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Histopathological examination of healing in bone defects in intermittent fasting: An experimental study

Examen histopatológico de la curación de defectos óseos en ayuno intermitente: un estudio experimental

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ABSTRACT

The aim of this study is to histopathologically examine the effect of intermittent fasting, which has been followed with interest by researchers recently, on the healing of bone defects created in rat tibias. Sixteen Sprague–Dawley rats were included in this experimental study. For this purpose, the rats were divided into two groups: a defect control group (n = 8) and a defect + fasting group (n = 8). In the defect groups, a 4 mm diameter and depth defect was created in the corticocancellous bone of the metaphyseal tibia. Intermittent fasting was applied to the fasting groups three days a week for eight weeks. All animals were sacrificed at the end of the process, and the tibias were decalcified and examined histopathologically, with new bone formation and callus were assessed. Data were analyzed statistically. Student's t–test was used for statistical analysis. The mean longitudinal defect size value for the control group was 1675.43, while that of the fasting group was 1594.29. The mean vertical defect size value for the control group was 576.86, while that of the fasting group was 528. And the mean callus size value for the control group was 145, while that of the fasting group was 154.14. Both bone formation and callus values were numerically higher in the fasting group compared to the control group. However, these differences were not statistically significant (P>0.05). Based on the limited results of this study, although intermittent fasting may have a potential biological effect in supporting bone healing, no statistically significant difference was found in this study.

Key words: Intermittent fasting; bone defect; bone healing; bone regeneration; rat tibia

RESUMEN

El objetivo de este estudio es examinar histopatológicamente el efecto del ayuno intermitente, que ha sido seguido con interés por los investigadores recientemente, en la curación de defectos óseos creados en tibias de ratas. Dieciséis ratas Sprague–Dawley se incluyeron en este estudio experimental. Para este propósito, las ratas se dividieron en dos grupos: un grupo de control del defecto (n = 8) y un grupo de defecto + ayuno (n = 8). En los grupos de defecto, se creó un defecto de 4 mm de diámetro y profundidad en el hueso corticoesponjoso de la tibia metafisaria. El ayuno intermitente se aplicó a los grupos de ayuno tres días a la semana durante ocho semanas. Todos los animales fueron sacrificados al final del proceso, y las tibias fueron descalcificadas y examinadas histopatológicamente, con nueva formación ósea y se evaluó el callo. Los datos se analizaron estadísticamente. La prueba t de Student se utilizó para el análisis estadístico. El tamaño medio del defecto longitudinal en el grupo control fue de 1675,43, mientras que el del grupo en ayunas fue de 1594,29. El tamaño medio del defecto vertical en el grupo control fue de 576,86, mientras que el del grupo en ayunas fue de 528. El tamaño medio del callo en el grupo control fue de 145, mientras que el del grupo en ayunas fue de 154,14. Tanto la formación ósea como los valores de callo fueron numéricamente más altos en el grupo de ayuno en comparación con el grupo control. Sin embargo, estas diferencias no fueron estadísticamente significativas (P>0,05). Dados los limitados resultados de este estudio, si bien el ayuno intermitente podría tener un posible efecto biológico en la consolidación ósea, no se encontraron diferencias estadísticamente significativas.

Palabras clave: Ayuno intermitente; defecto óseo; curación ósea; regeneración ósea; tibia de rata

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INTRODUCTION

Bone tissue is a biological nanocomposite with a porous, multilayered hierarchy composed of an organic extracellular matrix and inorganic hydroxyapatite crystals [1].

This structure consists of three main cell types: osteoblasts, osteoclasts, and osteocytes. Throughout life, there is a constant shift in the balance between bone resorption by osteoclasts and bone formation by osteoblasts. Bone remodeling is a dynamic process between the formation of new bone tissue by osteoblasts and the breakdown of existing bone tissue by osteoclasts [2, 3].

Bone healing is a dynamic and multi–stage sequence of events that begins after injury and involves the interaction of biological and biomechanical processes [4]. This process is defined by three basic phases, namely inflammation, repair, and remodeling, which partially overlap with each other [5, 6].

Studies have revealed that macrophages and bone marrow mesenchymal stem cells (BMSCs) play a critical role in regulating inflammation and ensuring tissue regeneration during the bone healing process [7, 8].

