PPARδ-mediated mitochondrial rewiring of osteoblasts determines bone mass
Dorothea I H Müller, Cornelia Stoll, Katrin Palumbo-Zerr, Christina Böhm, Brenda Krishnacoumar, Natacha Ipseiz, Jule Taubmann, Max Zimmermann, Martin Böttcher, Dimitrios Mougialakos, et al.

To cite this version:
Dorothea I H Müller, Cornelia Stoll, Katrin Palumbo-Zerr, Christina Böhm, Brenda Krishnacoumar, et al.. PPARδ-mediated mitochondrial rewiring of osteoblasts determines bone mass. Scientific Reports, Nature Publishing Group, 2020, 10 (1), pp.8428. 10.1038/s41598-020-65305-5. inserm-02869506

HAL Id: inserm-02869506
https://www.hal.inserm.fr/inserm-02869506
Submitted on 16 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
PPARδ-mediated mitochondrial rewiring of osteoblasts determines bone mass

Dorothea I. H. Müller1,2, Cornelia Stoll1,2, Katrin Palumbo-Zerr1,2, Christina Böhm1,2, Brenda Krishnacoumar1,2, Natacha Ipseiz3, Jule Taubmann1,2, Max Zimmermann1,2, Martin Böttcher4, Dimitrios Mougiakakos4, Jan Tuckermann5, Farida Djouad6, Georg Schett1, Carina Scholtysek1,2,7 & Gerhard Krönke1,2,7

Bone turnover, which is determined by osteoclast-mediated bone resorption and osteoblast-mediated bone formation, represents a highly energy consuming process. The metabolic requirements of osteoblast differentiation and mineralization, both essential for regular bone formation, however, remain incompletely understood. Here we identify the nuclear receptor peroxisome proliferator-activated receptor (PPAR) δ as key regulator of osteoblast metabolism. Induction of PPARδ was essential for the metabolic adaption and increased rate in mitochondrial respiration necessary for the differentiation and mineralization of osteoblasts. Osteoblast-specific deletion of PPARδ in mice, in turn, resulted in an altered energy homeostasis of osteoblasts, impaired mineralization and reduced bone mass. These data show that PPARδ acts as key regulator of osteoblast metabolism and highlight the relevance of cellular metabolic rewiring during osteoblast-mediated bone formation and bone-turnover.

Bone formation and bone remodeling represent highly energy demanding processes1. Especially the unique vascular architecture of long bones as well as large gradients in oxygen tension and nutrient supply impose metabolic challenges for bone resident cells2,3. Insights into the mechanisms that allow osteoblasts and osteoclasts the metabolic adaption to their specific microenvironment are therefore essential to understand both physiological and pathological bone turnover. Osteoblasts in long bones can sense low oxygen tension leading to a stabilization of hypoxia-induced factor (HIF) proteins, a process that controls both VEGF-dependent vascularization of bones and erythropoiesis within the bone marrow4,5. On a cellular level, HIF-1alpha-driven glycolysis in osteoblasts also directly contributes to bone formation6 and bone anabolic factors such as agonists of the Wnt signaling pathway can stimulate aerobic glycolysis in osteoblasts7. However, osteoblasts were also shown to consume and utilize large amounts of fatty acids8,9. In accordance, osteogenic differentiation of mesenchymal stem cells (MSCs) was reported to be paralleled by an increased mitochondrial respiration, where levels of ATP derived from oxidative phosphorylation peak in association with the accumulation of mitochondria that can directly support differentiation of osteoblasts10,11.

These findings show that especially differentiating and mineralizing osteoblasts utilize both aerobic glycolysis and oxidative phosphorylation, indicating that these cells need to adjust their bioenergetic machinery in order to adapt to transient metabolic challenges such as low and high oxygen and varying nutrient supply. The exact reason for the usage of divergent energy pathways as well as the in vivo relevance of the observed increase in oxidative phosphorylation in differentiating osteoblasts remains unclear. Notably, glucose transporter 1 (Glut1)-mediated glucose uptake has been recognized as an important signal that initiates early osteoblast commitment by regulating the stability of Runx-related transcription factor 2 (Runx2)12. The signals that control the induction of

