Palestinian Medical and Pharmaceutical Journal (PMPJ). 2024; 9(4): 509-520

A Randomized Controlled Pilot Study on the Short-Term Impact of Meal Frequency on Bone Remodeling in Healthy Adults

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Received: (27/1/2024), Accepted: (18/4/2024), Published: (1/12/2024)

Abstract

Background: Bone remodeling is a continuous process involving the actions of osteoclasts followed by osteoblasts, which mineralize the newly synthesized bone matrix. There is a growing recognition of the influence of dietary patterns on bone remodeling, with implications for overall bone health. Meal frequency is a crucial factor affecting bone metabolism. Thus, this study aimed to investigate the impact of meal frequency on Procollagen Type 1 N-terminal propeptide (P1NP), a marker of bone remodeling. **Methods:** A total of 30 healthy adult males aged 19 to 30 years from Jordan were recruited through informational flyers and participated in a randomized controlled intervention trial. Participants were randomly assigned to either three or eight meals per day for three consecutive days (Phase 1). After a one-week washout period, the participants were switched to the alternate meal frequency for another three days (Phase 2). Blood samples were collected at baseline and after 3 days of both Phase 1 and Phase 2, with P1NP levels measured using an enzyme-linked immunosorbent assay". **Results:** Changes in meal frequency significantly impacted the blood bone formation biomarker P1NP in both phases, as indicated by a notable decrease compared to baseline (20.40 ± 7.85 mcg/L and 21.12 ± 4.17 mcg/L versus 28.90 ± 9.06 mcg/L) ($P<0.05$). However, no significant differences were observed between the P1NP results for the 3-meal and 8-meal groups ($P=0.663$). Notably, despite the differences in the calculations (20.40 ± 7.85 mcg/L vs. 21.12 ± 4.17 mcg/L, respectively), we did not observe significant differences. **In conclusion:** This study demonstrated the significant impact of meal frequency on the blood bone formation biomarker P1NP, revealing a consistent decrease in both phases compared to baseline. While no substantial differences were observed between the 3-meal and 8-meal groups, these findings contribute valuable insights into the intricate relationship between dietary patterns and bone metabolism, emphasizing the need for further research to elucidate the nuanced dynamics of the effect of meal frequency on bone health.

Trial registration: ClinicalTrials.gov NCT06359483

Keywords: Bone Remodeling, P1NP, Bone Turnover Biomarkers, Meal Frequency.

INTRODUCTION

Bone is a metabolically active structure that is constantly changing throughout life [1]. Bone modeling is the process by which bones are molded or modified by the separate action of osteoblasts and osteoclasts. It is responsible for the formation and movement of bones and determines skeletal development and growth [2].

Even after skeletal maturity, the bone regeneration process continues through the periodic replacement of old bone with newly created bone at the same site, known as remodeling [3]. Bone remodeling is a lifelong process. This process involves the removal of mineralized bone by osteoclasts followed by the formation of bone matrix by osteoblasts that subsequently become mineralized [4].

Several biomolecules known as bone turnover markers (BTMs) are released into the blood and eliminated in the urine after peak bone mass and bone remodeling [5]. In addition, measuring BTMs yields a quantitative measure of current turnover,

which is used to determine the rate of bone remodeling [6]. BTMs are classified into two categories: enzymes secreted by osteoclasts and osteoblasts and structural proteins or fragments secreted by osteoblasts during bone formation or released by bone resorption when the collagen matrix is degraded [5].

Type 1 collagen breakdown products (N-telopeptide of type 1 collagen [NTX] and C-telopeptide of type 1 collagen [CTX]) are resorption-specific BTMs, while type 1 collagen synthesis markers (N- terminal propeptide type I procollagen [PINP]), osteoblast enzymes (bone-specific alkaline phosphatase [BAP]), or bone matrix proteins are formation-specific markers (osteocalcin) [7].

Recently, the serum CTX-I and PINP have been designated gold standard markers of bone resorption and formation, respectively [8].

