How to Cite:
Boun, S. M. A., Hani, K. M. B., Khreisat, R. F., Jaradat, R. Q., Alboun, A. M., & Al_Quraan, K. M. (2022). Low protein intake impacts on bone mineral density. *International Journal of Health Sciences*, *6*(S8), 6096–6110. https://doi.org/10.53730/ijhs.v6nS8.13716

Low protein intake impacts on bone mineral density

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***Abstract*---**Background/Aim: Protein may play a positive beneficial role in the augmenting Bone Mineral Density (BMD), mitigation bone resorption and consequently risk of osteoporosis. The effect of dietary protein may be different in older adults compared to younger adults, since this population has a greater need for protein. The aim of this study is to investigate the impact of a dietary protein intake below the Recommended Dietary Allowance (RDA) of 0.8 g/kg /day from any source (Group I) on Bone Mineral Density (BMD) to a higher dietary protein intake ≥0.8 g/kg/day in our Jordanian osteoporotic patients. Methods: This study trial was a single-center retrospective study, conducted for 206 participants who attended the rheumatology clinic between Sep 2021 and Nov 2021 at Prince Rashid Bin Al-Hasan

Manuscript submitted: 9 July 2022, Manuscript revised: 18 Sept 2022, Accepted for publication: 27 Oct 2022

Military Hospital under ethical approval of Ref #24, 1/2022. The results were expressed as Mean±SD and as Mean difference±SEM. The correlation strengths of binary categorical variables were also expressed as an odds ratio. FRAX score of 3% was used in our study to dichotomize the overall cohort into two cohorts. Univariate and Multivariate Linear Regression Tests were conducted sequentially to abstract the significant coefficients for predicting the 10-year $_{f}$OHF risk. Run participant's regression results were used to investigate the area under the ROC curve (AUROC) and to explore the optimal cut-off point. Results: The AUROC for the constructed ROC based on the run participant's composing data into our proposed multivariate linear regression equation against the 10-year risk of fHOP_FRAX score ≥3% (1) or <3% (0), was 0.992±0.05 (95% CI; 0.983-1). Also, we explored the optimal operating dichotomized level of 3.45% for our proposed regression equation to discriminate between lower risk and higher risk cohorts regarding the 10-Year risk of fHOP fracture with the prognosticating performance of 97.27%. Conclusion: In this study, we concluded that as long as the minimum PD didn't decrease below 2.5 g/100 Cal and the number of OsCal-D tablets was above 2 tablets per day in Jordanian participants who maintained regular Fruits/Vegetables consumption pattern and active daily lifestyle, the risk of osteoporotic fracture will be maximally 2.924%.

***Keywords*---**dietary protein, protein density, bone mineral density, osteoporosis, dual energy X-ray absorptiometry.

Introduction

In childhood and adolescence, there is a relatively large need for dietary calcium and protein. Peak bone density (PBD) is achieved after age 20 years and is maintained through the 30-40 years. During the women's menopause phase, there is an accelerated bone resorption owing to loss of estrogen which ultimately weaken post-menopausal bone architecture. [1-3] Oppositely, men have a much slower bone resorption rate, leading to osteoporotic related fracture at more advanced age. It is important to peak bone mineral density (BMD) as early as possible to mitigate the incidences of ageing related osteoporosis as much as possible. [4-5]

While Osteoporosis is generally characterized by reduced BMD and strength, deterioration in microarchitectural structures is consequently associated with increasing skeletal fragility and higher risk of fracture. Peak bone density and overall BMD status are affected by several modifiable and non-modifiable co-factors. [6] Although the genetic factors are the most important contributing factors for determining BMD, they are unmodifiable and therefore it is important for public health interventions to focus on modifiable factors, Hormonal, nutritional, and mechanical factors can positively modify BMD if they are early taking into consideration. [7] Replacement of sex hormones and insulin like growth factor in deficit affected patients, optimizing bone supporting nutrients intake in an adequately and a sustainable manners, and ensuring reasonable physical activities, are mandatory for ensuring maximizing BMD in early life. Of important, nutritional factors including dietary nutrients such as calcium, phosphorus, magnesium, potassium, and protein. [8-10]

Adequate protein ensures an optimal lean body mass (LBM). Protein intake stimulates the release of the hormone Insulin Growth Factor-1 (IGF-), which increases muscle mass and bone growth. This partially explains observations that higher LBM is associated with increased BMD. Many observational studies have shown positive clinical impacts of higher protein intake and improved bone health. A value of 0·8 g/kg/d are used by many nutritional agencies with some recommending higher values in certain circumstances. [11-13] It has been hypothesized that adequate protein intakes are associated with higher BMD. Previous studies yield conflicting results and thus we aimed to investigate the impact of a dietary protein intake below the Recommended Dietary Allowance (RDA) of 0.8 g/kg /day which is equivalent to 2.5 g/100 Cal (Group I) on Bone Mineral Density (BMD) to a higher dietary protein intake ≥0.8 g/kg/day in our Jordanian participants who attended the rheumatology clinic.

