



RESEARCH ARTICLE

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Murat TANRISEVER^{1, a}
Özmen İSTEK^{2, b}
Ümit Koray CAN^{3, c}
Erhan Cahit ÖZCAN^{4, d}
Burak KARABULUT^{5, e}
Müge BEREKET ALTINTAŞ^{6, f}
Serkan DÜNDAR^{5, g}
Türker GELİÇ^{1, h}

¹ Firat University,
Faculty of Veterinary
Medicine,
Department of Surgery,
Elazığ, TÜRKİYE

² Mus Alparslan University,
Faculty of Health Sciences,
Department of Nursing, Mus,
TÜRKİYE

³ Turkish Jockey Club Elazığ
Racecourse Horse Hospital,
Elazığ, TÜRKİYE

⁴ Firat University,
Faculty of Medicine,
Department of Plastic,
Reconstructive and Esthetic
Surgery,
Elazığ, TÜRKİYE

⁵ Firat University,
Faculty of Veterinary
Medicine,
Department of Pathology,
Elazığ, TÜRKİYE

⁶ Firat University,
Faculty of Dentistry,
Department of Peridontology,
Elazığ, TÜRKİYE

^a ORCID: 0000-0003-3815-8543

^b ORCID: 0000-0002-7536-7064

^c ORCID: 0000-0002-3272-6311

^d ORCID: 0000-0001-6513-0226

^e ORCID: 0000-0002-4907-6159

^f ORCID: 0009-0001-4810-6678

^g ORCID: 0000-0003-3944-1957

^h ORCID: 0009-0007-7623-8161

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Correspondence

Murat TANRISEVER
Firat University,
Faculty of Veterinary
Medicine,
Department of Surgery
Elazığ – TÜRKİYE
mtanrisever@firat.edu.tr

Evaluation of Bone Fracture Healing in Rats Subjected to Experimental Intermittent Fasting

Intermittent fasting has gained recognition as a promising approach for weight reduction and metabolic health enhancement; however, its impact on bone health remains insufficiently understood. In the present study, the impact of intermittent fasting on bone healing was histopathologically investigated using an experimental fracture model in rat tibiae. Sixteen female Sprague-Dawley rats were divided into two groups: a control group (n=8) and a fasting group (n=8). In the all group, bone cuts were performed on the right tibia using a rotating steel disc under serum cooling. The rats in fasting group underwent intermittent fasting three days per week for an eight-week experimental setup. Intermittent fasting was applied every other day (food intake was allowed on one day, food intake was not allowed on the next day, and water intake was allowed), and the rats were allowed free access to water. At the end of the eight-week experimental setup, all rats were euthanized. The tibia bone tissue containing the bone cut was removed, decalcified, and subjected to histopathological analysis. Histopathological analysis demonstrated that the newly formed bone tissue within the healing callus of the fasting group was more homogeneously and densely distributed than that of the control group. Quantitative assessment demonstrated that the difference in new bone formation between the fasting and control groups was not statistically significant ($p=0.084$).

Key Words: Intermittent fasting, fracture healing, rat, bone

Deneysel Aralıklı Oruca Tabi Tutulan Sıçanlarda Kemik Kırığı İyileşmesinin Değerlendirilmesi