1Department of Internal Medicine 3, Rheumatology and Immunology, Friedrich Alexander University Erlangen-Nurnberg and Universitätsklinikum Erlangen, Erlangen, Germany. 2Nikolaus Fiebiger Center of Molecular Medicine, University of Erlangen-Nuremberg, Erlangen, Germany. 3Systems Immunity Research Institute, Heath Park, Cardiff University, Cardiff, United Kingdom. 4Department of Internal Medicine 5, Hematology and Oncology, Friedrich Alexander University Erlangen-Nurnberg and Universitätsklinikum Erlangen, Erlangen, Germany. 5Institute of Comparative Molecular Endocrinology, University of Ulm, Ulm, Germany. 6IRMB, University Montpellier, INSERM, Montpellier, France. 7These authors contributed equally: Carina Scholtysek and Gerhard Krönke. ✉ e-mail: gerhard.kroenke@uk-erlangen.de
mitochondrial respiration dominating during later stages of osteoblast differentiation and mineralization, in turn, have remained elusive.

**Results**

Osteoblast differentiation is dependent on an increase in oxidative phosphorylation. To determine the metabolic requirements for regular osteoblast differentiation, we initially performed an extracellular flux analysis of in vitro cultured primary osteoblasts and their precursors. We compared the metabolic profiles of osteoblast precursors during steady state and upon initiation of osteoblast differentiation. These experiments confirmed a significantly increased oxygen consumption and oxidative phosphorylation of differentiating osteoblasts (Fig. 1A). Although the glycolytic activity slightly increased as well, this metabolic adaption resulted in an increase in the ratio between oxygen consumption rate (OCR, an indicator for mitochondrial respiration) and the extracellular acidification rate (ECAR, an indicator for glycolysis), suggestive of a robust metabolic rewiring of differentiating osteoblasts. In accordance, we observed a time-dependent increase in the expression of multiple genes involved in the control of mitochondrial respiration, mitochondrial biogenesis and oxygen-dependent energy provision such as Peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1α), mitochondrial transcription factor A (TFAM) or dynamin-1-like protein (DRP)1 in differentiating osteoblasts (Fig. 1B). Pharmacologic inhibition of mitochondrial biogenesis by tigecycline or of oxidative phosphorylation by rotenone, in turn, did not interfere with osteoblast viability, but dramatically diminished their differentiation and mineralization potential (Fig. 1C,D and Suppl. Fig. 1). These data indicated a global shift in the transcriptional program that controlled the cellular metabolic adaption during osteoblast differentiation and mineralization.
The nuclear receptor PPARδ mediates metabolic rewiring of osteoblasts. PGC1α is considered a master regulator of mitochondrial biogenesis and was among the most highly induced genes in differentiating osteoblasts. As PGC1αalpha serves as co-activator and interacts with various other transcription factors, we next assessed expression levels of genes encoding for proteins that represent known PGC1α interaction partners involved in cellular metabolism and oxidative phosphorylation. This analysis identified peroxisome proliferator-activated receptor (PPAR)δ as one of the prominently expressed transcription factors in differentiating osteoblasts (Fig. 2A–C). PPARδ belongs to the superfamily of nuclear receptors and acts as a ligand-dependent transcription factor that senses fatty acids and subsequently controls fatty acid oxidation, a process that primarily fuels mitochondrial respiration. We have previously identified PPARα as important regulator of Wnt signaling and osteoblast/osteoclast crosstalk. Our current analysis showed that expression of PPARα gradually increased during early osteogenic differentiation, whereas expression levels of its family members PPARα and PPARγ remained low (Fig. 2A–C). We additionally confirmed expression of PPARα on a protein level, which was induced during osteogenic differentiating of wild-type, but not in PPARδ-deficient MSCs (Fig. 2B). In accordance, induction of PPARα was paralleled by induction of several known PPARα target genes such as carnitine palmitoyltransferase (CPT)-1 or pyruvate dehydrogenase kinase (PDK)4, with expression levels likewise increasing during osteoblast differentiation (Fig. 2D). As expected, chromatin immunoprecipitation experiments confirmed direct binding of PPARα to these known target genes also in osteoblasts (Fig. 2E). Next, we sought to determine whether induction of PPARα indeed controlled the metabolic rewiring and increase in oxidative phosphorylation we had observed during osteogenic differentiation. We consequently analyzed the metabolic phenotype of PPARδ-deficient osteoblasts. Absence of PPARα resulted in suppression of the expression of metabolic key genes such as PGC1α (Fig. 2F). This finding indicated that PPARδ controlled a transcriptional program that regulated mitochondrial metabolism and oxidative phosphorylation during osteoblast differentiation. The expression levels of metabolic genes such as glucose transporter (Glut1) or Glut3, which are involved in glycolysis, in turn, were not suppressed, but even increased in PPARδ-deficient osteoblasts (Fig. 2G). The PPARδ-mediated regulation of genes involved in oxidative phosphorylation accordingly resulted in a reduced oxygen consumption and impaired oxidative phosphorylation in PPARδ-deficient osteoblasts, whereas glycolysis was increased (Fig. 2H).