Material and Methods

This study trial was a single-center, non-funded and non-sponsored retrospective study, conducted for 206 participants who attended the rheumatology clinic between Sep 2021 and Nov 2021 at Prince Rashid Bin Al-Hasan Military Hospital, Royal Medical Services, Irbid, Jordan. Ethical approval was signed by our Institutional Review Board (Ref #24, 1/2022). An informed consent form was waived owing to the study's retrospective design. Jordanian attended aged male (≥ 60 years) and post-menopausal female participants were included in this study. Participants with hip or vertebral anatomy malformity, subjects with a history of renal or non-renal related metabolic osteodystrophy, secondary osteoporosis, cancer affected patients with bone metastasis, participants' history of hip or vertebral osteoporotic fracture, and prior use of any bisphosphonate were excluded from the study.

Dual-emission X-ray absorptiometry (DEXA) scans of the proximal femoral hip and anteroposterior spine participant’s data were retrieved from the DEXA recorded database. Retrievable DEXA recorded database in our study included femoral Hip T-Score ($_f$HipT-Score), femoral Hip Z-Score ($_f$HipZ-Score), femoral Hip BMD in g per cm2 (f Hip BMD), Lumbar T-Score, Lumbar Z-Score, Lumbar BMD, the 10-year risk of femoral osteoporotic fracture (10-year $_f$OHF risk) FRAX score, and 10-year risk of major overall osteoporotic fracture FRAX score. A countable protein density was used in our study to dichotomize the overall cohort into two cohorts; Attended rheumatological clinic patients who were on Standard Protein Density (≥2.5 g/100 Cal) (Group I) and attended rheumatological clinic patients who were on Low Protein Density (<2.5 g/100 Cal). (Group II).

The comparative non-dichotomous variables between Group I and Group II were statistically analyzed by Independent T-Test and the results were expressed as Mean±SD and as Mean difference±SEM. While the comparative variables for the total sample were analyzed by One-Sample T-Test and the results were also expressed as Mean±SD. For dichotomous categorical data, a Chi-Square Test was used to express the analysis outcomes as Numbers (Percentages). The correlation strengths of binary categorical variables were also expressed as an odds ratio.

The FRAX score-based variables that were tested in our study include age, weight, height, sex, smoking, history of fractures, parental history of fractures, use of glucocorticoid medications, having secondary osteoporosis**,** drinking three or more units of alcohol per day, bone mineral density. Co-Morbidities were also tested in our study and including, Rheumatoid Arthritis (RA), Hypertension (HTN), Diabetes Mellitus (DM), Chronic Kidney Disease (CKD), Peptic Ulcer Disease (PUD), Cardiovascular Disease (CVD).

In addition to FRAX score-based composite variables, dietary and lifestyle independent variables were included in our study to explore their combined interactive explanation for the prediction of our target of interest (The 10-year fOHF risk). The density of protein consumed in grams per 100 Calories (PD), the consumption pattern of fruits/vegetables (FVCP), the number of daily calcium/Vit D tablet intake (OsCal-D), and the lifestyle of daily activities (ADLs) were the 4 tested variables that were run individually into Univariate Linear Regression and collectively into Multivariable Linear Regression analysis. Both the FVCP and ADLS were dichotomized into intermittent versus regular patterns and sedentary versus active lifestyles. The daily OsCal-D tablets were categorized into 1 Tab/day, 2 Tab/day, 3 Tab/day, or 4 Tab/day. Dietary PD in g/100 Cal was roughly estimated based on the attended rheumatology clinic participants' weekly average consumption quantities of foods that are mentioned in Figure 2. Also, the participants' PDs were dichotomized into either below 2.5 g/100 Cal or above 2.5 g/100 Cal.

Firstly, we pursued to conduct a Univariate Linear Regression Test for the 4 tested variables to individually explore the degree of correlation (R), how much of the total variations in the dependent variable can be explained by the independent variables and a proportion of variation accounted for by the regression model above and beyond the mean model (Coefficient of determination or R^2), how much the quality of the prediction of the dependent variable, and how the regression model is a good fit for the data (F-Ratio in the ANOVA table). Also, this test was conducted to abstract the necessary coefficients to individually predict the 10-year $_f$OHF risk. Thereafter, The Multivariate Linear Regression Test was followed to composite the predictive variables that were significantly correlated and to fit these significant explanatory variables into a proposed multivariate linear regression model, after abstracting the significant coefficients, to collectively predict the 10-year $_f$OHF risk.