Aralıklı oruç, kilo kaybı ve metabolik sağlığın iyileştirilmesi açısından umut verici bir strateji olarak öne çıkmaktadır; ancak kemik sağlığı üzerindeki etkileri henüz netlik kazanmamıştır. Bu çalışmada, rat tibiasında oluşturulan deneysel kırık modeli kullanılarak aralıklı orucun kemik iyileşmesi üzerindeki etkileri histopatolojik olarak incelenmiştir. Çalışmada, 16 dişi Sprague-Dawley rat; kontrol grubu (n=8) ve oruç grubu (n=8) olmak üzere iki gruba ayrılmıştır. Tüm gruplarda, sağ tibia üzerine serum soğutması altında döner çelik disk ile kemik kesisi uygulanmıştır. Oruç grubundaki sıçanlara sekiz haftalık deneysel süreç boyunca haftada üç gün aralıklı oruç uygulanmıştır. Aralıklı oruç, gün aşırı olacak şekilde (bir gün gıda alımına izin verilmiş, ertesi gün verilmemiş; suya ise serbest erişim sağlanmıştır) gerçekleştirilmiştir. Sekiz haftalık deneysel sürecin sonunda tüm hayvanlar ötenazi edilmiştir. Kesi bölgesini içeren tibia kemik dokusu çıkarılmış, dekalifiye edilerek histopatolojik analiz için hazırlanmıştır. Yapılan histopatolojik incelemede iyileşme kallusu içerisinde, oruç grubundaki yeni kemik dokusunun kontrol grubuna kıyasla daha homojen ve yoğun dağılım gösterdiği gözlemlenmiştir. Kantitatif değerlendirmeler yeni kemik oluşumu oranı açısından kontrol ve oruç grupları arasında anlamlı olarak istatistiksel bir fark bulunmadığını ortaya koymuştur ($p=0.084$).

Anahtar Kelimeler: Aralıklı oruç, kırık iyileşmesi, rat, kemik

Introduction

Fracture healing and bone repair are postnatal biological processes that closely parallel the ontogenetic events observed during embryonic skeletal development, and these mechanisms have been extensively studied in prior research (1, 2). Skeletal tissue repair and fracture healing initiate with an anabolic phase characterized by tissue growth, resulting from stem cell recruitment and differentiation into both skeletal and vascular lineages. Immediately after fracture, a cartilaginous callus adjacent to the fracture site as an initial stage of the repair process. Surrounding this central region, the periosteum undergoes hypertrophy at the margins of the newly formed cartilaginous tissue, initiating primary bone formation (3). Concurrently with the development of cartilaginous tissue, progenitor cells destined to form new blood vessels accumulate and differentiate within the surrounding muscle sheath to support the vascularization of the emerging bone (4, 5). The expansion of the vascular network surrounding the callus, followed by its invasion into the callus tissue, is evidenced by an increase in blood flow to the site of tissue repair. With the progression of chondrocyte differentiation, mineralization of the cartilage extracellular matrix occurs, culminating in the completion of the anabolic phase of fracture repair via chondrocyte apoptosis (6). The anabolic phase is succeeded by a prolonged period dominated by catabolic activity, characterized by a gradual reduction in the volume of the callus tissue. During this phase, catabolic processes such as cartilage resorption predominate; however, certain

anabolic activities also persist. As the cartilage is resorbed, secondary bone formation is initiated, and primary angiogenesis continues to support the replacement of cartilage with newly forming bone tissue. Subsequently, with the initiation of bone remodeling, the primary mineralized matrix is resorbed by osteoclasts, followed by the removal of the secondary bone that was deposited during the cartilage resorption phase (7). As resorption of the bony callus progresses, the process is characterized by coordinated osteoblastic and osteoclastic activity, known as 'coupled remodeling', which remodels the callus back to the original cortical architecture. During this time, the marrow cavity is re-established, and the original marrow composition of hematopoietic tissue and bone is restored. In the final stage of this catabolic phase, extensive vascular remodeling occurs, wherein the expanded vascular network regresses and the elevated blood flow returns to pre-injury levels (8).