Absence of PPARδ in osteoblasts alters their differentiation and mineralization. To understand whether osteoblast differentiation and/or mineralization were dependent on this PPARδ-mediated metabolic rewiring, we studied osteoblast differentiation in the absence of PPARδ. In comparison to wild-type osteoblast, PPARδ-deficient cells showed a defective upregulation of osteoblast differentiation markers such as Runx2, osteocalcin (OCN), osterix (Osx), and alkaline phosphatase (ALP) (Fig. 3A). PPARδ-deficient MSCs accordingly displayed a defective mineralization upon induction of osteogenic differentiation, demonstrating an important intrinsic role of the PPARδ-controlled metabolic adaption during osteoblast differentiation and function (Fig. 3B,C). To determine the in vivo relevance of the observed phenotype, we generated mice carrying an osteoblast-specific deletion of PPARδ. Analysis of these Runx2Δ osteoblast-specific deletion of PPARδ mice showed that these animals displayed a reduced bone mass in comparison to their wild type littermates with a significantly decreased bone volume/total volume, lower trabecular number and decreased bone mineral density in both tibia and spine (Fig. 3D–F). This low bone mass phenotype was associated with a reduced number of osteoblasts and a tendency towards a reduced mineral apposition and bone formation rate (Fig. 3G).

Discussion

Nuclear receptors including the members of the PPAR family control pleotropic processes throughout the body, including control of fatty acid and glucose metabolism in liver, adipose tissue and muscle. Previous data have identified PPARα as important regulator of bone turnover and musculoskeletal homeostasis. Although these studies determined a contribution of this nuclear receptor during muscle and bone biology as well as during osteoblast/osteoclast crosstalk, its exact molecular role during bone formation remained unclear. Our current data expand these insights on the involvement of this nuclear receptor in osteoblast biology and reveal an osteoblast-intrinsic role of this nuclear receptor during bone homeostasis in vivo. We show that PPARδ regulates the metabolic adaption and increased mitochondrial respiration, events that are required for efficient osteoblast differentiation and mineralization.

It remains to be determined to which extent the PPARδ-mediated control of energy homeostasis accounts for the reduced mineralization and bone mass observed in Runx2Δ osteoblast-specific deletion of PPARδ mice and whether additional effects exerted by this transcription factor contribute to this phenotype. Control of osteoblast-intrinsic mitochondrial respiration, however, seems to critically influence differentiation and mineralization of this cell type. Although mice that carry a Sox2-mediated complete depletion of PPARδ also showed an altered OPG/RANKL ratio and an increased osteoclast differentiation, the current Runx-2-mediated osteoblast-specific deletion of this nuclear receptor primarily resulted in osteoblast-intrinsic effects and reduced mineralization without increasing osteoclast differentiation, suggesting predominant effects of PPARδ on osteoblast metabolism, differentiation and mineralization. The exact reason for this discrepancy is unclear, but might be due an additional PPARδ-mediated control of the OPG/RANKL system in cells others than osteoblasts.