Osteoblasts are cells that play a critical role in bone tissue repair. They synthesize and mineralize bone matrix at the defect site, increasing bone formation [6]. However, to fulfill this role, they must differentiate from precursor cells into osteoblasts. Various transcription factors, most notably Runt–related transcription factor 2 (Runx2), play important roles in this differentiation process [9].

The healing of fractures and defects is a complex, multistage process in which bone tissue rebuilds and strengthens. Each stage plays an important role in bone healing [10].

The first phase of bone healing is the inflammatory process that begins immediately after the injury. During this phase, a hematoma forms at the fracture site. A hematoma is not only an accumulation of blood but also a medium that triggers an inflammatory response that initiates healing. Immune cells arrive at the site and release cytokines. These molecules attract mesenchymal stem cells (MSCs) to the area, promoting healing and preparing the healing environment for the subsequent stages [11].

Following inflammation, the healing process transitions to soft callus formation. This typically begins a few days after the fracture. During this phase, MSCs differentiate into chondrocytes and osteoblasts, producing a fibrocartilaginous callus. This soft callus temporarily stabilizes the fracture and serves as a scaffold for bone healing [12].

As healing progresses, the soft callus gradually transforms into a hard callus. This phase, which lasts several weeks, marks the beginning of bone remodeling. Osteoblasts continue to produce the extracellular matrix, which will then mineralize. This mineralized structure strengthens the fracture site, paving the way for the final phase of healing [13].

The final stage of bone healing is bone remodeling, which can last for months or even years. During this process, the hard callus transforms into lamellar bone, restoring the bone's original structure and strength. The collaborative efforts of osteoblasts, osteoclasts, and mesenchymal stem cells allow the bone to heal and regain its function [14].

Bone healing is also a dynamic process influenced by many systemic and environmental factors. Conditions such as nutritional deficiencies, smoking, diabetes, and aging negatively impact healing by impairing vascularization and cellular response. Parathyroid hormones have a regulatory effect on osteoblast and osteoclast balance. Local factors such as infection, fracture type, and inadequate cartilage formation can also hinder the healing process. Additionally, some medications and systemic diseases can delay bone regeneration and impair the repair process [15].

Long–term calorie restriction (CR) without causing malnutrition is considered one of the most consistent dietary approaches that improves health and prolongs lifespan across different species [16].

However, the sustainability of long–term daily calorie restriction practices and the consistent long–term maintenance by individuals are quite difficult in practical living conditions [17]. Intermittent fasting has emerged as an alternative intervention to long–term CR, offering similar benefits in body weight reduction and chronic disease control [18, 19].

Intermittent fasting is an increasingly popular dietary pattern that alternates periods of restricted energy intake with periods of free energy intake [20]. The term intermittent fasting (IF) is a broad concept encompassing a variety of different methods, making it difficult to interpret data in the literature. The most widely adopted and researched types of IF include the 5:2 diet, alternate–day fasting, alternate–day modified fasting, and time–restricted feeding/eating [21].

Intermittent fasting has been shown to provide a variety of health benefits, including reducing body weight, improving body composition, preventing cancer development, suppressing inflammatory responses, alleviating oxidative stress, improving insulin resistance, increasing nerve regeneration and lifespan, and supporting wound healing [22, 23, 24].

Theoretically, time–restricted feeding may affect bone health through changes in physiological and metabolic parameters or individual behaviors. In particular, maintaining a regular daily rhythm of eating and fasting may positively influence metabolic functioning and the circadian rhythm of metabolic pathways [25, 26].

Preclinical and clinical data demonstrate the determinant role of the circadian system in bone physiology and that disruption of this rhythm may increase bone fragility. These findings suggest a potential impact of intermittent fasting on bone health [27].

Several small–scale human studies conducted in recent years demonstrate that time–restricted feeding plays an important role in maintaining a healthy metabolism. Consistent with findings in animal models, these studies have demonstrated that intermittent fasting also provides multiple benefits on human metabolism. Time–restricted feeding led to significant reductions in energy intake, body weight, fat mass, blood pressure, blood glucose, triglyceride levels, glucose tolerance, and inflammatory markers [28, 29].

Because fasting has been shown to affect parathyroid hormone secretion, some researchers have suggested that it may be beneficial for bone health. However, data on the effects of IF on bone health are quite limited. Therefore, carefully designed studies evaluating the role of IF on bone metabolism are clearly needed [30].