Fasting has been a longstanding practice across diverse cultures, commonly observed during religious rituals (e.g., Ramadan), and also documented historically in contexts such as political hunger strikes, periods of famine, and therapeutic interventions for morbid obesity (9). However, over the past 10 to 15 years, short-term fasting has been increasingly adopted as a novel strategy for weight loss and the enhancement of metabolic function. After the release of Michael Mosley's 2013 book *The Fast Diet*, the concepts of 'intermittent fasting' (IF) and the '5:2 diet' became increasingly popular in the United Kingdom. Currently, IF is widely practiced as a strategy to reduce energy intake. According to a recent survey, one in four American adults reported having considered or attempted IF (10). Furthermore, publications on IF have increased exponentially over the past decade. Current evidence indicates that IF can promote weight loss and confer beneficial health effects, including enhanced insulin sensitivity, improved lipid profiles, and reduced blood pressure (11). Unlike conventional diets based on daily caloric restriction, IF involves complete or substantial energy restriction within specific time windows, while allowing adequate or ad libitum food intake during non-restricted periods. Although the health benefits of IF are well-documented, evidence concerning its effects on other physiological systems, particularly the skeletal system, remains limited (10). Weight reduction through continuous energy restriction—ranging from mild to severe calorie restriction, with or without micronutrient supplementation—and/or combined with exercise, has been associated with decreases in bone mass and detrimental alterations in bone microarchitecture (11). Multiple mechanisms have been suggested to account for these effects, including mechanical unloading, nutrient insufficiencies, and hormonal alterations (10). However, it remains uncertain whether IF elicits adverse effects on bone comparable to those observed with other weight loss strategies, or whether distinct characteristics of IF regimens might instead confer benefits for bone healing after fractures. For instance, IF has been hypothesized to influence metabolism by alternating between defined periods of prolonged fasting (catabolic

state) and shorter periods of food intake (anabolic state), and/or by aligning eating patterns with endogenous circadian rhythms (9). Improved metabolic regulation and enhanced circadian alignment are believed to support skeletal health (11). Nevertheless, the cumulative impact of these various features and mechanisms associated with IF on bone remains poorly understood.

Materials and Methods

Research and Publication Ethics: This study received approval from the Local Animal Experiments Ethics Committee of Firat University (protocol number: 2024/01-12, dated 09.01.2024) and was conducted at the Firat University Experimental Research Center in strict accordance with the Helsinki Declaration guidelines.

Animals and Study Design: The number of animals to be used in the experiments was determined through a power analysis; with an 8% deviation, a type I error (α) of 0.05, and a type II error (β) (Power = 0.80). In experiments where the animals were divided into groups, at least 7 animals were required per group. To account for potential mortality due to surgical procedures and during the experimental period, each group was composed of 8 rats.

This study utilized sixteen female Sprague-Dawley rats, aged 3 to 4 months and weighing between 250 and 300 grams. Care was taken to ensure that all subjects were in the same estrus period for standardization of the study. The rats were randomly assigned to two groups: A control group (n=8) and a fasting group (n=8). In all groups, bone cuts were performed on the right tibia using a rotating steel disc under physiological serum (0.9% NaCl) cooling. The rats in the fasting group underwent IF three days per week for an eight-week experimental setup. Intermittent fasting was applied every other day (food was provided ad libitum on one day, withheld on the following day, while water intake was permitted throughout), and the rats were allowed free access to water. Upon completion of the eight-week experimental period, all rats were humanely euthanized. The tibia bone tissue containing the bone cut was removed, decalcified, and subjected to histopathological analysis.

Surgical Procedures: All surgical interventions were conducted under sterile conditions. General anesthesia was induced via intraperitoneal administration of xylazine (10 mg/kg; Rompun, Bayer, Germany) and ketamine (40 mg/kg) (Ketasol, Richter Pharma, Wels, Austria). Prior to surgery, the operative area was shaved and disinfected using povidone-iodine solution. Approximately 1.5 cm incisions were made on the tibial crest to create a bone fracture, and the soft tissues and periosteal tissue were removed with an elevator. Bone cuts were made in the diaphysis of the tibia using a rotating steel disc cooled with serum, and the fracture fragments were fixed with Kirshner wires (Figure 1). In all cases, the soft tissues were repositioned and sutured with 3-0 suture material to

facilitate healing after surgical interventions. Postoperatively, antibiotics (cefazolin sodium, 40 mg/kg) and analgesics (tramadol hydrochloride, 1 mg/kg) were administered intramuscularly to all subjects for three days for infection and pain control.

Histopathological Analysis: Following euthanasia procedures, the tibiae were kept in 10% neutral buffered formalin for 3 days. Subsequently, surrounding soft tissues such as muscle, tendon, and fascia were carefully removed, and the bones were decalcified in 10% formic acid solution for approximately one week. The samples were then processed in an automatic tissue processor (Leica TP 1020, Germany) through a series of graded alcohols, xylene, and paraffin, and longitudinally embedded in paraffin blocks (Leica EG1150 H-C, Germany). Sections with a thickness of 3 micrometers were obtained using a rotary microtome (Leica RM2125 RTS, Germany) and stained with hematoxylin and eosin (Leica Autostainer XL). Histological evaluation was performed under a conventional light microscope (Olympus BX42, Japan).