PPARδ belongs to the nuclear receptor superfamily of transcription factors. Its close family member PPARγ acts as key factor during adipocyte differentiation. Although both PPARα and PPARδ sense a repertoire of lipophilic compounds and subsequently act as ligand-activated transcription factors, they dictate partially opposing cellular pathways such as fatty acid synthesis and fatty acid oxidation. Moreover, PPARα and PPARδ are differentially expressed in distinct cell types such as adipocytes and osteoblasts. Ligand-induced activation of these different PPAR family members accordingly exerts contrasting effects on both systemic energy and bone metabolism. These findings highlight the importance of such metabolic sensors during cellular fate decision processes and
Figure 2. PPARδ-mediated metabolic rewiring of osteoblasts. (A) Quantitative real-time PCR analysis of PPAR isoforms (α, δ and γ) normalized to β-actin in calvarial osteoblasts cultured in differentiation media for 24 hours (n = 3). (B) Immunofluorescence microscopy of PPARδ wildtype and PPARδ-deficient MSCs cultured in differentiation medium for 7 days (β-actin, green; DAPI, blue; PPARδ, red; representative for 3 independent experiments). (C) Quantitative real-time PCR analysis of PPARδ mRNA expression normalized to β-actin of osteoblasts cultured in regular growth medium (black) and differentiation media (grey) for 24 and 72 hours (n = 3). (D) Real-time PCR analysis of mRNA expressions of CPT, PDK4 and UCP3 normalized to β-actin in calvarial osteoblasts cultured in regular growth media (black) and differentiation media (grey) for 24 and 72 hours (n = 3). (E) Chromatin immunoprecipitation (ChIP) assays determining binding of PPARδ to the promoters of indicated genes in calvarial osteoblasts cultured in regular growth media (black) and differentiation media (grey) for 72 hours. (F,G) Real-time PCR analysis of mRNA expressions of (F) PGC1alpha and (G) Glut1 and Glut3 normalized to β-actin in PPARδ wildtype (black and grey) and PPARδ-deficient (dark and light blue) calvarial osteoblasts cultured in either regular growth media or differentiation media (n = 3). (H) OCR, ECAR, basal and maximal respiration rate measured in freshly isolated PPARδ wildtype (grey) and PPARδ-deficient (light blue) calvarial osteoblasts cultured in differentiation medium for 24 hours (n = 3).

*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.005, ****P ≤ 0.001 using two-tailed Student’s t-test.
Figure 3. Absence of PPARδ in osteoblasts alters their differentiation and mineralization. (A) Real-time PCR analysis of mRNA expressions of Runx2, OCN, Osx and ALP normalized to β-actin in PPARδ wildtype (black and grey) and PPARδ-deficient (dark and light blue) calvarial osteoblasts cultured in either regular growth medium or differentiation media for 6, 24 and 72 hours (n = 3). (B) Alizarine Red staining of PPARδ wildtype and PPARδ-deficient MSCs cultured in osteoblastic differentiation media supplemented with 10 ng/ml Wnt3a at day 3, 7 and 10 of culture (n = 3). (C) Quantitative analysis of mineralized areas of alizarine red staining of PPARδ wildtype (black) and PPARδ-deficient (blue) MSCs cultured in osteoblastic differentiation medium for 10 days (n = 3). (D,E) µCT images of (D) tibial and (E) vertebral bone of female wild type mice (PPARδfl/fl) or their female littermates carrying an osteoblast-specific deletion of PPARδ (Runx2crePPARδfl/fl) at 12 weeks of age. (F) µCT-based analysis of structural bone parameters including bone volume per total volume (BV/TV), trabecular number (Trab.Numb.), trabecular separation (Trab.Sp.) and bone mineral density (BMD) of tibial (upper panel) and vertebral (lower panel) bone of female wild type mice (black) or their female littermates carrying an osteoblast-specific deletion of PPARδ (Runx2crePPARδfl/fl) at 12 weeks of age (n = 6–9). µCT analysis was performed using the operating system "Open VMS" by SCANCO Medical (Version 6.1). (G) Histomorphometric analysis of percentage of surfaces covered by osteoblast (OB.S/BS), osteoblast number (N.OB/B.PM), osteoclast number (N.Oc/B.PS), mineral apposition rate (MAR) and ratio of bone formation to bone surface (BFR/BS) counted in the proximal tibia of wild type mice (black) or their female littermates carrying an osteoblast-specific deletion of PPARδ (blue) at 12 weeks of age (n = 3). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.005 using two-tailed Student’s t-test.
were dyed with 2% Alizarin Red S (Merck #A5533) in H2O (pH adjusted between 4 and 4.3) for 3 to 5 minutes. (PPARfl/fl) were kindly supplied from Béatrice Desvergne, Center of Integrative Genomics, Faculty of Biology and Medicine, University of Lausanne, Switzerland. Runx2-CreERT2 mice were a gift from Jan Tuckermann, Institute of Comparative Molecular Endocrinology, University of Ulm, Germany.

**Bone analysis.** We measured the structure of tibial and vertebral bones with a SCANCO Medical µCT 40 scanner to produce the images. Results were analyzed with SCANCO evaluation software for segmentation, three-dimensional morphometric analysis, density and distance parameters (SCANCO Medical AG). We performed histomorphometric analysis with the OsteoMeasure Analysis System (Osteo-Metrics) to determine osteoclast and osteoblast parameters as previously described.