After the multivariable linear regression model was constructed, we ran participant composed data into this constructed proposed multivariable linear regression equation into receiver operating characteristic (ROC) and sensitivity analyses to investigate the area under the ROC curve (AUROC) and to explore the optimal cut-off point, sensitivity, specificity, positive and negative predictive values, Youden and accuracy indices, and the negative likelihood ratio for the 10-Year Risk of f Hip OPF percentages based on our proposed multivariable regression equation results. Statistical analysis was performed using Statistical

Package for Social Science (SPSS) software version 23.0. Statistical significance was set at 5%.

Results

This study included a total of 206 participants who attended the rheumatology clinic between Sep 2021 and Nov 2021 at Prince Rashid Bin Al-Hasan Military Hospital, Royal Medical Services, Irbid, Jordan. 51.94% of the tested cohort (107 participants) had an estimated protein density ≥2.5 g/100 Cal and so they were allocated to Group I, while the remaining 48.06% of the tested cohort (99 participants) had an estimated protein density <2.5 g/100 Cal and so they were grouped to the other comparative group (Group II). The overall mean age of participants was 59.88±1.673 years. Group I participants were insignificantly older than Group II participants [59.99±2.00 years versus 59.76±1.22 years, respectively, +0.23±0.23years, P-value=0.32).

Most of the attended rheumatological clinic participants in our study belonged to the female gender. Female to male ratio (F: M) was 5.87: 1 [176 (85.4%) versus 30 (14.6%), respectively, P-value=0.426] in which 14% (15 Men) and 86.0% (92 Women) belonged to Group I compared to 15.2% (15 Men) and 84.8% (84 Women) in Group II. The menopausal onset means age for female participants was 48.41±4.41 years which was also insignificantly distributed across Group I-II [48.47±4.67 years vs 48.35±4.15 years, respectively, +0.12±0.67 years, p-value=0.86]. Anthropometrically, all assessed and calculated participants' anthropometrics were insignificantly distributed between the two comparative groups. Bodyweight (BW), height, body mass index (BMI), ideal body weight (IBW), and adjusted body weight (Adj_BW) were 76.80±7.89 Kg, 160.71±8.65 cm, 30.11±5.27 Kg/m^2, 56.33±7.29 Kg, and 64.52±4.48 Kg vs 76.91±7.47 Kg, 160.11±7.20 cm, 30.21±4.43 Kg/m^2, 55.89±6.29 Kg, and 64.27±4.46 Kg, respectively, p-value>0.05.

All retrievable DEXA recorded database of f HipT-Score, f Hip Z-Score, f Hip BMD, Lumbar T-Score, Lumbar Z-Score, and Lumbar BMD were significantly higher in attended rheumatological clinic participants who were belonged to Group I comparative to participants who had lower protein density (Group II) -1.24±0.19, -1.15±0.18, 0.78±0.02 g/cm^2, 0.69±0.58, 0.62±0.53, and 1.05±0.09 g/cm^2 versus -1.78±0.22, -1.63±0.20, 0.72±0.02 g/cm2, -0.87±0.63, -0.79±0.57, and 0.82±0.10 g/cm^2] with Mean differences±SEM of +0.53±0.03, +0.48±0.03, +0.06±0.00 g/cm^2 , +1.56±0.08, +1.42±0.08, and +0.23±0.01 g/cm^2 , respectively, p-value<0.05.

The 10-year hip fracture risk based on the FRAX score was significantly lower in Group I compared to Group II [2.2%±0.6% vs 3.8%±0.6%, -1.6%±0.1%, p-value=0.000]. Similarly, the 10-year major osteoporotic fracture (OPF) was significantly lower in Group I compared to Group II [9.7%±4.1% vs 20.8%±4.5%, -11.1%±0.6%, respectively, p-value=0.000]. Overall weekly consumption quantity of CaC03 was 2619±1138 mg/week which was significantly higher in Group I than in Group II [3319±1161 mg/week vs 1863±370 mg/week, +1455±122 mg/week, respectively, p-value=0.000]. Also, 25-OH-Cholecalciferol (Vit D) level was significantly higher in Group I than in Group II by +6.11±0.33 ng/ml [18.53±2.27 ng/ml vs 12.42±2.47 ng/ml, respectively, p-value=0.000]. The daily

OsCal-D tablet consumption rates were significantly distributed across the two tested groups for which the rates of 3 tab/day and 4 tab/day were allocated in Group I in percentages of 48.6% and 51.4%, respectively. While the OsCal-D consumption rate of 1 tab/day, 2 tab/day, and 3 tab/day were allocated in Group II in percentages of 21.2%, 75.8%, and 3% respectively.

