclast activation, especially after the application of compressive forces during orthodontics. However, more clinical evidence, as well as additionally research using primary human cementoblasts, is necessary to further prove the translatability of the results. In the present study, mRNA analysis was performed using β-Actin as housekeeping gene. Prior to RT-PCR analysis, the OCCM-30 cells were tested for their expression of β-Actin and GAPDH, showing a stable and strong expression of both genes with similar results as in primary mouse osteoblasts. However, considering that cementoblasts are part of the conglomerate of dental and periodontal tissues, for future studies, it could be interesting to test the gene expression of PPIB and YWHAZ, as these genes have been shown to be the more stably expressed genes in these tissues [31].

Several studies have described increased levels of circulating leptin in obese individuals [32, 33]. Gröschl et al. 2001 have established a positive correlation between plasma and salivary leptin levels [34]. For obese children, a fourfold increase in salivary leptin levels in comparison with normal weight children has been described (40.4 ng/ml ± 28.8 vs. 9.58 ± 3.1) [35]. During orthodontic tooth movement, the salivary leptin levels in obese individuals may even increase to 89.58 ± 45.4 ng/ml, 1 h after orthodontic forces application, almost doubling the levels measured in lean individuals [36]. At the same time, the rate of orthodontically induced tooth movement was decreased in overweight individuals [36].

Previous studies indicate that leptin can induce apoptosis in osteoblast-lineage human bone marrow (hBMSC) cells via the ERK/cPLA2/cytochrome C pathway (Kim, Hong et al. 2003). We found that leptin can interact with cementoblasts in a similar way (Scheme 1), it strongly induces ERK1/2 phosphorylation, even at lower concentrations that mimic biological levels of leptin in saliva (20 to 50 ng/ml) [35, 37].

In accordance with the data shown by Kim el al. (2002) in their experiment using hBMSC, we did not observe phosphorylation of P38 or JNK induced by leptin on cementoblasts [38]. However, we observed that leptin strongly induces STAT3 as well as STAT1 phosphorylation on OCCM-30 cells.

Furthermore, it was observed that exogenous leptin added to the cells promotes PGE2 release as well as cell apoptosis. Both effects are probably regulated by ERK1/2 commitment, as the pharmacological blockade of ERK1/2 seems to have a protective effect on cementoblasts against apoptosis reducing cPLA2 and the release of cytochrome C from the mitochondria. At the same time, the secretion of PGE2 was efficiently counteracted by ERK1/2 blockade. Depending upon the cell type or stimulus, activated ERK1/2 catalyzes several cytoplasmatic and nuclear substrates including regulatory

Scheme 1  Scheme showing the proposed molecular mechanism induced by leptin on OCCM cells
molecules and transcription factors that regulate several cell processes including differentiation, migration, adhesion, survival, inflammation, or even cell apoptosis [39–41]. Hoshi et al. (2014) showed that compressive forces induce osteocyte apoptosis through the ERK1/2 pathway [42]. Another study indicated that ERK1/2 was also activated on MC3T3 osteoblasts after peroxide-induced cell death upstreaming the mitochondria-dependent pathway to initiate the apoptotic signal [43]. Activation of ERK1/2 has been described to promote apoptosis throughout caspase 3 on renal epithelial cells [44]. In addition, animal studies demonstrated that ERK1/2 is activated after ischemic and septic renal injury, proposing that this molecule can activate STAT3 [45]. In vitro experiments using liver cancer cells underline that STAT3 regulates cell survival and that inhibition of this molecule blocks the anti-apoptotic activity of IL-6 on these cells [46]. Interestingly, Kim et al. (2002) showed that STAT1 but not STAT3 was activated by leptin on hBMSC and that STAT1 can directly interact with MEK to induce apoptosis [38]. Other studies using human osteoblasts have shown that phosphorylated STAT1, induced by dexamethasone, decreases the expression of BCL2 and increases in cytochrome C release as well as activates caspase 9 and caspase 3 [47]. In vivo, this mechanism has been demonstrated to be responsible for the progression of steroid-induced avascular necrosis of femoral heads on rats [47]. Other studies using transgenic animals demonstrated that STAT1 deletion can rescue from FGF-induced chondrocyte apoptosis [48].