**Cell culture.** Isolation of calvarial osteoblasts was previously described. For osteoblast monocultures, we differentiated freshly isolated calvarial osteoblast precursors in the presence of 5 mM β-glycerolphosphate (CALBIOCHEM #35675) and 100 mg/ml ascorbic acid (Sigma-Aldrich #A0278). MSCs were isolated and fully characterized from PPARδ wildtype and PPARδ-deficient mice bone-marrow as described previously confirming the mesenchymal stem cell minimal criteria. We plated both MSC and osteoblast cell suspensions (0.5 × 10^6 cells cm^-2) in α-MEM (GIBCO by life technologies #32571-028) supplemented with 10% FBS (Biowest #S1580), 100 U/ml penicillin and 10 mg/ml streptomycin (PAN Biotech GmbH #P06-07100), at 37 °C in humidified atmosphere containing 5% CO2 in air. Culture media were changed every 2 days. At confluence, we plated cells at a density of 5 000 cells cm^-2. MSC were used between passages 7 and 12.

**Immunohistochemistry.** PPARδ immunohistochimical expression was evaluated MSC at day 7 after induction of osteoblast differentiation We used polyclonal anti-PPARδ from rabbit (Santa Cruz Biotechnology, #sc-7197) in a dilution of 1:100 in 2% BSA/PBS as previously described. Slides were viewed under the fluorescence microscope ECLIPSE Ni-Series microscope, Nikon, USA.

**Mineralization assay.** To boost mineralization osteoblasts were cultured in a medium containing 10 ng/ml recombinant mouse Wnt3a protein (R&D #NP_149122), β-glycerolphosphate (CALBIOCHEM #35675) and 100 mg/ml ascorbic acid (Sigma-Aldrich #A0278).

**Real-time PCR analysis.** We isolated total RNA from cells by using TRIzol reagent (Invitrogen). Concentrations were measured via NanoDrop analysis. 1 μg of isolated RNA was used for the first-strand complementary DNA synthesis (Amersham Biosciences), which was then used for SYBR Green–based quantitative cell culture.

Following real-time PCR primer sequences were used: ALP, 5′-CAGCGATGCAACACACTCCAG-3′ (sense) and 5′-GCATGTCGCCGGCTCAAGA-3′ (antisense); CPT1, 5′-CCAGGCTACAGGACAGCTT-3′ (sense) and 5′-GAACCTGCCATGTCTGGT-3′ (antisense); DRP1, 5′-CTGACCTTTGACTCGGATTCC-3′ (sense) and 5′-GCCCTTCCAATACTCCG-3′ (antisense); GLUT1, 5′-TCAACGACGCGGCTTCACTG-3′ (sense) and 5′-CACGATGCTCAAGATGACAC-3′ (antisense); Glut3, 5′-TCTGTGTCGGAATGCTTCC-3′ (sense) and 5′-GAACTGCCATGTCTGGT-3′ (antisense); OCN, 5′-ACCGCTGTGCTCGTCTGCTCGT-3′ (sense) and 5′-GAGGCTTTGGGGGGGCTTCC-3′ (antisense); Oxt, 5′-GAGGCGACACAAAGGAGGCATGGC-3′ (sense) and 5′-TCGACAGAGGAGAGGATCCTAG-3′ (antisense); PDK4, 5′-CCTTACAATCTCCATT-3′ (sense) and 5′-AAA GAG GGC GTC AGT ACT TG-3′ (antisense); PGClalpha, 5′-TATGAGTGACATAGTGTCTG-3′ (sense) and 5′-CAGTATCATTCCCAGCAGG-3′ (antisense); Ppara, 5′-GACGCTTTGCGCAGCTGGT-3′ (sense) and 5′-TCGGACCTCTGCTCTGTATG-3′ (antisense); Ppard, 5′-CTGAGGAGGAGGGGTAGAG-3′ (sense) and 5′-CAGTCTGGATGCTGCTACA-3′ (antisense); Pparg, 5′-CATTTCTGGCCACCACTCTC-3′ (sense) and 5′-TCAAGAAATGCGATGGTCTTCT-3′ (antisense); Runx2, 5′-GAGGCCAAGAGTTTACCC-3′ (sense) and 5′-GGAGCGTTTACCAGCTT-3′ (antisense); TFAM, 5′-GATTGGGCTTTCGCG-3′ (sense) and 5′-TGGATAGCCATCCAGTGAGGA-3′ (antisense) and UCP3, 5′-CCTACGACCATCATCAAGGAGAAGT-3′ (sense) and 5′-TCAAAAGGCGAGAACAAATG3′
(antisense). Normalized gene expression values for each sample were calculated as the ratio of expression of mRNA of the gene of interest to the expression of mRNA for β-actin.