The biological effects of IF on bone tissue have not been adequately investigated, particularly in the context of regenerative processes such as bone fracture and defect healing. This highlights the need for systematic and controlled experimental studies evaluating the potential effects of this nutritional model on bone regeneration.

Therefore, the aim of this study was to histopathologically evaluate the effects of intermittent fasting on the healing process in bone defect models created in rat tibias. The anticipated findings are expected to fill a gap in the literature regarding the effects of IF on bone tissue and provide a scientific basis for future experimental and clinical research.

MATERIAL AND METHODS

This study was approved by the Firat University Animal Experiments Local Ethics Committee (Protocol No: 2024/01–12, Date: 09 January 2024). All procedures were carried out in accordance with the ethical principles of the Helsinki Declaration and the "Guide for the Use of Experimental Animals," and experimental applications were carried out at the Firat University Experimental Research Center.

Animals and study design

Sixteen female Sprague–Dawley rats (*Rattus norvegicus*), 3.5–4 months old and weighing 250–300 grams, (WL, Shimadzu, Japan) were used in this study. Subjects were provided by Firat University Experimental Research Center. All animals were housed in specially ventilated rooms throughout the experiment, under a 12–hour (h) light/12–h dark cycle, at 25 ± 2°C, with free access to water and food.

Care was taken to ensure that all subjects were in the same estrus period for standardization of the study. The study was designed based on previous scientific findings to investigate the effects of intermittent fasting on bone defect healing in rat tibias. Subjects were divided into two groups: a defect control group (n = 8) and a defect + IF group (n = 8).

Surgical procedures

All surgical procedures were performed under sterile conditions. General anesthesia was achieved with 10 mg·kg^{-1} Xylazine (Rompun, Bayer, Germany) and 40 mg·kg^{-1} Ketamine (Ketasol, Richter Pharma, Austria) administered intraperitoneally. Before surgery, the surgical area was shaved and disinfected with povidone–iodine solution. Surgical procedures were initiated in accordance with aseptic and antiseptic guidelines.

During the surgical procedures, incisions were made in the skin and soft tissues of the diaphyseal region of the right tibia using a number 15 scalpel. After periosteal elevation, a standard bone defect, 4 mm in diameter and 4 mm deep, was created in the corticocancellous bone region of the metaphyseal region of the right tibia using a rotary instrument. No grafts or implants were placed in the defect areas. To prevent thermal necrosis, the surgical field was regularly cooled with physiological saline irrigation during the defect creation process. Following all surgical procedures, the incised soft tissues were primarily closed with 4/0 resorbable polyglactin suture material. Postoperatively, antibiotics (Cefazolin sodium, 40 mg·kg^{-1}) and analgesics (Tramadol hydrochloride, 1 mg·kg^{-1}) were administered intramuscularly to all subjects for three days (d) for infection and pain control. Since one rat died in both the experimental and control groups immediately after the surgical procedures, they were excluded from the study and analyses were performed on 7 rats each.

Histopathological analysis

Histopathological evaluations were carried out in the laboratory of the Pathology Department of Firat University, Faculty of Veterinary Medicine. Tibias obtained after euthanasia were stored in 10% neutral formalin solution for 3 d. They were then cleaned of surrounding soft tissues such as muscle, tendon, and fascia and decalcified in 10% formic acid solution for approximately 1 week.

They were then processed through ascending alcohol, xylene, and paraffin series using an automatic tissue processing device (Leica TP 1020, Germany) and embedded longitudinally in paraffin (Leica EG1150 H–C, Germany). 3–micron–thick sections were obtained using a rotary microtome (Leica RM2125 RTS, Germany) and stained with hematoxylin and eosin (Leica Autostainer XL).

Examination was performed under a standard light microscope (Olympus BX42, Japan). Measurements were obtained by measuring the widest and deepest points of the defected areas longitudinally and vertically, and by measuring the thickest part of the callus tissue transversely (cellSens Standart, Japan).

Statistical analysis

Statistical analyses of the data obtained from histopathological evaluations were performed using IBM SPSS Statistics (Version 22.0, SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation (mean ± SD). The conformity of the data to normal distribution was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Student's t–test was used for data with parametric distribution in comparisons between groups. In all statistical analyses, $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Histopathological analyses revealed that new bone formation values were numerically higher in the IF groups compared to the control groups. However, these differences did not reach statistical significance ($P>0.05$). When the vertical and horizontal length measurements of the defects were evaluated, bone regeneration in the fasting group was more advanced than in the control group, but this did not reach significance (FIG. 1). Mature callus formation was observed to be more regular in the fasting group in all subjects, but this was not statistically significant ($P>0.05$) (TABLE I), (FIG. 2.).