Bone healing was assessed based on the formation of new bone tissue. For this purpose, the entire area of the healing tissue at the fracture site was measured. The area of new bone formation was then measured and expressed as a ratio to the total healing tissue area. This allowed for the determination of the percentage of new bone formation within the healing region for each sample (12).

Statistical Analysis: Statistical analyses of histopathological data were conducted using IBM SPSS Statistics software (Version 22.0, SPSS Inc., Chicago, IL, USA). Data are presented as mean $\pm$ standard deviation (mean $\pm$ SD). The assumption of normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk goodness-of-fit tests. Since the data did not exhibit a normal distribution, non-parametric tests were employed.

The Mann-Whitney U test was used for comparisons between groups with non-parametric data. A p-value less than 0.05 was considered statistically significant in all analyses.



Figure 1. Fixation of fracture fragments with Kirshner wires

Results

In both the control and fasting groups, callus tissue was observed at the fracture site, bridging the fracture gap. The newly generated bone tissue within the callus of the fasting group appeared to be more uniformly and densely arranged than in the control group. Quantitative assessment indicated that the difference in new bone formation between the fasting and control groups was not statistically significant (Table 1, Figures 2 and 3).

Table 1. New Bone Formation (NBF) ratios of the groups after experimental period in fracture line

Parameter	Groups	N	Mean (%)	Minumum	Maximum	p*
NBF	Control	8	48.25	46	53	>0.05 (0.084)
	Fasting	8	51.25	49	54	

* Mann Whitney U Test

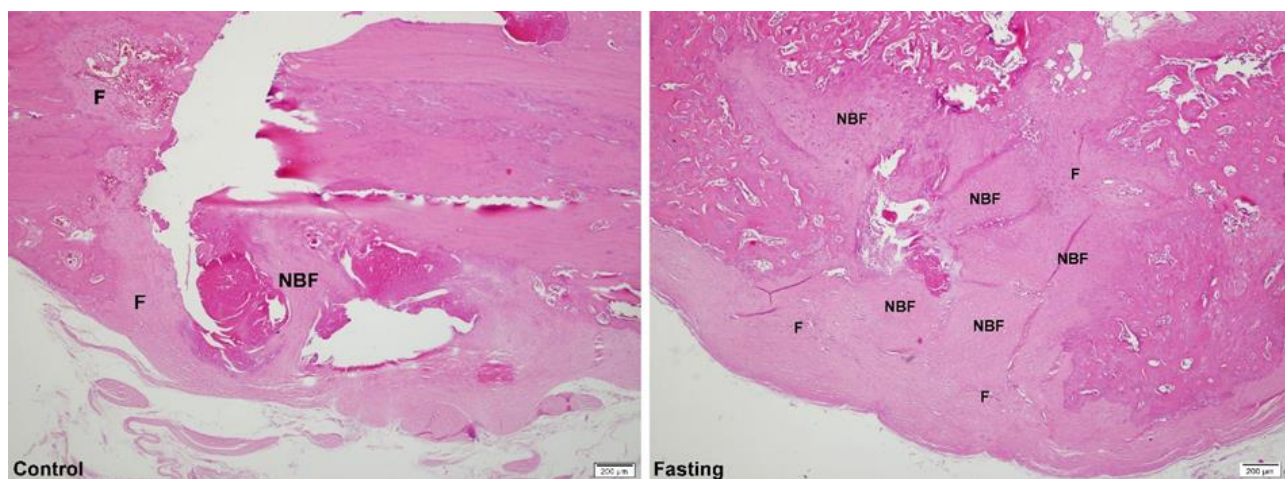


Figure 2. Areas of fibrosis (F) and new bone formation (NBF) in control and fasting groups. 4X magnification, H&E staining