Nutrition is a modifiable factor that plays a crucial role in optimizing bone health [9]. There is a growing body of evidence indicating that various dietary factors, including meal composition, meal size, caloric intake, meal timing, and feeding frequency, can induce acute changes in bone turnover biomarkers [5]. Calcium (Ca) and phosphorus constitute between 80 and 90% of the mineral content of bones. Another vital nutrient is protein, which is incorporated into the organic matrix of bone to form collagen, initiating the mineralization process. While calcium has been the primary focus of much-related research, normal bone metabolism relies on other minerals, such as magnesium, fluoride, zinc, copper, iron, selenium, and vitamins D, A, C, K, and folate [32]. The observed acute reduction in bone metabolic markers during feeding underscores the notion that bone is a tissue responsive to nutritional modulation [10].

Furthermore, given the rapid increase in the incidence of osteoporosis, elucidating the effects of a specific number of days following a certain meal frequency intake on bone remodeling and turnover is highly important [11]. Blood PINP has been proposed as one of the reference measures of bone turnover for monitoring the treatment of osteoporosis and fracture risk prediction [31].

Therefore, this study hypothesizes the following: A meal frequency of 8 meals/day will lead to significant alterations in blood bone formation biomarker levels compared to a meal frequency of 3 meals/day while maintaining an equivalent total energy intake in healthy adults aged between 19 and 30 years. Furthermore, if three days of specific meal frequency intake have a significant impact on the P1NP biomarker associated with bone health, it suggests a rapid responsiveness of bone formation to changes in dietary patterns.

This study addresses a significant gap in the literature regarding the impact of meal frequency on blood bone formation biomarker levels in healthy adults. Although various aspects of diet and bone health have been studied, little is known about how meal frequency in particular affects biomarkers linked to bone formation. By focusing on the comparison between a meal frequency of 8 meals/day and 3 meals/day, while keeping total energy intake constant, this study sheds light on the potential effects of meal timing and frequency on bone health biomarkers. Moreover, by examining the effects over three days, the study aims to clarify the promptness with which bone production reacts to dietary patterns, a novel concept that has not been thoroughly discussed in previous research. Therefore, this study covers a significant knowledge gap in the field by providing insightful information about the complex relationship between meal frequency and biomarkers associated with bone health.

METHODS

Research Design

A randomized controlled intervention pilot trial was conducted among a group of healthy Jordanian males aged between 19 and 30 years to investigate the impact of meal frequency on the blood procollagen biomarker P1NP for bone remodeling (Figure 1). Given the variability of BTMs daily, a one-week washout period was implemented to allow P1NP levels to return to baseline. Subsequently, the two groups underwent reciprocal switching for three consecutive days.

In this study, the researcher opted for a 3-day trial period due to several considerations. Firstly, a thorough review of existing literature on bone biomarkers showed that most of the studies used 2- or 3-day trial lengths, consistently yielding significant outcomes within this timeframe [11] [26] [30]. Secondly, given the dynamic nature of bone biomarkers, and sensitivity to daily fluctuations, a 3-day trial period was considered suitable for capturing any potential alterations and trends over a short timeframe [37]. Moreover, financial limitations and other practical considerations necessitated a short period, to guarantee that participants would have access to necessary supplies, such as food.

As a result, the choice to implement a 3-day trial period was cautiously considered, intending to reach a balance between practical constraints and scientific rigor.

Three fasting blood samples were collected in the morning, marking the baseline at the start of the study. Subsequent samples were obtained after the 3rd day of the first phase, and the last sample was taken following the 3rd day of the second phase. The designated one-week washout period was deemed adequate for P1NP patients to return to baseline, facilitating a comprehensive investigation of the impact of meal frequency on bone remodeling in the subsequent three-day switch between the two groups.

We verify that all methods in this study were executed in strict adherence to the pertinent guidelines and regulations established by the Ethical Committee Institutional Review Board (IRB) with the assigned number 108/2021. The experimental protocols employed in this research received approval from The University of Jordan. The study's purpose, objectives, methodology, and confidentiality were verbally communicated to all participants, who were also informed of their right to withdraw from the study at any point. Informed consent was obtained from all subjects as an integral component of our ethical research practices.

Experimental Protocol

Participants and randomization

In early June 2021, a sample of 130 participants of adult males aged between 19-30 years was recruited randomly using recruitment forms posted on social media platforms (Facebook, Instagram, and LinkedIn). Participants were assessed for eligibility to participate in this study. Out of the initial pool, 50 healthy adult males met the inclusion criteria. One hundred participants were excluded from the study based on various criteria: thirty-five participants were shift workers, ten adhered to specific dietary patterns, seven were diagnosed with hypothyroidism, two had diabetes, nine had experienced a fractured bone within the last six months, ten exhibited irregular sleeping patterns, twenty were currently using calcium and vitamin D supplements, and seven expressed a lack of willingness to continue participation in the trial. Only 30 participants were recruited due to financial constraints related to laboratory testing and food preparation.