The differentiation of MSCs and subsequent bone formation at the fracture and defect site are mediated by various microenvironmental signals. These signals include growth factors released from the bone matrix, changes in oxygen levels, and the mechanical microenvironment [31].

TABLE I
Bone measurement results obtained using three different parameters of the groups after the experimental protocols

Parameters	Groups	N	Mean (µm)	Minimum	Maximum	*P**
LDS	Fasting	7	1594.29	1311	2064	> 0.05
	Control	7	1675.43	1307	2041	
VDS	Fasting	7	528	442	600	
	Control	7	576.86	520	665	
CLS	Fasting	7	154.14	105	230	
	Control	7	145	108	200	

LDS: Longitudinal Defect Size, VDS: Vertical defect size, CLS: Callus. *Mann Whitney U tests. LDS‣ *P*: 0.602, VDS‣ *P*: 0.151, CLS‣ *P*: 0.679

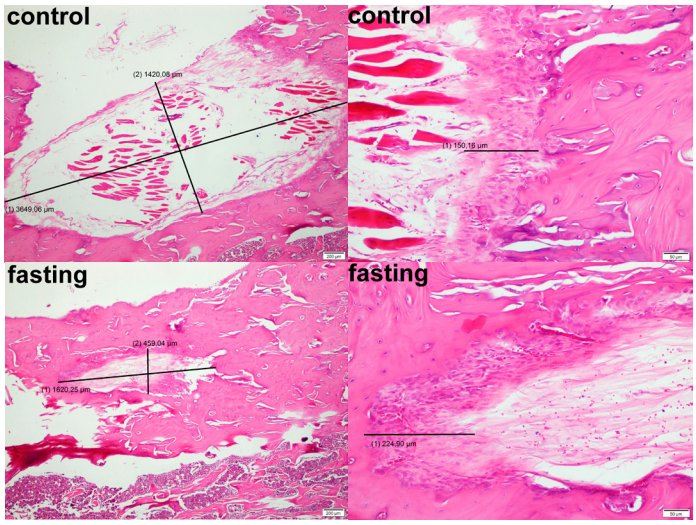

FIGURE 1. Defect area width and callus tissue measurements in the control and fasting groups show that the fasting group has a smaller defect area and wider callus formation

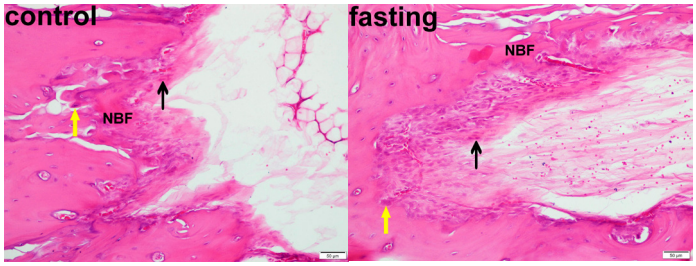

FIGURE 2. Osteoclasts in the control and fasting groups are shown with yellow arrows. Osteoblasts are shown with black arrows. Areas of new bone formation (NBF) are also seen in figure

Osteoblasts, osteocytes, osteoclasts, and osteogenic precursor cells in bone tissue, along with type I collagen fibers and various non–collagen components, play a fundamental role in bone formation and structural integrity.

Bone remodeling is a dynamic process regulated by complex interactions between hormones, cytokines, growth factors, and other biological molecules [32]. Dalle–Carbonare *et al.* [33] emphasize that the bone microenvironment is a dynamic system where components such as MSCs, immune cells, intercellular signaling, and extracellular vesicles come together to form a complex communication network. This system shapes the biological environment that influences the differentiation of MSCs into osteoblasts and the osteogenesis process.

Sheen *et al.* [34] clearly define bone fracture healing as a dynamic process involving hematoma formation, granulation tissue, hard callus formation, and long–term remodeling phases. The initial stages of this healing cascade begin with inflammation and the migration of MSCs to the fracture site; granulation tissue and osteogenesis occur in the subsequent process, ultimately resulting in bone remodeling through mechanical stress responses.