All attended rheumatology clinic participants in Group I had a regular consumption pattern for fruits and vegetables while participants in Group II had an intermittent consumption pattern rate of 21.2% and a regular consumption pattern rate of 78.8%. the odd ratio for FVCP was 2.37 (95% CI; 2.00-2.81). Contrarily, all participants in Group II had a sedentary lifestyle of daily activities while participants in Group I had a sedentary ADLS rate of 48.6% and an active ADLS rate of 51.4%. The odd ratio of ADLS for our tested attended rheumatology clinic participants was 0.344 (95% CI; 0.276-0.429). The overall protein density intake in the study was 3.19±1.59 g/100 Cal which was significantly higher in Group I participants compared to Group II participants by +2.820±0.102 g/100 Cal [4.546±0.998 g/100 Cal vs 1.726±0.213 g/100 Cal, respectively, p-value=0.000]. The participant's comorbidities distributions across the two tested groups and all aforementioned comparatively variables for cohort people who attended the rheumatology clinic between Sep 2021 and Nov 2021 at Prince Rashid bin Al-Hasan Military Hospital, Royal Medical Services, Irbid, Jordan, among who were on Standard Protein Cohort I (Cohort I) compared to who were on Low Protein Cohort (Cohort II) were summarized in Table 1-3.

The degree of correlations (R) and the coefficient of determination (R^2) results for the 4 tested variables from the conducted Univariate Linear Regression Test were 0.894 and 0.799, 0.600 and 0.361, 0.732 and 0.536, and 0.930 and 0.865 for PD (g/100 Cal), FVCP (Intermittent/Regular), ADLS (Sedentary/Active), and OsCal-D (1-4 Tab/day), respectively. The abstracted individual coefficients (B± SEM) of each aforementioned tested variable for the 10-year $_f$OHF risk prediction were -0.563±0.020, -1.983±0.185, -1.653±0.108, and -0.954±0.026, respectively. The necessary coefficients to collectively predict the 10-year Hip Fracture Risk and to present the final form of our proposed multivariate linear regression model for the tested osteoporosis patients can be formulated as follows.

10-Year Osteoporosis related Fracture Risk (%) =5.406-0.325×PD-0.885×FVCP-0.447*ADLS-0.169*OsCal-D

Samer Alboun et al proposed a multivariable linear regression model for prognosticating the % 10-Year Osteoporosis related Fracture Risk based on 4 tested dietary patterns and lifestyle factors, including PD: Protein density in g per 100 Cal, FVCP: Fruits/Vegetables consumption pattern (Intermittent pattern=0, Regular pattern=1), ADLS: Activities of daily lifestyle (Sedentary life style=0, Active life style=1), and OsCal-D: Supplementary tablet which contains 600 mg CaC03 and 400 IU Vit D3.

After the multivariable linear regression model was constructed, we incorporated receiver operating characteristic (ROC) and sensitivity analyses to investigate the area under ROC curve (AUROC) and to explore the optimal cut-off point, sensitivity, specificity, positive and negative predictive values, Youden and accuracy indices, and the negative likelihood ratio for the 10-Year Risk of f Hip OPF percentages based on our proposed multivariable regression equation results. Statistical analysis was performed using Statistical Package for Social Science (SPSS) software version 23.0. Statistical significance was set at 5%.

The AUROC for the constructed ROC based on the run participant's composing data into our proposed multivariate linear regression equation against the 10-year risk of fHOP_FRAX score ≥3% (1) or <3% (0), was 0.992±0.05 (95% CI; 0.983-1). Also, we explored the optimal operating dichotomized level of 3.45% for our proposed regression equation to discriminate between lower risk and higher risk cohorts regarding the 10-Year risk of fHOP fracture with the prognosticating performance of 97.27%.

Discussion

The National Institutes of Health consensus conference defined osteoporosis as an aging-related increased skeletal fragility accompanied by low BMD diseases. Low BMD is numerically defined as a T score below −2.5 and the preferred diagnostic sites for calculating the T score are the hip, either at the total hip or the femoral neck [14-16]. Even though it would be beneficial to conduct routine osteoporosis screening tests, it is not feasible in most countries including our country due to the restricted availability of DEXA machines and their associated high-cost expenditure. So, it is therefore not feasible to screen all postmenopausal Jordanian women and aged males using DEXA screening.

The availability of a variety of osteoporosis-related effective pharmacotherapeutics emphasized the recommendation of T score assessment for patients considered at high risk. Various risk assessment tools have been developed for focusing on subjects who are at increased risk. so, they can be referred for BMD measurement. [17-18] The OST, ORAI, SCORE, and OSIRIS indices were derived according to the algorithms suggested by their developers, and the following operating discriminative cut-offs were used: <2 for OST, >7 for SCORE, >8 for ORAI, and <1 for OSIRIS. [19-20]

Additionally, sensitivity was low when DEXA screening of the spine was used for analysis. This can be explained by the bone density within the spine would be increased in the presence of multiple vertebral fractures giving falsely overestimated T scores without affecting the overall osteoporotic statuses. [21-22] A fracture risk assessment tool (FRAX) is developed based on the use of clinical risk factors that were previously mentioned, with DEXA screening to differentiate the screened patients with a debatable osteoporotic risk into a more meaningful way for proactively prescribing osteoporosis-related effective drugs when it is exceeded 3%. [23-24]