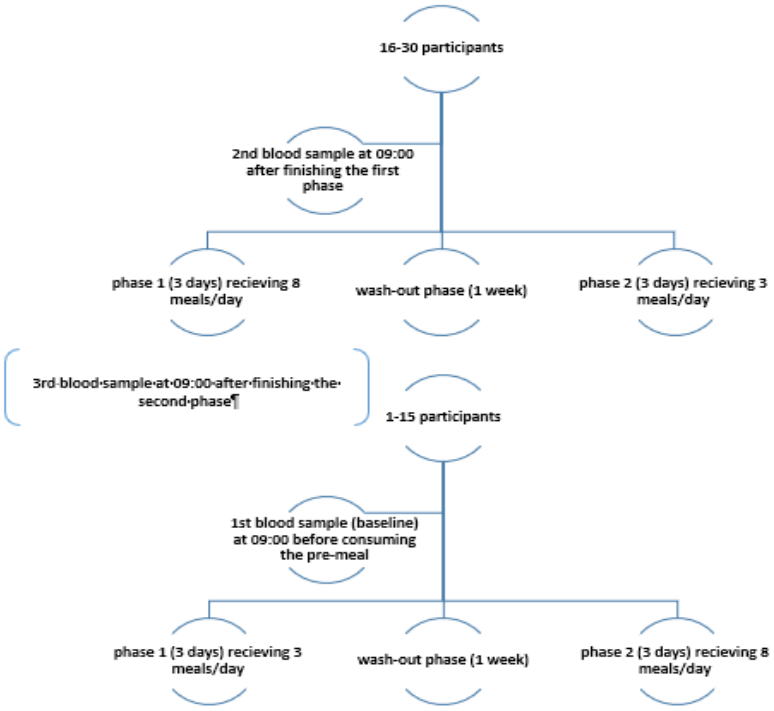

Figure (1): Research design.

The inclusion criterion for participants was healthy adult males between the ages of 19 and 30 years, while the exclusion criterion included individuals with medical conditions affecting bone remodeling, such as hyper/hypothyroidism, diabetes, cancer, renal problems, Paget's disease, Cushing's disease, multiple myeloma, rickets, osteomalacia, hypogonadism, osteoporosis, metastatic carcinoma, Gaucher's disease, and hairy cell leukemia. Additionally, individuals with abnormal food habits, including night eating or frequent diet changes, as well as shift workers, daytime sleepers, and those with irregular sleeping patterns, were excluded. The exclusion criteria further extended to individuals taking medications or supplements impacting bone remodeling, calcium homeostasis, or sleep patterns and those who had experienced a broken or fractured bone within the last 6 months before the study.

The rationale behind exclusively enlisting male volunteers was to eliminate the potential influence of maternal hormones, such as estrogen, on bone remodeling. This choice is rooted in the known direct actions of estrogen on osteocytes, osteoclasts, and osteoblasts, which inhibit bone remodeling, reduce bone resorption, and maintain bone creation, respectively [33]

Each participant was assigned a different registration number, and using a random assignment method, even-numbered participants were allocated to the group receiving 3 meals/day, while odd-numbered participants were assigned to the group receiving 8 meals/day. The decision to opt for 8 meals/day aligns with the Clinical Nutrition guidelines, which recommend small, frequent meals within the range of 6-10 meals [34].

Meals

The meals in both phases were standardized to contain an equivalent amount of energy and macronutrients and were monitored following sex-specific and dietary guidelines outlined in the 2015–2020 Dietary Guidelines for Americans [35]. To address the potential for hunger and ensure participants' compliance with meal composition, average total energy requirements were calculated, incorporating an additional 15%. Specifically, the total energy was set at 2200 + 15% equals 2530 calories, the protein content was 158 grams (25%), the carbohydrate content was 284 grams (45%), and the fat content was 84 grams (30%) [36].