In this study, a numerical increase in the rate of new bone formation was observed in the intermittent fasting groups, but this difference was not statistically significant. According to Sheen *et al.* [34] model, this numerical trend may be biologically significant because the signals involved in post–hematoma MSCs migration and granulation are related to metabolic status. In this context, there is significant conceptual overlap between the classical healing phases described in the literature and the numerical trend in this study.

Sequeira [35] demonstrated that fracture healing in aged mice can be revitalized by a combination of systemic and local methods. Specifically, IF reversed the aging–related loss of cellular function, bringing the bone repair process closer to youthful levels [36]. This approach improved mitochondrial function and significantly increased osteogenesis capacity in osteoprogenitor cells, demonstrating that even marginal histopathological changes can be biologically significant.

In this study, a numerical increase in bone healing was observed in the IF groups, but this difference was not statistically significant. The findings of Sequeira [35] strongly indicate that the observed numerical increases have biological potential. Specifically, these studies reported that IF induced functional recovery and increased remodeling activity in osteoprogenitor cell populations. In this context, the increases that fell short of significance in this studies findings may reflect the positive molecular effects that systemic fasting allows at the cellular level.

The potential for IF to support bone healing can be explained by several biological mechanisms, including metabolic regulation,

activation of intracellular autophagy, and suppression of oxidative stress. This process may encourage MSCs in the bone microenvironment to more effectively differentiate into osteoblasts, thereby accelerating bone regeneration. Furthermore, the suppression of inflammatory responses by systemic fasting may contribute to bone repair by reducing osteoclastic resorption [33, 37, 38, 39].

The numerical increase in new bone formation observed in the IF groups in this study supports the potential impact of these biological processes on bone regeneration. However, the fact that these effects did not reach statistical significance suggests that these mechanisms have not yet reached histopathologically measurable levels.

Animal models are indispensable tools in scientific research to better understand the physiological and pathological processes of human biology. Rodent models, which provide controlled and reproducible experimental conditions, are frequently preferred, particularly for investigating bone healing, regenerative mechanisms, and the effects of systemic factors. Rats are widely used in preclinical studies due to their small body size, short reproductive cycle, economical maintenance, and genetically standardizable characteristics [40].

Sprague–Dawley rats are among the most preferred laboratory animals in the biomedical field due to their high growth potential, physiology amenable to experimental interventions, and genetic homogeneity. These characteristics make them ideal models for studies evaluating the effects of both tissue engineering and systemic interventions [41, 42]. Considering all these scientific and practical advantages, Sprague–Dawley rats were used as experimental animal models in this study.

In this study, the tibia was chosen as the experimental model. The tibia is a frequently used bone in studies investigating bone healing due to its endochondral ossification properties, morphological suitability, and suitability for histopathological evaluation. The tibia defect model provides a microenvironment similar to the natural healing process, allowing the assessment of the regenerative response within physiological limits. Bone defects used in experimental studies must be reproducible, standardizable, and capable of healing within a specific timeframe. Corticocancellous defects created on the tibia largely meet these criteria [43]. The current study, which combines an IF protocol with an experimental fracture and defect model in the tibia, which exhibits endochondral ossification, presents a unique experimental approach.

Second, the evaluations were limited to histopathological analyses. While histological examinations are useful for assessing parameters such as new bone formation, they may be insufficient to determine details such as bone density, degree of mineralization, and microstructural integrity. The omission of micro–CT, immunohistochemistry, and molecular biomarker analyses limited the scope of the results.

Third, the study used only one IF protocol (fasting three d a week, every other d). Because protocols of different durations and intensities were not tested, the most effective fasting regimen could not be determined.

Finally, the study only conducted short–term follow–up. However, bone healing is a process that changes over time. Because longer–term follow–up was not conducted, late–term effects could not be assessed.

CONCLUSION

In this study, new bone formation was histopathologically evaluated in the tibia bone defect models in groups treated with and without an IF protocol. Based on the limited findings, it was concluded that IF may have a positive biological effect on bone healing, but this effect was not statistically significant. However, the numerical differences observed between the groups suggest the potential impact of systemic metabolic factors on bone regeneration.

According to this study findings, the IF groups achieved numerically higher new bone formation values compared to the control group, although this difference was not statistically significant. However, improvement was observed in all groups, and biological signs were obtained that IF may positively affect the regenerative process. These results suggest that systemic metabolic factors may be effective in bone healing and that protocols such as IF may enhance the osteogenic response.

These results suggest that systemic practices such as IF can be considered a strategy to support bone healing, but further studies are needed to more clearly demonstrate this effect.