Our results indicate that the release of PGE2 induced by leptin was partially counteracted by ERK1/2 inhibition. On periodontal cells, stimulation with IL-1 upregulates COX2 that further drives the release of PGE2, and that, its expression can be counteracted by ERK inhibition [49]. Upregulated PGE2 further promotes the release of RANKL and the TRAP⁺ cell formation [49, 50]. Other in vitro studies indicate that leptin alone or in the presence of IL-1 induces COX2 and PGE2 by mitogen-activated protein kinase (MAPK) pathway activation in osteoarthritis cartilage [51]. The COX2-PGE2 augmentation induced by long-term leptin administration has been described to exert a pro-apoptotic effect on renal tubular cells dose-dependently via caspase 3 commitment [52]. One study performed using OCCM cementoblasts revealed that cells exposed to compressive forces can strongly release PGE2 and that this effect can be effectively counteracted using a COX2 inhibitor. In addition, it was observed that high levels of exogenous PGE2 added to OCCM cultures completely abolish mineralization [29]. When we performed the analysis of PGE2 release after the application of compressive forces in the presence or absence of leptin, we observed that the pre-treatment of cells with an ERK1/2 antagonist can only partially counteract the release of PGE2 on cells exposed to compressive forces, suggesting that additional factors, as for example, oxygen reduction during compression, could play a role. Kaidi et al. (2006) have demonstrated that hypoxia-inducible factor (HIF-1) directly binds the COX2 promoter in carcinoma cell lines causing PGE2 upregulation. This mechanism facilitates the adaptation to cellular stress imposed by hypoxia [53].

In summary, we conclude that in vitro leptin exerts a deleterious effect on cementoblasts, increasing the release of PGE2 as well as the rate of apoptosis, particularly in cells exposed to compression. The blockade of ERK1/2 partially reverses these processes.

Acknowledgments We sincerely thank Prof. J. Deschner and Dr. M. Nokhbehsaim, Department of Periodontology, University of Bonn, Germany, to facilitate the sending of OCCM cells and Dr. P. Roemer, Department of Orthodontics, University of Regensburg, Germany, for his help in the establishment of the cell culture compression method in our laboratory.

Funding information Open Access funding provided by Projekt DEAL.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. World Health Organisation (WHO). https://www.who.int/topics/obesity/en/. Accessed 14.05.2019
2. Adamczak M,Wiecek A (2013) The adipose tissue as an endocrine organ. Semin Nephrol 33:2–13. https://doi.org/10.1016/j.semnephrol.2012.12.008
3. Krysik R,Handzik-Orlik G,Opokien B (2012) The role of adipokines in connective tissue diseases. Eur J Nutr 51:513–528. https://doi.org/10.1007/s00394-012-0370-0
4. Raucci R,Rusolo F,Sharma A,Colonna G, Castello G, Costamini S (2013) Functional and structural features of adipokine family. Cytokine 61:1–14. https://doi.org/10.1016/j.cyto.2012.08.036
5. Malfeti M,Halaas J,Ravussin E,Pratley RE, Lee GH,Zhang Y,Fei H,Kim S, Iallon R,Ranganathan S,Kern PA,Friedman JM (1995) Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1:1155–1161
6. Nokhbehsaim M,Keser S,Nogueira AV,Jager A,Jepsen S,Cirelli JA, Bourauel C,Eick S,Deschner J (2014) Leptin effects on the regenerative capacity of human periodontal cells. Int J Endocrinol 2014:180304–180313. https://doi.org/10.1155/2014/180304
37. Aydin S, Halifeoglu I, Ozercean IH, Erman F, Kilic N, Ilhan N, Ozkan Y, Akpolat N, Sert L, Caylak E (2005) A comparison of leptin and ghrelin levels in plasma and saliva of young healthy subjects. Peptides 26:647–652. https://doi.org/10.1016/j.peptides.2004.11.008

38. Kim GS, Hong JS, Kim SW, Koh JM, An CS, Choi JY, Cheng SL (2003) Leptin induces apoptosis via ERK/cPLA2/cytokrome c pathway in human bone marrow stromal cells. J Biol Chem 278: 21920–21929. https://doi.org/10.1074/jbc.M204598200

39. Roskoski R Jr (2012) MEK1/2 dual-specificity protein kinases: structure and regulation. Biochem Biophys Res Commun 417:5–10. https://doi.org/10.1016/j.bbrc.2011.11.145