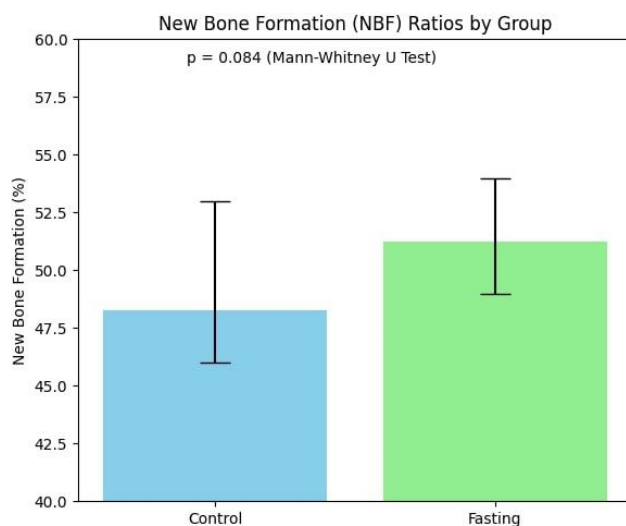


Figure 3. Graph showing the statistical comparison of the groups

Discussion

Intermittent fasting has gained recognition as a potential approach for weight loss and metabolic health enhancement; nevertheless, its impact on bone health is not yet well understood (13). In this study, the impact of IF on bone healing was histopathologically investigated using an experimental fracture model in rat tibiae. Although the impact of IF on fracture healing did not achieve statistical significance compared to controls, within the constraints of this study, the IF group demonstrated numerically enhanced bone healing.

Evidence indicates that sustained caloric restriction, whether mild or severe and with or without micronutrient supplementation, alone or combined with physical exercise, results in reductions in bone mass and deterioration of bone microarchitecture. The underlying mechanisms are thought to involve factors such as mechanical unloading, inadequate nutrient intake, and hormonal alterations (13, 14). The extent to

which IF is associated with detrimental effects on bone, akin to those seen with standard weight loss methods, versus its potential to promote bone health and offset skeletal deterioration remains uncertain. For example, IF is suggested to modulate metabolism through repeated cycles of extended fasting (catabolic phases) alternating with defined feeding periods (anabolic phases), and/or by aligning eating patterns with endogenous circadian rhythms. Enhanced metabolic regulation and alignment with circadian rhythms are considered advantageous for skeletal health; however, the overall impact of these factors and diverse IF protocols on bone remains incompletely understood (13-15).

Hisatomi et al. (16) reported that a 96-hour fast did not cause any major macroscopic changes in bone structure but resulted in a decrease in bone mineral density. Although our findings suggest that fasting does not adversely affect fracture healing, they appear to contradict those of Hisatomi et al., despite the implementation of a similar dietary intervention. These inconsistent findings may be explained by differences in fasting duration or by the specific methodologies employed to evaluate bone healing and bone mineral density. Contrary to the findings of Hisatomi et al. (16) that indicated a negative impact of fasting on bone health, our study showed that histopathological bone healing scores in the fasting group were numerically higher than those in the control group. However, this difference was not statistically significant, suggesting that IF does not adversely affect bone metabolism.

Kaya et al. (17) investigated the osseointegration mechanism of titanium implants—which is similar to the bone fracture healing process—using a biomechanical approach in a model involving IF and a high-fat diet. In their study, they found no statistically significant difference in osseointegration among the groups: IF, high-fat diet, high-fat diet combined with IF, and control. Kaya et al. (17) reported that IF does not impair bone metabolism or implant osseointegration and does not have a detrimental effect on bone tissue. Additionally, they found that a high-fat diet did not negatively affect osseointegration or bone tissue. Examination of the data

from this study indicates that there was no statistically significant difference in fracture healing between the control and fasting groups, suggesting that the fasting diet has neither a positive nor negative effect on fracture healing.

Yavuz et al. (18) investigated the effects of IF and a high-fat diet on bone healing using a biomechanical approach in rat tibiae with bone defects created around titanium implants, employing a guided bone regeneration model that shares a similar healing mechanism to bone fractures. Their data showed no statistically significant differences in healing outcomes between the IF, high-fat diet, combined high-fat diet and IF groups compared to

the control group. Moreover, they reported that neither the high-fat diet nor IF combined with a high-fat diet had a detrimental effect on bone healing. The findings from this study align with those of Kaya et al. (17), reinforcing similar conclusions regarding the lack of negative effects of these interventions on bone healing.