Food quality was rigorously controlled, with all participants receiving the same type of food throughout the study. The sole variable under consideration in this investigation was the frequency of meals. The original three meals were restructured into eight pieces, consisting of breakfast, lunch (divided into two portions), and dinner (divided into four portions). This meticulous approach to controlling variables ensures that any observed effects on the outcome can be attributed specifically to changes in meal frequency.

Meals were cooked and prepared as follows: breakfast consisted of cheese manousheh, thyme manousheh, cucumber, tomato, and a medium banana. Lunch consisted of cooked rice with chicken breast, yogurt, and a bar of chocolate. Dinner comprised cheese and turkey sandwiches, cucumber, and tomato, the food was prepared by researchers at the University of Jordan's food preparation laboratory, which is subjected to the health system, food quality control, and hygienic standards.

Meals were delivered in thermogenic plastic bags by a delivery man to the participants the day before, to consume them at the scheduled time regardless of location. Scales used to weigh food are calibrated to the nearest (0.1).

Three Meals/Day Phase: In this phase, each meal consisted of 840-850 calories. The first pre-meal (Dinner) was served at 8:00 pm the day before. Day 1, 2, and 3: the first meal (Breakfast) at 10:00 am, the next meal (Lunch) at 3:00 pm, the third meal (Dinner) at 8:00 pm. All blood samples were collected at 9:00 AM while fasting. The first blood sample was collected the day before starting the study (at baseline) before consuming the pre-meal. The second blood collection was after the last day of phase 1. The third blood sample was collected after the last day of phase 2.

Eight Meals/Day Phase: In this phase, each meal consisted of 310-320 calories. The first pre-meal (4 portions of the dinner) was served at 5:00 pm, 7:00 pm, 9:00 pm, and 11:00 pm the day before. Day 1, 2, and 3: the first meal (2 portions of breakfast) at 9:00 am and 11:00 am, the next meal (2 portions of lunch) at 1:00 pm and 3:00 pm, the third meal (4 portions of dinner) at 5:00 pm, 7:00 pm, 9:00 pm, and 11:00 pm. All blood samples were collected at 9:00 am while fasting. The first blood sample was collected the day before starting the study (at baseline) before consuming the pre-meal. The second blood collection was after the last day of phase 1. The third blood sample was collected after the last day of phase 2.

Sample collection and analysis

The participants were scheduled to attend the laboratory on three occasions throughout the study: the day preceding the commencement of the study (baseline), the day after the first phase, and the day following the second phase. Blood samples were acquired by a licensed laboratory technician after an overnight fast at 9:00 am. Blood samples were collected in serum separator tubes, allowed to clot for two hours at room temperature or overnight at 4°C, and then subjected to centrifugation for 20 minutes at approximately 1000 ×g. The resultant serum was aliquoted and stored at -80°C until analysis.

P1NP analysis was conducted utilizing a standard Enzyme-linked Immunosorbent Assay Kit for P1NP (Multiskan Go Spectrophotometer, Model 1510; Thermo Fisher, UK) following the manufacturer's instructions. The assay kits, including the standard, detection reagent A, detection reagent B, and 96-well strip plate reagents, were stored at -20°C upon receipt, while the other components were stored at 4°C. Before use, all the kit components and samples were heated to room temperature (18-25°C).

Statistical analysis

All the statistical analyses were conducted using SPSS software version 27 (IBM, Armonk, NY, USA). The data were tested for normality, confirming a normal distribution. Descriptive statistics are presented for sociodemographic data, and values for blood serum tests are expressed as the mean ± standard deviation (SD).

To explore potential relationships between phases and assess the impact of sociodemographic data on P1NP values in phases 1 and 2, correlation tests were performed. Paired sample T-tests were used to

ascertain significant differences between baseline and phase 1 P1NP values and between baseline and phase 2 P1NP values. To compare P1NP values across the three phases, ANOVA was employed, followed by post hoc analysis using the LSD test to identify the specific phases contributing to any observed differences. A P value of <0.05 was considered to indicate statistical significance.

RESULTS

General characteristics and the sociodemographic data of the study sample

The general characteristics and the sociodemographic data of the study sample are listed in Table 1. Age, weight, height, and BMI were recorded. In addition, the participants were encouraged to smoke and sleep.

Table (1): General characteristics and sociodemographic data of the study sample.