40. Roskoski R Jr (2012) ERK1/2 MAP kinases: structure, function, and regulation. Pharmacol Res 66:105–143. https://doi.org/10.1016/j.phrs.2012.04.005

41. Zhuang S, Schnellmann RG (2006) A death-promoting role for extracellular signal-regulated kinase. J Pharmacol Exp Ther 319: 991–997. https://doi.org/10.1124/jpet.106.107367

42. Hoshi K, Kawaki H, Takahashi I, Takeshita N, Seityu M, Munshid SA, Masuda T, Anada T, Kato R, Kitaura H, Suzuki O, Takano-Yamamoto T (2014) Compressive force-produced CCN2 induces osteocyte apoptosis through ERK1/2 pathway. J Bone Miner Res 29:1244–1257. https://doi.org/10.1002/jbmr.2115

43. Park BG, Yoo CI, Kim HT, Kwon CH, Kim YK (2005) Role of mitogen-activated protein kinases in hydrogen peroxide-induced cell death in osteoblastic cells. Toxicology 215:115–125. https://doi.org/10.1016/j.tox.2005.07.003

44. Zhuang S, Yan Y, Daubert RA, Han J, Schnellmann RG (2007) ERK promotes hydrogen peroxide-induced apoptosis through caspase-3 activation and inhibition of Akt in renal epithelial cells. Am J Physiol Ren Physiol 292:F440–F447. https://doi.org/10.1152/ajprenal.00170.2006

45. Collier JB, Schnellmann RG (2017) Extracellular signal-regulated kinase 1/2 regulates mouse kidney injury molecule-1 expression physiologically and following ischemic and septic renal injury. J Pharmacol Exp Ther 363:419–427. https://doi.org/10.1124/jpet.117.244152

46. Chung J, Uchida E, Grammer TC, Blenis J (1997) STAT3 serine phosphorylation by ERK-dependent and -independent pathways negatively modulates its tyrosine phosphorylation. Mol Cell Biol 17:6508–6516. https://doi.org/10.1128/mcb.17.11.6508

47. Feng Z, Zheng W, Tang Q, Cheng L, Li H, Ni W, Pan X (2017) Fludarabine inhibits STAT1-mediated up-regulation of caspase-3 expression in dexamethasone-induced osteoblasts apoptosis and slows the progression of steroid-induced avascular necrosis of the femoral head in rats. Apoptosis 22:1001–1012. https://doi.org/10.1007/s10495-017-1383-1

48. Sahni M, Raz R, Coffin JD, Levy D, Basilio C (2001) STAT1 mediates the increased apoptosis and reduced chondrocyte proliferation in mice overexpressing FGF2. Development 128:2119–2129

49. Fukushima H, Jimi E, Okamoto F, Motokawa W, Okabe K (2005) IL-1-induced receptor activator of NF-kappa B ligand in human periodontal ligament cells involves ERK-dependent PGE2 production. Bone 36:267–275. https://doi.org/10.1016/j.bone.2004.09.011

50. Park HJ, Baek K, Baek JH, Kim HR (2017) TNFalpha increases RANKL expression via PGE(2)-induced activation of NFATc1. Int J Mol Sci 18. https://doi.org/10.3390/ijms18030495

51. Vuolteenaho K, Koskinen A, Kuukkonen M, Nieminen R, Paivarinta U, Moilanen T, Moilanen E (2009) Leptin enhances synthesis of proinflammatory mediators in human osteoarthritic cartilage–mediator role of NO in leptin-induced PGE2, IL-6, and IL-8 production. Mediat Inflamm 2009:345838–345810. https://doi.org/10.1155/2009/345838

52. Hsu YH, Cheng CY, Chen YC, Chen TH, Sue YM, Tsa W L, Chen CH (2012) Long-term leptin treatment exerts a pro-apoptotic effect on renal tubular cells via prostaglandin E2 augmentation. Eur J Pharmacol 689:65–71. https://doi.org/10.1016/j.ejphar.2012.06.008

53. Kaidi A, Qualtrough D, Williams AC, Paraskeva C (2006) Direct transcriptional up-regulation of cyclooxygenase-2 by hypoxia-inducible factor (HIF)-1 promotes colorectal tumor cell survival and enhances HIF-1 transcriptional activity during hypoxia. Cancer Res 66:6683–6691. https://doi.org/10.1158/0008-5472.CAN-06-0425

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.