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Ethics approval and consent to participate

The present study was performed in line with the principles of The Declaration of Helsinki. Approval was granted by the Firat University Experimental Animal Ethics Committee (09 January 2024, Protocol no: 2024/01–12; Elazig, Türkiye).

Conflict of interest

The authors declarate there is no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Lelo I, Calabrese G, De Luca G, Conoci S. Recent advances in hydroxyapatite–based biocomposites for bone tissue regeneration in orthopedics. Int. J. Mol. Sci. [Internet]. 2022; 23(17):9721. doi: https://doi.org/g8rzn6

[2] Omi M, Mishina Y. Roles of osteoclasts in alveolar bone remodeling. Genesis [Internet]. 2022; 60(8–9):e23490. doi: https://doi.org/gtz76m

[3] Sun J, Xie W, Wu Y, Li Z, Li Y. Accelerated bone healing via electrical stimulation. Adv. Sci. [Internet]. 2025; 12(24):e2404190 doi: https://doi.org/g8694w

[4] Fan S, Sun X, Su C, Xue Y, Song X, Deng R. Macrophages–bone marrow mesenchymal stem cells crosstalk in bone healing. Front. Cell Dev. Biol. [Internet]. 2023; 11:1193765. doi: https://doi.org/gtg2ph

[5] Maruyama M, Rhee C, Utsunomiya T, Zhang N, Ueno M, Yao Z, Goodman SB. Modulation of the inflammatory response and bone healing. Front. Endocrinol. [Internet]. 2020; 11:386. doi: https://doi.org/gjtsz6

[6] Moriarty TF, Metsemakers WJ, Morgenstern M, Hofstee MI, Diaz AV, Cassat JE, Wildemann B, Depypere M, Schwarz EM, Richards RG. Fracture–related infection. Nat. Rev. Dis. Primers. [Internet]. 2022; 8(1):67. doi: https://doi.org/mq9t

[7] Wang YH, Zhao CZ, Wang RY, Du QX, Liu JY, Pan J. The crosstalk between macrophages and bone marrow mesenchymal stem cells in bone healing. Stem Cell Res. Ther. [Internet]. 2022; 13(1):511. doi:https://doi.org/qmxz

[8] Zhao Q, Liu X, Yu C, Xiao Y. Macrophages and bone marrow–derived mesenchymal stem cells work in concert to promote fracture healing: A brief review. DNA Cell Biol. [Internet]. 2022; 41(3):276–284. doi: https://doi.org/grs8x6

[9] Khotib J, Gani MA, Budiatin AS, Lestari MLAD, Rahadiansyah E, Ardianto C. Signaling pathway and transcriptional regulation in osteoblasts during bone healing: Direct involvement of hydroxyapatite as a biomaterial. Pharmaceuticals [Internet]. 2021; 14(7):615. doi: https://doi.org/gp2cwb

[10] Molitoris KH, Huang M, Baht GS. Osteoimmunology of fracture healing. Curr. Osteoporos. Rep. [Internet]. 2024; 22(3):330–339. doi: https://doi.org/qmx2

[11] Shu LZ, Zhang XL, Ding YD, Lin H. From inflammation to bone formation: the intricate role of neutrophils in skeletal muscle injury and traumatic heterotopic ossification. Exp. Mol. Med. [Internet]. 2024; 56(7):1523–1530. doi: https://doi.org/g8xfdh

[12] Camarena A, Kang L, Mirando AJ, Augustine E, McMillian NS, Stinson NC, Agarwal SM, Becker ML, Hilton MJ, Fernandez–Moure JS. Platelet–rich plasma enhances rib fracture strength and callus formation *in vivo*. J. Trauma Acute Care Surg. [Internet]. 2024; 97(6):884–890. doi: https://doi.org/qmx3

[13] Trompet D, Melis S, Chagin AS, Maes C. Skeletal stem and progenitor cells in bone development and repair. J. Bone Miner. Res. [Internet]. 2024; 39(6):633–654. doi: https://doi.org/gt2h8d

[14] Hente RW, Perren SM. Tissue deformation controlling fracture healing. J. Biomech. [Internet]. 2021; 125:110576. doi: https://doi.org/qmx4

[15] Kondi S, Gowda SR. Principles of bone healing. Surgery [Internet]. 2023; 41(10):625–631. doi: https://doi.org/qmx5