General Characteristics	Mean ± S.D.
age (years)	22.07 (19, 29)
weight (kg)	76.49 (58, 100)
height (cm)	1.77 (1.5, 1.97)
BMI (kg/m²)	24.61 (17.9, 34.2)
Smoking	**N (%)**
Smokers (5-<10 cig/day)	9 (30.0%)
Nonsmokers	21 (70.0%)
Sleep pattern N (%)	
Regular (7-9 hrs/night/week)	9 (30.0%)
Irregular*	2 (6.7%)
Sometimes (7-9 hrs/night-3-4 days a week)	19 (63.3%)

*General characteristic data is presented as the mean ± standard deviation (SD). BMI: body mass index. Smoking and sleep pattern data are presented by sample size (N) and frequency (%). Sleep pattern (Irregular) means that participants do not sleep between 7-9 hours per night the whole week.

Correlations between General Characteristics and the Sociodemographic Data of the Study Sample and P1NP

Table 2 shows the correlations between the study characteristics and sociodemographic data and between the study characteristics and blood P1NP results in the three phases. There was no significant correlation between any of the data collected and blood P1NP levels in any phase (P>0.05).

Table (2): Correlations between General Characteristics and the Sociodemographic Data of the Study Sample and P1NP.

P1NP Results	Age	BMI	Smoking	Sleep pattern
Baseline (28.90 ± 9.06)	0.046 (0.810)	-0.136 (0.473)	0.051 (0.787)	-0.237 (0.207)
3 meals (20.40 ± 7.85)	-0.266 (0.155)	0.253 (0.177)	0.079 (0.679)	-0.255 (0.173)
8 meals (21.12 ± 4.17)	-0.174 (0.358)	0.204 (0.279)	-0.284 (0.129)	0.220 (0.243)

Correlation is statistically significant at p< 0.05. BMI; body mass index.

Blood Bone Formation Biomarker Measures for the Study Sample

Figure 4 shows the blood bone formation biomarker (P1NP). The figure shows the mean values for the biomarkers in the three groups of the study and the significant differences between them. The analysis revealed statistically significant differences among the three groups, with a p-value of **0.001*** (P<0.05). Significantly lower values were found for 3 meals (P1NP: 20.40 ± 7.85 mcg/L) and 8 meals (P1NP: 21.12 ± 4.17 mcg/L) than for their baseline measures (28.90 ± 9.06 mcg/L, P value <0.05).

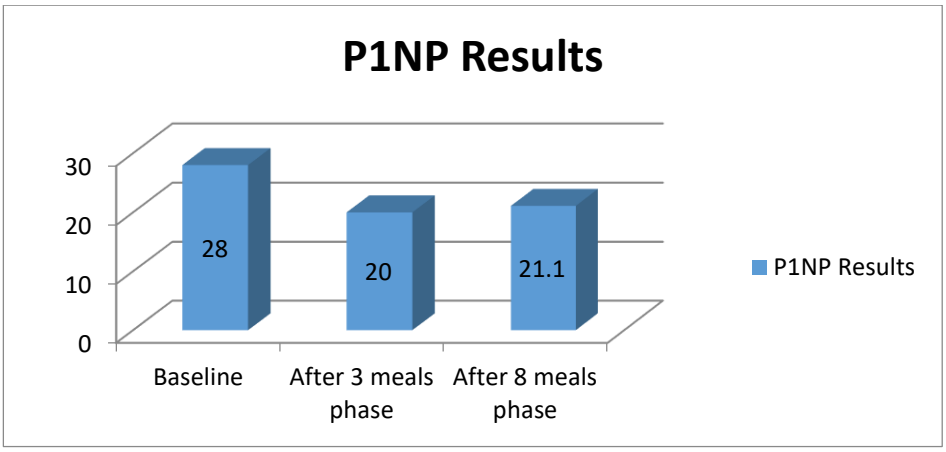

Figure (2): Blood Biomarker Measures for the Study Sample. *

*P1NP results are presented as the mean ± standard deviation (SD) and were considered to be significantly different at p<0.05.