**Metabolic analysis.** The cells’ bioenergetics were assessed using an XFe96 Extracellular Flux Analyzer ( Seahorse Bioscience, North Billerica, MA) as well as corresponding kits (Agilent, Santa Clara, California, USA). Cells were seeded in an optimized concentration of 15,000 cells per well. One hour before performance of sea- horse experiment, cells were incubated at 37°C in a CO₂-free atmosphere. XF Mitochondrial Stress Test Kits (Agilent, #103015) and XF Glycolysis Stress Test Kits (Agilent, #103020) were utilized according to the user guide (Seahorse Bioscience) and as described before31. For the Mitochondrial Stress Test, basal oxygen consumption rate (OCR) (an indicator for mitochondrial respiration) and extracellular acidification rate (ECAR) (an indicator for lactic acid production or glycolysis) were analyzed. Next, OCR and ECAR responses toward the application of oligomycin (1 µM), FCCP (2.5 µM), and the combination of antimycin (3 µM) and rotenone (3 µM) were evaluated. For Glycolysis Stress Test, OCR and ECAR were detected. Then, OCR and ECAR responses toward the application of Glucose (100 mM), Oligomycin (100 µM) and 2-DG (500 mM) were evaluated.

**Chromatin immunoprecipitation (ChIP) assays.** To verify the binding of PPARδ on selected promoters, ChIP assays were performed according to manufacturer instructions using the ChIP-IT® Express Chromatin Immunoprecipitation Kit (#530008, Active Motif, CA, USA). 25 µg of sonicated chromatin extract were incubated with 3.4 µg specific antibodies against PPARdelta (#ab178866, Abcam, Cambridge, MA, USA) or normal rabbit IgG antibodies (#sc-2027, Santa Cruz Biotechnology, CA, USA). After purification of DNA using Chromatin IP DNA Purification Kit (Active Motif, #58002) qPCR reactions were carried out on specific genomic regions using SYBR Green (Bio- Rad, USA). The resulting signals were normalized for primer efficiency by carrying out qPCR for each primer pair using the genomic input DNA. For positive control ChIP H3K36me3 antibody was used.

The following primers were used in these ChIP assays: PPRE on UC3P promoter 5′-TGTGTGTGCAAGAAGATGG-3′ (sense) and 5′-ACCCCTTGTTTTACAAAGCACG-3′ (antisense); PPRE on CTP1 promoter 5′-TGTAGATGAGTCGTGAG-3′ (sense) and 5′-CTTGAACCTCAAACTTCGCT-3′ (antisense) and PPRE on PDK4 promoter 5′-GTCCAATACAGGAGCAC-3′ (sense) and 5′-GAAATCATGATGACGGTGG-3′ (antisense).

**Statistical analyses.** All data are presented as mean ± SEM. Tests for statistical significance were performed with Student’s t test using GraphPad Prism Version 5 (GraphPad Prism Software Inc. La Jolla, California, USA). P < 0.05 was considered as significant.

Received: 3 December 2019; Accepted: 27 April 2020; Published online: 21 May 2020