However, most of the tools have been developed for the Western population and the risk of osteoporotic fractures varies widely between populations. Thus, population-specific data are required to predict the risk of fracture in each population. However, few studies have developed an assessment model from the dietary and lifestyle risk factors of osteoporotic fractures which gives the uniqueness of our study. [25-26]

While the consumption of animal proteins-based sulfur amino acids and grains phytate-based phosphate increase physiological acidity, the consumption of fruits and green vegetables increases alkalinity owing to high alkaline potassium salts of weak organic acids contents. A higher protein: potassium (PRO: K) ratio is undesirable, as demonstrated by the finding that it is associated with higher renal net acid excretion. Oppositely to high PRO: K, a low PRO: K-based diet is associated with a lower potential for renal acid and calcium load which can be achieved by consuming a balanced diet.[27-30] Calcium intake may also be important. For example, in the Framingham study, the increased fracture risk associated with higher sulfur amino acids intake was only present in the participants with lower calcium intake (<800mg/d) while contrarily no association between higher sulfur amino acids and fracture risk when calcium intake was sufficient (≥800 mg/d) which suggests adequate calcium intake may offset any detrimental effects of high sulfur amino acids. [31-33]

Indeed, there is an argument for a whole diet approach for bone health, which includes a balanced intake of nutrients such as protein, potassium, calcium, and phosphate. As discussed earlier, one way of increasing potassium intake is to consume more fruit and vegetables. Adequate calcium intake may also help compensate for any sulfur amino acid-induced bone loss. Adequate protein intake ensures enough amino acids for the growth and repair of body tissues but should not be in excess. [40-45] In this study, we revealed in our dietary pattern and lifestyle derived proposed multivariate regression model that for each 1 g/100 Cal increment in PD, the 10-Year Osteoporosis related Fracture Risk (%) was decreased from baseline constant (5.406%) by 1.826%, 2.151%, 2.476%, and 2.801 %, respectively, as long as the tested participant maintained regular FVCP, active ADLS, and OsCal-D of at least 1 tab per day. Also, we mathematically extrapolated the optimal PD (g/100 Cal) and OsCal-D (tablet/day) to state the 10-Year Osteoporosis related Fracture Risk (%) below 3% in tested participants who maintained regular FVCP and active ADLS.

Conclusion

In this study, we concluded that as long as the minimum PD didn't decrease below 2.5 g/100 Cal and the number of OsCal-D tablets was above 2 tablets per day in Jordanian participants who maintained regular Fruits/Vegetables consumption pattern and active daily lifestyle, the risk of osteoporotic fracture will be maximally 2.924%.

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Table 1. Comparatively, variables for cohort people who attended the rheumatology clinic between Sep 2021 and Nov 2021 at Prince Rashid bin Al-Hasan Military Hospital, Royal Medical Services, Irbid, Jordan, among who were on Standard Protein Cohort I (Cohort I) compared to who were on Low Protein Cohort (Cohort II).

Variables		Overall 206 Mean±SD	Cohort I 107, 51.94% Mean±SD	Cohort II 99, 48.06% Mean±SD	Mean diff±SEM or OD	p-Value
Age (Yrs)		59.88±1.673	59.99±2.00	59.76±1.22	0.23±0.23	0.32
Gender	Female	176 (85.4%)	92 (86.0%)	84 (84.8%)	1.09 (95% CI; 0.51-2.38)	0.486
	Male	30 (14.6%)	15 (14.0%)	15 (15.2%)		
	F: M	5.87: 1	6.13: 1	5.6: 1		
BW (Kg)		76.85±7.672	76.80±7.89	76.91±7.47	-0.11±1.07	0.92
Height (cm)		160.42±7.97	160.71±8.65	160.11±7.20	0.60±1.11	0.59
BMI (Kg/m²)		30.16±4.87	30.11±5.27	30.21±4.43	-0.10±0.68	0.89
IBW (Kg)		56.12±6.815	56.33±7.29	55.89±6.29	0.44±0.95	0.64
Adj_BW (Kg)		64.40±4.464	64.52±4.48	64.27±4.46	0.25±0.62	0.69
Hx of parental fracture	No	181 (87.9%)	104 (97.2%)	77 (77.8%)	9.91 (95% CI; 2.86-34.29)	0.000
	Yes	25 (12.1%)	3 (2.8%)	22 (22.2%)		
Co-Morbidities	<3	156 (75.7%)	107 (100.0%)	49 (49.5%)	0.31 (95% CI; 0.25-0.39)	0.00
	≥3	50 (24.3%)	0 (0.0%)	50 (50.5%)		
RA	No	189 (91.7%)	104 (97.2%)	85 (85.9%)	5.71 (95% CI; 1.59-20.53)	0.003
	Yes	17 (8.3%)	3 (2.8%)	14 (14.1%)		
HTN	No	101 (49.0%)	98 (91.6%)	3 (3.0%)	348 (9%CI; 92-1326)	0.00
	Yes	105 (51.0%)	9 (8.4%)	96 (97.0%)		
DM	No	140 (68.0%)	5 (4.7%)	61 (61.6%)	32.75 (95% CI; 12.23-87.68)	0.00
	Yes	66 (32.0%)	5 (4.5%)	61 (63.5%)		
CKD	No	183 (88.8%)	105 (98.1%)	78 (78.8%)	14.14 (95% CI; 3.22-62.07)	0.00
	Yes	23 (11.2%)	2 (1.9%)	21 (21.2%)		
PUD	No	142 (68.9%)	95 (88.8%)	47 (47.5%)	8.76 (95% CI; 4.27-17.97)	0.00
	Yes	64 (31.1%)	12 (11.2%)	52 (52.5%)		
CVD	No	177 (85.9%)	101 (94.4%)	76 (76.8%)	5.09 (95% CI; 1.98-13.13)	0.00
	Yes	29 (14.1%)	6 (5.6%)	23 (23.2%)		