Comparison of Blood Bone Formation Biomarkers

Table 3 presents the comparison of P1NP results among the three groups. The table shows significant differences between the baseline P1NP (28.90 ± 9.06 mcg/L) and 3-meal P1NP results (20.40 ± 7.85 mcg/L) (P=0.001). Moreover, a significant difference was found between the baseline P1NP level (28.90 ± 9.06 mcg/L) and the P1NP level (21.12 ± 4.17 mcg/L) (P=0.001). No significant differences were detected between the P1NP results for 3 meals and the P1NP results for 8 meals (P=0.663), although there were differences (20.40 ± 7.85 mcg/L) (21.12 ± 4.17 mcg/L).

Table (3): Comparison of Blood Bone Formation Biomarkers*.

Variable	Groups	t-test (P value)
PINP	Baseline (28.90 ± 9.06 mcg/L) vs. 3 meals (20.40 ± 7.85 mcg/L)	0.001*
PINP	Baseline (28.90 ± 9.06 mcg/L) vs. 8 meals (21.12 ± 4.17 mcg/L)	0.001*
PINP	3 meals (20.40 ± 7.85 mcg/L) vs. 8 meals (21.12 ± 4.17 mcg/L)	0.663

*P1NP results are presented as the mean ± standard deviation (SD) and were considered to be significantly different at p<0.05. The test used was the paired-sample t-test.

DISCUSSION

The evidence has demonstrated the dynamic nature of bone remodeling, a continuous interplay between osteoblasts and osteoclasts crucial for maintaining a healthy bone matrix [2][4]. Given the increasing impact of dietary habits on bone health [9], particularly on bone remodeling [5], this study delves into the specific influence of meal frequency on bone metabolism.

In this randomized controlled intervention trial involving thirty healthy adult males aged 19 to 30 years, participants were assigned to consume either three or eight meals a day for three consecutive days (Phase 1). Subsequently, they switched to the alternative meal frequency for an additional three days (Phase 2) after a one-week washout period. Blood samples collected at baseline and after 3 days in each phase provided insights into the impact of meal frequency on P1NP, a key marker of bone remodeling, as assessed through an enzyme-linked immunosorbent assay.

The results revealed a significant decrease in P1NP levels during both phases compared to baseline, suggesting the substantial influence of meal frequency on this blood-bone formation biomarker. Despite the observed differences between the 3-meal and 8-meal groups, no significant differences were evident, prompting further exploration into the intricate dynamics of meal frequency and its implications for bone health.

Previous research has shown that feeding can impact bone remodeling by suppressing

both bone resorption and formation markers. Feeding has been associated with a reduction in bone resorption markers of approximately 20–40%, while the suppression of formation markers is less pronounced, typically falling below 10% [12][13][14]. This acute reduction in bone metabolic markers upon feeding underscores the notion that bone is responsive to nutritional modulation, with formation markers being influenced to a lesser extent than resorption markers [10].

To our knowledge, this study represents the first exploration of the relationship between meal frequency and bone formation biomarkers in humans. Bone biomarkers exhibit day-to-day variations [15]. The hypothesis that altering meal frequency affects bone turnover biomarkers was validated by the significant differences observed in blood bone formation biomarkers, particularly P1NP, following changes in specific meal frequencies over three days. P1NP levels were significantly lower in both Phase 1 (3 meals) and Phase 2 (8 meals) than they were at baseline ($P<0.05$).

Despite a notable decrease in P1NP levels during both meal frequency phases, the three-meal-a-day phase presented lower P1NP levels than did the 8-meal-a-day phase. Even though there was no statistically significant difference in P1NP levels between the 3-meal and 8-meal groups, these observed differences may still have practical implications and need more research. These differences could direct potential trends or hypotheses for future research into the effects of meal frequency on bone remodeling, even if the effects were not statistically significant in this study.

Adopting a small, frequent meal may help reduce bone resorption and enhance bone balance, as evidenced by a study in mice showing a decrease in bone resorption following meal consumption when solid food and liquid were separated, resulting in increased bone mass after 30 days [16]. Therefore, adopting a regimen of small, frequent meals is recommended for supporting bone health.

However, it is essential to note that research on the impact of meal frequency on bone turnover biomarkers, especially bone formation markers, in humans is limited [19]. While dietary composition and quality are often considered in nutritional discussions, the frequency of meals is a significant factor [20].

Given the rising prevalence of bone-related conditions, particularly osteoporosis [21], our study highlights the benefits of distributing meals throughout the day to enhance bone formation. In contrast, a study showed that two daily hypoenergetic meals improved insulin sensitivity compared to six daily hypoenergetic meals, potentially contributing to improved bone strength [22][23]. However, additional research is needed to explore the impact of meal frequency on bone turnover biomarkers in humans.