[16] Duregon E, Pomatto–Watson LCDD, Bernier M, Price NL, de Cabo R. Intermittent fasting: from calories to time restriction. Geroscience [Internet]. 2021; 43(3):1083–1092. doi: https://doi.org/gm5crm

[17] Hwangbo DS, Lee HY, Abozaid LS, Min KJ. Mechanisms of lifespan regulation by calorie restriction and intermittent fasting in model organisms. Nutrients [Internet]. 2020; 12(4):1194. doi: https://doi.org/g6xkh6

[18] Hu D, Xie Z, Ye Y, Bahijri S, Chen M. The beneficial effects of intermittent fasting: an update on mechanism, and the role of circadian rhythm and gut microbiota. Hepatobiliary Surg. Nutr. [Internet]. 2020; 9(5):597–602. doi: https://doi.org/qmx6

[19] Song DK, Kim YW. Beneficial effects of intermittent fasting: a narrative review. J. Yeungnam Med. Sci. [Internet]. 2023; 40(1):4–11. doi: https://doi.org/qmx7

[20] Elsworth RL, Monge A, Perry R, Hinton EC, Flynn AN, Whitmarsh A, Hamilton–Shield JP, Lawrence NS, Brunstrom JM. the effect of intermittent fasting on appetite: A systematic review and meta–analysis. Nutrients [Internet]. 2023; 15(11):2604. doi: https://doi.org/qmx8

[21] Clayton DJ, Varley I, Papageorgiou M. Intermittent fasting and bone health: a bone of contention? Br. J. Nutr. [Internet]. 2023; 130(9):1487–1499. doi: https://doi.org/qmx9

[22] Varady KA, Cienfuegos S, Ezpeleta M, Gabel K. Clinical application of intermittent fasting for weight loss: progress and future directions. Nat. Rev. Endocrinol. [Internet]. 2022; 18(5):309–321. doi: https://doi.org/gqrts3

[23] Serger E, Luengo–Gutierrez L, Chadwick JS, Kong G, Zhou L, Crawford G, Danzi MC, Myridakis A, Brandis A, Bello AT, Müller F, Sanchez–Vassopoulos A, De Virgiliis F, Liddell P, Dumas ME, Strid J, Mani S, Dodd D, Di Giovanni S. The gut metabolite indole–3 propionate promotes nerve regeneration and repair. Nature [Internet]. 2022; 607(7919):585–592. doi: https://doi.org/gqd3nr

[24] Minciuna I, Gallage S, Heikenwalder M, Zelber–Sagi S, Dufour JF. Intermittent fasting–the future treatment in NASH patients? Hepatology [Internet]. 2023; 78(4):1290–1305. doi: https://doi.org/gs86nz

[25] Zeb F, Wu X, Chen L, Fatima S, Haq IU, Chen A, Majeed F, Feng Q, Li M. Effect of time–restricted feeding on metabolic risk and circadian rhythm associated with gut microbiome in healthy males. Br. J. Nutr. [Internet]. 2020; 123(11):1216–1226. doi: https://doi.org/qmzb

[26] Wilkinson MJ, Manoogian ENC, Zadourian A, Lo H, Fakhouri S, Shoghi A, Wang X, Fleischer JG, Navlakha S, Panda S, Taub PR. Ten–Hour time–restricted eating reduces weight, blood pressure, and atherogenic lipids in patients with metabolic syndrome. Cell Metab. [Internet]. 2020; 31(1):92–104.e5. doi: https://doi.org/gghdcg

[27] Papageorgiou M, Biver E, Mareschal J, Phillips NE, Hemmer A, Biolley E, Schwab N, Manoogian ENC, Gonzalez Rodriguez E, Aeberli D, Hans D, Pot C, Panda S, Rodondi N, Ferrari SL, Collet TH. The effects of time–restricted eating and weight loss on bone metabolism and health: a 6–month randomized controlled trial. Obesity (Silver Spring). [Internet]. 2023; 31(1):85–95. doi: https://doi.org/qmzc

[28] McAllister MJ, Pigg BL, Renteria LI, Waldman HS. Time–restricted feeding improves markers of cardiometabolic health in physically active college–age men: a 4–week randomized pre–post pilot study. Nutr. Res. [Internet]. 2020; 75:32–43. doi: https://doi.org/gpnnf2