The comparative non-dichotomous variables between Group I and Group II were statistically analyzed by Independent T- Test and the results were expressed as Mean±SD and as Mean difference±SEM. While the comparative variables for the total sample were analyzed by One Sample T-Test and the results were also expressed as Mean±SD. For dichotomous data, a Chi Square Test was used to express the analysis outcomes as Number (Percentage). The correlation strengths of binary categorical variables were expressed as odd ratio (OD). (At p-value< 0.05*).

- Group I: Attended rheumatological clinic patients who were on Standard Protein Density (≥2.5 g/100 Cal).
- Group II: Attended rheumatological clinic patients who were on Low Protein Density (<2.5 g/100 Cal).

OD: Odd ratio.	RA: Rheumatoid arthritis.
IBW: Ideal body weight.	HTN: Hypertension.
ABW: Actual body weight.	DM: Diabetes mellitus.
Adj_BW: Adjusted body weight.	CKD: Chronic kidney disease.
BMI: Body mass index.	PUD: Peptic ulcer disease.
BSA: Body surface area.	CVD: Cardiovascular disease.

Table 2. Comparatively, variables for cohort people who attended the rheumatology clinic between Sep 2021 and Nov 2021 at Prince Rashid bin Al-Hasan Military Hospital, Royal Medical Services, Irbid, Jordan, among who were on Standard Protein Cohort I (Cohort I) compared to who were on Low Protein Cohort (Cohort II).

Variables		Overall 206 Mean±SD	Group I 107, 51.94% Mean±SD	Group II 99, 48.06% Mean±SD	Mean diff±SEM or OD	p-Value
On Thyroxine	No	158 (76.7%)	100 (93.5%)	58 (58.6%)	10.09 (95% CI; 4.26-23.97)	0.00
	Yes	48 (23.3%)	7 (6.5%)	41 (41.4%)		
FVCP	Intermittent	21 (10.2%)	0 (0.0%)	21 (21.2%)	2.37 (95% CI; 2.00-2.81)	0.000
	Regular	185 (89.8%)	107 (100.0%)	78 (78.8%)		
ADLS	Sedentary	151 (73.3%)	52 (48.6%)	99 (100.0%)	0.344 (95% CI; 0.276-0.429)	0.000
	Active	55 (26.7%)	55 (51.4%)	0 (0.0%)		
Post-Menopausal age		48.41±4.41	48.47±4.67	48.35±4.15	0.12±0.67	0.86
f HipT-Score		-1.50±0.34	-1.24±0.19	-1.78±0.22	0.53±0.03	0.00
f Hip Z-Score		-1.38±0.31	-1.15±0.18	-1.63±0.20	0.48±0.03	0.00
f Hip BMD (g/cm²)		0.75±0.038	0.78±0.02	0.72±0.02	0.06±0.00	0.00
Lumbar T-Score		-0.062±0.987	0.69±0.58	-0.87±0.63	1.56±0.08	0.00
Lumbar Z-Score		-0.06±0.89	0.62±0.53	-0.79±0.57	1.42±0.08	0.00
Lumbar BMD (g/cm²)		0.94±0.15	1.05±0.09	0.82±0.10	0.23±0.01	0.00

The comparative non-dichotomous variables between Group I and Group II were statistically analyzed by Independent T- Test and the results were expressed as Mean±SD and as Mean difference±SEM. While the comparative variables for the total sample were analyzed by One Sample T-Test and the results were also expressed as Mean±SD. For dichotomous data, a Chi Square Test was used to express the analysis outcomes as Number (Percentage). The correlation strengths of binary categorical variables were expressed as odd ratio (OD). (At p-value< 0.05*).