In conclusion, within the constraints of this study, intermittent fasting appears to exert neither beneficial nor detrimental effects on fracture healing. Further comprehensive studies are warranted to explore the relationship between fracture healing and intermittent fasting, including diverse clinical contexts and molecular-level analyses.

References

1. Wang C, Li C. Modern fixation techniques versus traditional tension band wiring for olecranon fractures: A systematic review and meta-analysis of functional outcomes, healing time, and complications. *J Orthop Surg Res* 2025;20(1):726.
2. Papadakis SA, Ampadiotaki MM, Pallis D, et al. Intramedullary fixation of tibial, femoral, and humeral fractures using an expandable nail: Case series and systematic review of the existing literature. *J Long Term Eff Med Implants* 2025;35(3):77-89.
3. He Q, Lu J, Liang Q, et al. Prg4+ fibroadipogenic progenitors in muscle are crucial for bone fracture repair. *Proc Natl Acad Sci USA* 2025;122(31):e2417806122.
4. Hausman MR, Schaffler MB, Majeska RJ. Prevention of fracture healing in rats by an inhibitor of angiogenesis. *Bone* 2001;29(6): 560-564.
5. Kurdy NM, Weiss JB, Bate A. Endothelial stimulating angiogenic factor in early fracture healing. *Injury* 1996; 27(2):143-145.
6. Gerstenfeld LC, Cho TJ, Kon T, et al. Impaired fracture healing in the absence of TNF-alpha signaling: The role of TNF-alpha in endochondral cartilage resorption. *Journal of bone and mineral research: The Official Journal of the American Society for Bone and Mineral Research* 2003;18(9):1584-1592.
7. Melnyk M, Henke T, Claes L, Augat P. Revascularisation during fracture healing with soft tissue injury. *Archives of Orthopaedic and Trauma Surgery* 2008;128(10):1159-1165.
8. Holstein JH, Karabin-Kehl B, Scheuer C, et al. Endostatin inhibits callus remodeling during fracture healing in mice. *Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society* 2013;31(10):1579-1584.
9. Ye Y, Lin Z, Chen Y, et al. Effects of intermittent fasting and calorie-restricted diet combined with moderate exercise in adults with overweight and obesity. *Nutrition* 2025;140:112904.
10. Sequeira I. Restoring bone healing potential. *Elife* 2025;14:e105420.
11. Liu X, Wu Y, Bennett S, et al. The effects of different dietary patterns on bone health. *Nutrients* 2024;16(14):2289.
12. Tanrisever M, Tekin B, Can UK, et al. The effect of local melatonin application on bone fracture healing in rat tibias. *Medicina* 2025;61:146.
13. Clayton DJ, Varley I, Papageorgiou M. Intermittent fasting and bone health: A bone of contention? *Br J Nutr* 2023; 130(9):1487-1499.
14. Papageorgiou M, Kersch-Schindl K, Sathyapalan T, Pietschmann P. Is weight loss harmful for skeletal health in obese older adults? *Gerontology* 2020;66(1):2-14.
15. Varady KA, Cienfuegos S, Ezpeleta M, Gabel K. Clinical application of intermittent fasting for weight loss: Progress and future directions. *Nat Rev Endocrinol* 2022;18(5):309-321.
16. Hisatomi Y, Kugino K. Changes in bone density and bone quality caused by single fasting for 96 hours in rats. *Peer J* 2019; 6: e6161.
17. Kaya CA, Guler R, Yavuz MC, et al. Does fasting and high-fatty diet effect osseointegration: An experimental study. *Niger J Clin Pract* 2025;28(1):19-26.
18. Yavuz MC, Guler R, Ozcan EC, et al. The investigation of bone-implant connection and new bone formation in fasting and high-fatty diet rats. *Niger J Clin Pract* 2024; 27(1):95-101.