[29] Martens CR, Rossman MJ, Mazzo MR, Jankowski LR, Nagy EE, Denman BA, Richey JJ, Johnson SA, Ziemba BP, Wang Y, Peterson CM, Chonchol M, Seals DR. Short–term time–restricted feeding is safe and feasible in non–obese healthy midlife and older adults. Geroscience [Internet]. 2020; 42(2):667–686. doi: https://doi.org/qmzd

[30] Veronese N, Reginster JY. The effects of calorie restriction, intermittent fasting and vegetarian diets on bone health. Aging Clin. Exp. Res. [Internet]. 2019; 31(6):753–758. doi: https://doi.org/gfzjjg

[31] Pajarinen J, Lin T, Gibon E, Kohno Y, Maruyama M, Nathan K, Lu L, Yao Z, Goodman SB. Mesenchymal stem cell–macrophage crosstalk and bone healing. Biomaterials [Internet]. 2019; 196:80–89. doi: https://doi.org/gh4vp4

[32] Tanrisever M, Tekin B, Can UK, Istek O, Ozcan EC, Ozercan IH, Gelic T, Dundar S.The effect of local melatonin application on bone fracture healing in Rat tibias. Medicina [Internet]. 2025; 61(1):146. doi: https://doi.org/qmzf

[33] Dalle–Carbonare L, Cominacini M, Trabetti E, Bombieri C, Pessoa J, Romanelli MG, Valenti MT.The bone microenvironment: new insights into the role of stem cells and cell communication in bone regeneration. Stem Cell Res. Ther. [Internet]. 2025; 16(1):169. doi: https://doi.org/qmzg

[34] Sheen JR, Mabrouk A, Garla VV. Fracture Healing Overview. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 (cited Oct 26, 2025). Available in: https://goo.su/H0c5t

[35] Sequeira I. Aging: Restoring bone healing potential. Elife [Internet]. 2025; 14:105420. doi: https://doi.org/qmzh

[36] Reeves J, Tournier P, Becquart P, Carton R, Tang Y, Vigilante A, Fang D, Habib SJ.Rejuvenating aged osteoprogenitors for bone repair. Elife [Internet]. 2024; 13:104068. doi: https://doi.org/qmzj

[37] Liang W, Zhou C, Liu X, Xie Q, Xia L, Liu L, Bao W, Lin H, Xiong X, Zhang H, Zheng Z, Zhao J.Current status of nano–embedded growth factors and stem cells delivery to bone for targeted repair and regeneration. J. Orthop. Transl. [Internet]. 2025; 50:257–273. doi: https://doi.org/qmzk

[38] He T, Qin L, Chen S, Huo S, Li J, Zhang F, Yi W, Mei Y, Xiao G. Bone–derived factors mediate crosstalk between skeletal and extra–skeletal organs. Bone Res. [Internet]. 2025; 13(1):49. doi: https://doi.org/qmzm

[39] Iyer SS. Epigenetic regulation of bone healing: implications for fracture repair and clinical treatment strategies. Yale J. Biol. Med. [Internet]. 2025; 98(2):159–170. doi: https://doi.org/qmzn

[40] Bahadoran Z, Mirmiran P, Kashfi K, Ghasemi A. Importance of systematic reviews and meta–analyses of animal studies: Challenges for animal–to–human translation. J. Am. Assoc. Lab. Anim. Sci. [Internet]. 2020; 59(5):469–477. doi: https://doi.org/gg637h

[41] Gileta AF, Fitzpatrick CJ, Chitre AS, St Pierre CL, Joyce EV, Maguire RJ, McLeod AM, Gonzales NM, Williams AE, Morrow JD, Robinson TE, Flagel SB, Palmer AA. Genetic characterization of outbred Sprague Dawley rats and utility for genome–wide association studies. PLoS Genet. [Internet]. 2022; 18(5):e1010234. doi: https://doi.org/g9stfs

[42] Polat E, Bostan–Yoru HG, Kalay Y, Akca S. The importance of screening programs in laboratory animals and investigation of several important viral agents. J. Lab. Anim. Sci. Pract. [Internet]. 2024; 4(2):91–100. doi: https://doi.org/qmzp

[43] Durmaz B, Gunes N, Koparal M, Gul M, Dundar S, Bingul MB. Investigation of the effects of quercetin and xenograft on the healing of bone defects: An experimental study. J. Oral Biol. Craniofac. Res. [Internet]. 2023; 13(1):22–27. doi: https://doi.org/pmp9