- Group I: Attended rheumatological clinic patients who were on Standard Protein Density (≥2.5 g/100 Cal).
- Group II: Attended rheumatological clinic patients who were on Low Protein Density (<2.5 g/100 Cal).

*Cs: Corticosteroidal agents of at least Prednisolone 7.5 mg/day or equivalent for 3 months in the past year.

fHip T-Score: T-Score for femoral neck of the hip.
BMD: Bone mineral density in g per cm².
Cs: Corticosteroids.
$CaCO_3$: Weekly average of calcium carbonate supplement.

NA: Not mathematically applicable and can't be statistically computed.
Vit D: 25-OH-Cholecalciferol (Vit D3 level) in ng per ml.
OPF: Osteoporotic fracture risk.

Table 3. Comparatively, variables for cohort people who attended the rheumatology clinic between Sep 2021 and Nov 2021 at Prince Rashid bin Al-Hasan Military Hospital, Royal Medical Services, Irbid, Jordan, among who were on Standard Protein Cohort I (Cohort I) compared to who were on Low Protein Cohort (Cohort II).

Variables		Overall 206 Mean±SD	Group I 107, 51.94% Mean±SD	Group II 99, 48.06% Mean±SD	Mean diff±SEM or OD	p-Value
Cs*	No	176 (85.4%)	103 (96.3%)	73 (73.7%)	9.17 (95% CI; 3.07-27.40)	0.00
	Yes	30 (14.6%)	4 (3.7%)	26 (26.3%)		
Vit D (ng/ml)		15.59±3.86	18.53±2.27	12.42±2.47	6.11±0.33	0.00
Smoking Status	No	162 (78.6%)	101 (94.4%)	61 (61.6%)	10.49 (95% CI; 4.19-26.26)	0.000
	Yes	44 (21.4%)	6 (5.6%)	38 (38.4%)		
$CaCO_3$ (mg/wk)		2619±1138	3319±1161	1863±370	1455±122	0.00
OsCal-D	1 Tab/day	21 (10.2%)	0 (0.0%)	21 (21.2%)	NA	0.000
	2 Tabs/day	75 (36.4%)	0 (0.0%)	75 (75.8%)		
	3 Tab/day	55 (26.7%)	52 (48.6%)	3 (3.0%)		
	4 Tab/day	55 (26.7%)	55 (51.4%)	0 (0.0%)		
PD (g/100 Cal)		3.191±1.591	4.546±0.998	1.726±0.213	+2.820±0.102	0.000
10-year Hip fracture risk		2.99%±1.0%	2.2%±0.6%	3.8%±0.6%	-1.6%±0.1%	0.00
10-year major OPF		15.02%±7.04%	9.7%±4.1%	20.8%±4.5%	-11.1%±0.6%	0.000

The comparative non-dichotomous variables between Group I and Group II were statistically analyzed by Independent T- Test and the results were expressed as Mean±SD and as Mean difference±SEM. While the comparative variables for the total sample were analyzed by One Sample T-Test and the results were also expressed as Mean±SD. For dichotomous data, a Chi Square Test was used to express the analysis outcomes as Number (Percentage). The correlation strengths of binary categorical variables were expressed as odd ratio (OD). (At p-value< 0.05*).

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fHip T-Score: T-Score for femoral neck of the hip.
BMD: Bone mineral density in g per cm².
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$CaCO_3$: Weekly average of calcium carbonate supplement.

NA: Not mathematically applicable and can't be statistically computed.
Vit D: 25-OH-Cholecalciferol (Vit D3 level) in ng per ml.
OPF: Osteoporotic fracture risk.

Table 4. Univariate regression analysis results for the 4 tested variables regarding 10-year Hip Risk Fracture percentages among attended rheumatology clinic patients between Sep 2021 and Nov 2021 at Prince Rashid Bin Al-Hasan Military Hospital, Royal Medical Services, Irbid, Jordan.

Tested variables	Model summary		F-ANOVA	Coefficient's summary			p-Value
	R	R^2		Constant±SEM	B±SEM	Beta	
PD (g/100 Cal)	0.894	0.799	808.896	4.795±0.070	-0.563±0.020	-0.894	0.000
FVCP	0.600	0.361	115.048	4.780±0.175	-1.983±0.185	-0.600	0.000
ADLS	0.732	0.536	235.479	3.441±0.056	-1.653±0.108	-0.732	0.000
OsCal-D (Tab/day)	0.930	0.865	1304.124	5.575±0.076	-0.954±0.026	-0.930	0.000

The Univariate Linear Regression Test was conducted to explore the degree of correlation, how much of the total variation in the dependent variable can be explained by the independent variable, and the quality of the prediction of the dependent variable. Also, this test was conducted to abstract the necessary coefficients to individually predict the 10-year Hip Fracture Risk.