The daily rhythm in BTMs suggests that disruptions in sleep physiology and circadian rhythmicity may negatively affect bone health [25]. Although our findings revealed no significant correlation between participants' sociodemographic data and P1NP results in either phase, it is important to note that the lack of significant differences in our data may be attributed to the circadian pattern of bone remodeling, with high bone resorption occurring at night and peak bone formation during the day [26], and to the relatively lower sensitivity of bone formation markers than resorption markers. Additionally, urinary markers exhibit more variability than serum markers [27]. Moreover, most BTMs exhibit significant intrasubject fluctuations, posing a significant challenge in the practical application of bone markers [5].

Despite these insights, this study has several limitations. The sample size was relatively small, limiting the generalizability of the findings to larger populations. However, small sample sizes are associated with lower statistical power, which refers to the probability of detecting a true effect if it exists. Studies with low statistical power are more likely to yield inconclusive results or fail to detect significant effects, even when they genuinely exist [39]. In addition, budget constraints also led to the measurement of only one blood bone formation biomarker P1NP rather than all three formation biomarkers. The intervention period was limited to three days due to budget constraints. Short intervention durations may not allow

sufficient time to observe significant changes or effects. Many interventions, especially those targeting behavioral or lifestyle changes, require time for participants to adapt and for the effects to manifest. Moreover, Short interventions might yield immediate results, but their long-term sustainability may remain uncertain [40]. Thus further research with longer durations is necessary.

This study is the first to investigate the impact of food frequency on blood bone formation markers utilizing a recognized reference marker P1NP for assessing fracture risk and monitoring therapy. The inclusion of light smokers as a demographic factor in bone biomarker research further contributes to a more comprehensive understanding of these factors. These results have clinical significance demonstrating the importance of considering meal timing in dietary recommendations for bone health management.

CONCLUSIONS

In conclusion, our investigation elucidated the substantial impact of meal frequency on the blood bone formation biomarker P1NP, which was consistently lower during both phases than at baseline. Although no significant differences were found between the groups consuming 3 meals and those consuming 8 meals, these results emphasize the complex relationship between dietary patterns and bone metabolism. Further research with longer durations is necessary to fully understand the complex dynamics of meal frequency and its unique effects on bone health, given the observed numerical differences in the analyses.

Ethical Approval and Consent to participate.

This study was approved by the Ethical Committee Institutional Review Board (IRB) (number 108/2021), and the investigators were assessed if they met established ethical standards.

Clinical Trial Registration

This study was approved and registered by Clinical Trials. gov number (NCT06359483)

Consent for publication

Patients provided signed informed consent for the publication of their data and photographs.

Availability of data and material

Available upon request. You can contact Dr. Hadeel Ali Ghazzawi at h.ghazzawi@ju.edu.jo

Authors' contributions

Areen Haddad: Conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis, and wrote the paper. **Hadeel Ghazzawi:** Project administration, supervision, validation, and writing review & editing. **Laila Albardan:** Writing review & editing. **Haitham Jahrami:** Supervision, revise the manuscript. **Adam Tawfiq Amawi**: recruit participants and help in the editing.

Conflict of Interest:

No conflicts of interest exist.

Funding

This study was funded by the Deanship of Scientific Research at the University of Jordan.

Acknowledgments

– We would like to extend our sincere gratitude to everyone who assisted in this research attempt and the preparation of this manuscript.

– We would like to thank the participants who generously volunteered their time and insights, without whom this study would not have been possible. Their collaboration and ability to share their experiences helped us to make our findings more insightful and applicable.

– Financial support from the University of Jordan is gratefully acknowledged. This research was made possible through their kind funding, which enabled us to purchase the necessary equipment and resources for our experiments.

– We also like to thank the faculty and facilities of the University of Jordan for helping us with different parts of our

research. Their knowledge and resources made our work much easier.

– Finally, we want to express our gratitude to our friends and family for their tolerance, inspiration, and moral support during the long hours of research and writing.

– Without the teamwork of everyone indicated above, this research would not have been possible. Any mistakes or omissions in this document are our own, and they should not be taken to be an indication of the contributions of those acknowledged.

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