Statistical Analysis Plan
Title: Utilizing Protein During Weight Loss to Impact Physical Function and Bone
NCT03819478
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Statistical Analysis Plan

We expect to enroll 225 participants in the study. Retention is expected to be ≥90% at 6 months (≥67 completers/group) and ≥80% at 18 months (≥60 completers/group) based on compliance rates of prior weight loss and exercise trials conducted at the Wake Forest School of Medicine (WFSM). All statistical analyses will be performed in SAS or R. Before performing hypothesis testing, data distributions will be examined to determine if transformations are necessary and if nonparametric approaches should be used. Analyses will conform to intent-to-treat principles, indicating that participants will be included in analyses according to the groups to which they were randomized. The models will include a random effect for wave to account for the potential correlation of participants within waves.

Volumetric BMD and Cortical Thickness Outcome Measures at Primary Endpoint of 18 months

The study is ≥80% powered (N=180, 2-sided α=0.025) to detect differences in change between groups of 0.016 g/cm^3 (SD=0.028 g/cm^3) and 0.007 g/cm^3 (SD=0.013 g/cm^3) volumetric bone mineral density (vBMD) and 0.063 mm (SD=0.111 mm) and 0.018 mm (SD=0.032 mm) cortical thickness at 18 months at the spine and hip, respectively (SDs of change from preliminary data).[1] Mean group differences and effect sizes at 80% power are reported in **Table 1** for the expected 18-month sample size (N=60/group), as well as smaller sample sizes in the event of shortfalls in enrollment or retention (N=30, 40, and 50/group). Effect sizes ≥0.80 have been observed in prior trials that have detected statistically significant differences in lumbar spine BMD (0.92-1.60 effect sizes)[2-4] and hip BMD (0.80 effect size)[2,3] between higher versus lower protein groups, suggesting that our study is adequately powered to detect differences even with N=30/group.

We will use analysis of covariance (ANCOVA) to compare group effects on vertebral and femoral vBMD and cortical thickness at the primary endpoint of 18 months, with adjustment for baseline values. Age, sex, race, and baseline value of the outcome of interest will be adjusted for as covariates in these analyses.

Table 1. Power to detect significant differences in mean change between three groups at 18 months with ANCOVA (2-sided α=0.025 after Bonferroni correction for each outcome), assuming similar SD between groups.

Outcome Measures	SD of Change	Power	Mean Group Difference (Effect Size; Diff/SD)			
			N=30/group	N=40/group	N=50/group	N=60/group
Lumbar vBMD (g/cm^3)	0.028	0.80	0.023 (0.82)	0.020 (0.70)	0.018 (0.63)	0.016 (0.57)
Hip vBMD (g/cm^3)	0.013		0.011 (0.81)	0.009 (0.70)	0.008 (0.63)	0.007 (0.57)
Hip cortical thickness (mm)	0.032		0.026 (0.81)	0.022 (0.70)	0.020 (0.63)	0.018 (0.57)

Volumetric BMD and Cortical Thickness Outcome Measures at Secondary Endpoint of 6 months

Preliminary data for the vBMD and cortical thickness outcomes at 6 months is not available. However, preliminary data on hip areal BMD shows that at 80% power (2-sided α=0.05), the study can detect a 0.006 g/cm^2 difference in change in hip areal BMD (aBMD) at 6 months between the higher (N=120) and lower (N=60) protein groups (SD=0.013 g/cm^2; R01 AG012161, PI: Shapses, unpublished data), which relates to a 6% difference in fracture risk.[5] Assuming the SDs of change for vBMD and cortical thickness are similar at 6 and 18 months, the study is powered at 80% to detect the differences in mean change between groups at 6 months as reported in **Table 2**.

Table 2. Power to detect significant differences in mean change between high and low protein groups at 6 months with ANCOVA (2-sided α=0.05), assuming similar SD between groups.

Outcome Measures	SD of Change	Power	Mean Group Difference (Effect Size; Diff/SD)			
			N=30 vs. 60	N=40 vs. 80	N=50 vs.100	N=60 vs. 120
Lumbar vBMD (g/cm^3)	0.028	0.80	0.018 (0.63)	0.015 (0.55)	0.014 (0.49)	0.012 (0.45)
Hip vBMD (g/cm^3)	0.013		0.008 (0.63)	0.007 (0.55)	0.006 (0.49)	0.006 (0.45)
Hip cortical thickness (mm)	0.032		0.020 (0.63)	0.018 (0.55)	0.016 (0.49)	0.014 (0.45)

To examine the trajectory of change over time, we will compare vertebral and femoral vBMD and cortical thickness by group and time using mixed model ANCOVA with a random subject effect and a time-by-group interaction will be used to evaluate changes at 6 months and 18 months, using linear contrasts to compare the three groups at each time point. We will adjust for age, sex, race, and baseline values of the outcome of interest as covariates in these analyses.