- PD: Protein density in g per 100 Cal.
- FVCP: Fruits/Vegetables consumption pattern (Intermittent pattern=0, Regular pattern=1).
- ADLS: Activities of daily life style (Sedentary life style=0, Active life style=1).
- OsCal-D: supplementary tablet which contains 600 mg CaC03 and 400 IU Vit D3.

Table 5. Multivariate linear regression analysis results for the 4 tested variables regarding 10-year Hip Risk Fracture percentages among attended rheumatology clinic patients between Sep 2021 and Nov 2021 at Prince Rashid Bin Al-Hasan Military Hospital, Royal Medical Services, Irbid, Jordan.

Tested variables	Model summary		F-ANOVA	Coefficient's summary		p-Value
	R	R^2		B±SEM	Beta	
	0.959	0.920	579.539			
Constant				5.406±0.084		0.000
PD (g/100 Cal)				-0.325±0.029	-0.516	0.000
FVCP				-0.885±0.111	-0.268	0.000
ADLS				-0.447±0.099	-0.198	0.000
OsCal-D (Tab/day)				-0.169±0.081	-0.165	0.038

The Multivariate Linear Regression Test was conducted to explore the degree of correlations, how much of the total variations in the dependent variable can be explained by the independent variables, and the quality of the prediction of the dependent variable. Also, this test was conducted to abstract the necessary coefficients to collectively predict the 10-year Hip Fracture Risk and to present the final form of our proposed multivariate logistic regression model for the tested osteoporosis patients which can be formulated as follows.

10-Year Osteoporosis related Fracture Risk (%) =5.406-0.325×PD-0.885×FVCP-0.447*ADLS-0.169*OsCal-D

- PD: Protein density in g per 100 Cal.
- FVCP: Fruits/Vegetables consumption pattern (Intermittent pattern=0, Regular pattern=1).
- ADLS: Activities of daily life style (Sedentary life style=0, Active life style=1).
- OsCal-D: supplementary tablet which contains 600 mg CaC03 and 400 IU Vit D3.

ROC curve for the 10-Year Risk of f Hip OPF percentages based on our proposed multivariable regression equation results among attended rheumatology clinic patients between Sep 2021 and Nov 2021 at Prince Rashid Bin Al-Hasan Military Hospital, Royal Medical Services, Irbid, Jordan.

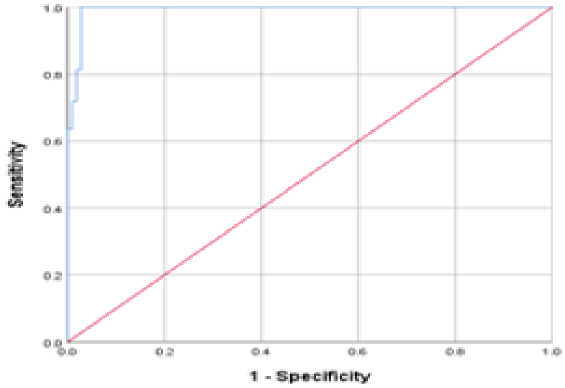

Fig 1. The area under the receiver operating characteristic (ROC) curve for our proposed multivariable regression equation result (AUROC) when they were constructed aginst 10-Year risk of fHOP ≥3% (1) or <3% (0). The Area±SEM (95% CI; Range) was investigated at 0.992±0.005 (95% CI; 0.983-1)

Table 6. The optimal cut-off points, sensitivities, specificities, positive and negative predictive values, Youden and accuracy indices, and the negative likelihood ratios for the 10-Year Risk of f Hip OPF percentages based on our proposed multivariable regression equation results among attended rheumatology clinic patients between Sep 2021 and Nov 2021 at Prince Rashid Bin Al-Hasan Military Hospital, Royal Medical Services, Irbid, Jordan.

Prognostic Indicator	Cut-off	TPR	FPR	YI	TNR	PPV	NPV	NLR	AI
10-Year risk of fHOP fracture	3.45%	100%	2.7%	97.27%	97.27%	96.97%	100.00%	0.00%	98.54%

- The area under the receiver operating characteristic (ROC) analysis was constructed against the 10-Year risk of fHOP_FRAX score ≥3% (1) or <3% (0). Sensitivity analysis was processed on a total of 206 processed cases, 96-case were processed as positive actual state, and 110-case were processed as a negative actual state. 3 processed cases were dealt with as missing data. higher values of the test result variable(s) indicate stronger evidence for a positive actual state. The positive actual state is the 10-Year risk of fHOP fracture based on our proposed multivariate linear regression analysis.
- fHOP: Femoral hip osteoporosis.

TPR: True positive rate (sensitivity).
FPR: False positive rate.
YI: Youden index.
TNR: True negative ratio (specificity).

PPV: Positive predictive value.
NPV: Negative predictive value.
NLR: Negative likelihood ratio.
AI: Accuracy index.

Figure 2. Calorie and protein chart of common foods