**References**

1. Karner, C. M. & Long, F. Wnt signaling and cellular metabolism in osteoblasts. Cell Mol. Life Sci. 74(9), 1649–1657 (2017).
2. Wan, C. et al. Role of HIF-1alpha in skeletal development. Am. N. Y. Acad. Sci. 1192, 322–6 (2010).
3. Grüneboom, A. et al. A network of trans-cortical capillaries as mainstay for blood circulation in long bones. Nat. Metab. 1(2), 236–250 (2019).
4. Wang, Y. et al. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. J. Clin. Invest. 117(6), 1616–26 (2007).
5. Wan, C. et al. Activation of the hypoxia-inducible factor-1alpha pathway accelerates bone regeneration. Proc. Natl Acad. Sci. USA 105(2), 686–91 (2008).
6. Regan, J. N. et al. Up-regulation of glycolytic metabolism is required for HIF1alpha-driven bone formation. Proc. Natl Acad. Sci. USA 111(23), 8673–8 (2014).
7. Essen, E. et al. WNT-LRP5 signaling induces Warburg effect through mTORC2 activation during osteoblast differentiation. Cell Metab. 17(5), 745–55 (2013).
8. Adamek, G. et al. Fatty acid oxidation in bone tissue and bone cells in culture. Charact. Hormonal Influences. Biochim. J. 248(1), 129–37 (1987).
9. Niemeier, A. et al. Uptake of postprandial lipoproteins into bone in vivo: impact on osteoblast function. Bone 43(2), 230–7 (2008).
10. Chen, C. T. et al. Coordinated changes of mitochondrial biogenesis and antioxidant enzymes during osteogenic differentiation of human mesenchymal stem cells. Stem Cell 26(4), 960–6 (2008).
11. Komarova, S. V., Ataullakhanov, F. I. & Globus, R. K. Bioenergetics and mitochondrial transmembrane potential during differentiation of cultured osteoblasts. Am. J. Physiol. Cell Physiol 279(4), C1220–9 (2000).
12. Wei, J. et al. Glucose Uptake and Runx2 Synergize to Orchestrate Osteoblast Differentiation and Bone Formation. Cell 161(7), 1576–1591 (2015).
13. Skrlec, M. et al. Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. Cancer Cell 20(5), 674–88 (2011).
14. Scholz, S., et al. PPARbeta/delta governs Wnt signaling and bone turnover. Nat. Med. 19(5), 608–13 (2013).
15. Ipeiza, N. et al. Adopted orphans as regulators of inflammation, immunity and skeletal homeostasis. Swiss Med. Wkly. 144, w14055 (2014).
16. Barish, G. D., Narkar, V. A. & Evans, R. M. PPAR delta: a dagger in the heart of the metabolic syndrome. J. Clin. Invest. 116(3), 590–7 (2006).
17. Fu, H. et al. Impaired musculoskeletal response to age and exercise in PPARbeta(-/-) diabetic mice. Endocrinology 155(12), 4866–96 (2014).
18. Kleyer, A. et al. Liver X receptors orchestrate osteoblast/osteoclast crosstalk and counteract pathologic bone loss. J. Bone Miner. Res. 27(12), 2442–51 (2012).
19. Boufi, C. et al. IL-6-dependent PGE2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis. PLoS One 5(12), e14247 (2010).
20. Dominici, M. et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4), 315–7 (2006).
21. Kronke, G. et al. R-spondin 1 protects against inflammatory bone damage during murine arthritis by modulating the Wnt pathway. Arthritis Rheum. 62(8), 2303–12 (2010).
22. Kronke, G. et al. Oxidized phospholipids induce expression of human heme oxygenase-1 involving activation of cAMP-responsive element-binding protein. *J. Biol. Chem.* **278**(51), 51006–14 (2003).

23. Jitschin, R. et al. Mitochondrial metabolism contributes to oxidative stress and reveals therapeutic targets in chronic lymphocytic leukemia. *Blood* **123**(17), 2663–72 (2014).

**Acknowledgements**

We thank A. Klej for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (DFG – FG 2886 “PANDORA” – TP05/03 to G.K., D.M. and G.S., the CRC1181-A03/ A01 to G.K. and G.S.), CRC 1149 to J.T. (Tu220/14-1), the Emerging Field Initiative (EFI) of the Friedrich-Alexander University Erlangen-Nürnberg (FAU) and the STAEDTLER Stiftung (EFI_Verbund_Med_05_MIRACLE to G.K.), the Bundesministerium für Bildung und Forschung (BMBF) (METARTHROS to G.K. and G.S.) and the European Union (Horizon 2020 ERC-2014-StG 640087 - SOS to G.K and Horizon 2020 ERC-2018-SyG nanoSCOPE and RTCure to G.S.).

**Author contributions**

D.M. designed the study, performed and interpreted experiments and wrote the manuscript. C.S., C.B., J.T., B.K. and M.Z. performed experiments, collected, and interpreted data. N.I. and F.D. provided important expertise and established the MSC culture. D.M. and M.B. established and performed the metabolic characterization of osteoblasts. J.T., G.S., C.S. and G.K. designed the study and experiments and wrote the manuscript. All authors read and commented on the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-65305-5.

Correspondence and requests for materials should be addressed to G.K.

Reprints and permissions information is available at www.nature.com/reprints.

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

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 license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license 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 license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020