Bone Marrow Adipose Tissue Outcome Measures

Bone marrow adipose tissue outcomes have never been measured with moderate weight loss in a randomized controlled trial of older adults; thus, we do not have prior studies from which we can estimate power and effect sizes for the bone marrow adipose tissue secondary outcomes. However, as noted in **Tables 1 and 2**, we will have 80% power to detect effect sizes of 0.57 when comparing 3 groups of 60 individuals, and of 0.45 when comparing two groups of 60 and 120 individuals.

Analyses of will use ANCOVA to compare the group effects on bone marrow adipose tissue fraction and marrow-corrected vBMD at 18 months, with adjustment for baseline values. To examine the trajectory of change over time, we will compare bone marrow adipose tissue fraction and marrow-corrected vBMD by group and time using mixed-model ANCOVA with a random subject effect and a time-by-group interaction will be used to evaluate changes at 6 months and 18 months, using linear contrasts to compare the three groups at each time point. We will adjust for age, sex, race, and baseline values of the outcome of interest as covariates in these analyses.

Bone Strength Outcome Measures

Bone strength and fracture risk outcomes are predicted from finite element modeling. The study is ≥80% powered (N=180, 2-sided α=0.025) to detect differences in change between groups of 0.037 kN (SD=0.065 kN) for femoral bone strength (SDs of change from preliminary data). Mean group differences and effect sizes at 80% power are reported in **Table 3** for the expected 18-month sample size (N=60/group), as well as smaller sample sizes in the event of shortfalls in enrollment or retention (N=30, 40, and 50/group). Fracture risk is a dichotomous outcome, where fracture is predicted in the finite element modeling simulations when elements exceed an effective plastic strain threshold; thus, estimated power and effect sizes are not provided for the fracture risk secondary outcomes.

Analyses of will be analogous to the other outcome analyses described earlier. ANCOVA will be used to compare the group effects on femoral bone strength and fracture risk at 18 months, with adjustment for baseline values. To examine the trajectory of change over time, we will compare femoral bone strength and fracture risk by group and time using mixed-model ANCOVA with a random subject effect and a time-by-group interaction will be used to evaluate changes at 6 months and 18 months, using linear contrasts to compare the three groups at each time point. We will adjust for age, sex, race, and baseline values of the outcome of interest as covariates in these analyses.

Table 3. Power to detect significant differences in mean change between groups at 18 months with ANCOVA (2-sided α=0.025 after Bonferroni correction for each outcome), assuming similar SD between groups.

Outcome Measures	SD of Change	Power	Mean Group Difference (Effect Size; Diff/SD)			
			n=30/group	n=40/group	n=50/group	n=60/group
Femoral bone strength (kN)	0.065		0.053 (0.81)	0.046 (0.70)	0.041 (0.63)	0.037 (0.57)

Exploratory Analyses

We will use the mixed model framework to evaluate associations between weight loss and changes in bone phenotypes of interest, adjusting for age, sex, intervention group, and baseline values.

References

1. Schoell SL, Weaver AA, Beavers DP, Lenchik L, Marsh AP, Rejeski WJ, Stitzel JD, Beavers KM. Development of Subject-Specific Proximal Femur Finite Element Models Of Older Adults with Obesity to Evaluate the Effects of Weight Loss on Bone Strength. Journal of Osteoporosis & Physical Activity. 2018;6(1):(In Press).

2. Sukumar D, Ambia-Sobhan H, Zurfluh R, Schlussel Y, Stahl TJ, Gordon CL, Shapses SA. Areal and volumetric bone mineral density and geometry at two levels of protein intake during caloric restriction: a randomized, controlled trial. J Bone Miner Res. 2011;26(6):1339-48. PubMed PMID: 21611972; PMCID: PMC3312759.

3. Shams-White MM, Chung M, Du M, Fu Z, Insogna KL, Karlsen MC, LeBoff MS, Shapses SA, Sackey J, Wallace TC, Weaver CM. Dietary protein and bone health: a systematic review and meta-analysis from the National Osteoporosis Foundation. Am J Clin Nutr. 2017. PubMed PMID: 28404575.

4. Kukuljan S, Nowson CA, Bass SL, Sanders K, Nicholson GC, Seibel MJ, Salmon J, Daly RM. Effects of a multi-component exercise program and calcium-vitamin-D3-fortified milk on bone mineral density in older men: a randomised controlled trial. Osteoporos Int. 2009;20(7):1241-51. PubMed PMID: 18958384.

5. Zibellini J, Seimon RV, Lee CM, Gibson AA, Hsu MS, Shapses SA, Nguyen TV, Sainsbury A. Does Diet-Induced Weight Loss Lead to Bone Loss in Overweight or Obese Adults? A Systematic Review and Meta-Analysis of Clinical Trials. J Bone Miner Res. 2015;30(12):2168-78. Epub 20150616. PubMed PMID: 26012544.

6. Kanis JA, McCloskey EV, Johansson H, Oden A, Strom O, Borgstrom F. Development and use of FRAX in osteoporosis. Osteoporos Int. 2010;21 Suppl 2:S407-13. Epub 2010/05/22. PubMed PMID: 20464374.