Protocol #: 21-3163
Project Title: Enhancing Skeletal Adaptation to Exercise by Attenuating the Acute Disruption of Calcium Homeostasis During Exercise
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Data Analysis Plan

The primary outcome for **Aim 1** is the change in CTX (ΔCTX) from before exercise to the peak value observed during 4 h of recovery, measured during the 1st, 8th, and 16th exercise sessions. The primary hypothesis is that there will be a significant ΔCTX for all three exercise sessions. The changes will be tested based on a repeated measures model to test the statistical hypotheses H0: $\Delta CTX_j=0$ vs H1: $\Delta CTX_j\neq0$, where j denotes the exercise session. A single test will be conducted based on ΔCTX_j, and differences and dependency across measures will be accounted for using an unstructured covariance matrix. This will enable testing of a single hypothesis to make decisions regarding ΔCTX.

Power (Aim 1): Attrition in our previous studies has been minimal (<10%), we have accounted for 20% attrition. The plan to enroll 30 participants (based on Aim 2) will ensure >99% power to address the hypothesis for Aim 1 in the full cohort and will also support secondary analyses of the sex specificity of the response. Based on our previous studies of older adults, ΔCTX is expected to be 0.15 ng/mL and the standard deviation (**SD**) is expected to be 0.12 ng/mL, which corresponds to a standardized effect size of 1.25 (Cohen's d=1.25). With statistical significance defined as $p\leq0.05$, a sample size of 24 for a paired t-test will provide >99% power. Alternatively, with 90% power, we will be able to detect an effect size of 0.69. In addition, because the test will utilize a repeated measured model, it will have higher power than the paired t-test. The goal is to enroll 15 women and 15 men and expanding the age range to include Veterans aged 25 to 45 y will enhance the recruitment of women Veterans. We have not observed sex differences in the CTX response to exercise in previous studies, so an imbalance in sex should not influence the results. Our expectation is that the increase in serum CTX that we[1-7] and others[8-12] have observed in response to 1 or 2 exercise session in participants across a wide range of fitness (young, older, normally active, highly trained) will occur during the 1st, 8th, and 16th exercise sessions. A single test across all three time points will provide more power than multiple paired t-tests to evaluate the change in CTX at these time points. A significant test will be interpreted as evidence that exercise has an acute *catabolic* effect on bone that persists with exercise training.

The main outcome for **Aim 2** is the change in pre-exercise serum P1NP ($\Delta P1NP_{PRE}$) from the 1st to the 16th exercise session. If the exercise-induced increase in PTH is anabolic, the pre-exercise P1NP values are expected to increase over the 16 exercise bouts, just as basal P1NP increases in response to PTH analog therapy (Fig 12-14). The observed change will be tested using a 2-sided paired t-test to test the statistical hypotheses H0: $\Delta P1NP_{PRE}=0$ vs H1: $\Delta P1NP_{PRE}\neq0$. This test will be conducted only if the test for Aim 1 is significant. The relative increase in P1NP after 4 wk of teriparatide or abaloparatide therapy ranged from 80% to 138% in several studies, and estimated effect sizes ranged from 1.3 to 2.6.[13-19] We will take a conservative approach to finding an anabolic signal with exercise and use an effect size that is 53% smaller than the smallest effect size for change in P1NP in response to teriparatide or abaloparatide (0.69). Under these conditions, a sample size of 24 will enable us to detect a change in $\Delta P1NP_{PRE}$ from the 1st to the 16th exercise session with 90% power. We will enroll 30 Veterans to allow for 20% attrition.

We considered correcting the significance level (alpha) for multiple tests because we are conducting two tests in the POC study (CTX, P1NP). However, because the second hypothesis test of P1NP will be conducted only if the first hypothesis test of ΔCTX is significant and a hierarchy of tests has been defined *a priori*, we chose to not correct the significance level.

The distributions of the outcome measures will be evaluated. All analyses will be performed using SAS 9.4 software (SAS Institute Inc., Cary, NC). If there are severe departures from a normal distribution, transformations (e.g., natural logarithm) will be applied as appropriate to achieve an endpoint that seems normally distributed. To minimize missing data, all participants will be informed of the importance of complete follow-up and encouraged to complete the study per the protocol. We will make substantial efforts to prevent missed exercise tests and samples. Monthly reports will be generated that summarize recruitment, accrual, and completeness of follow-up to allow research assistants to proactively follow-up with participants in a timely

manner. Prior to beginning analyses described below to address study hypotheses, we will examine the data carefully to assess whether patterns of missing seem ignorable (MAR=missing at random) or non-ignorable (MNAR= missing not at random).[20-22] If the former, we will employ likelihood–based methods that utilize all available data, adjusting for covariates that are associated with missingness. If missingness seems non-ignorable we will consider, as data permit, pattern mixture models.[21,23] Sensitivity analyses may be carried out using multiple imputation approaches.[21]

The primary analyses for Aims 1 and 2 utilize only a portion of the data that will be collected. Secondary analyses will take advantage of the rich data set generated during the POC phase and can be used to help guide decision-making for the RCT. A linear mixed model based on maximum likelihood with all available data will be conducted to evaluate changes in CTX, P1NP, and other secondary outcomes (iCa, tCa, PTH, PO4, Hct, urinary Ca excretion) during exercise and recovery and how responses change from the 1st to the 8th to the 16th exercise session. Secondary analyses will also be conducted to investigate, for example, whether responses are influenced by age, sex, bone turnover marker level at baseline, or absolute exercise intensity. Interaction terms of treatment variable and variable of interest will be included to test for heterogeneity of treatment effect.

REFERENCES

1 Barry, D. W. & Kohrt, W. M. Acute effects of 2 hours of moderate-intensity cycling on serum parathyroid hormone and calcium. *Calcif. Tissue Int.* **80**, 359–365 (2007).

2 Barry, D. W. *et al.* Acute calcium ingestion attenuates exercise-induced disruption of calcium homeostasis. *Med. Sci. Sports Exerc.* **43**, 617–623 (2011).

3 Shea, K. L. *et al.* Calcium supplementation and parathyroid hormone response to vigorous walking in postmenopausal women. *Med Sci Sports Exerc* **46**, 2007–2013 (2014). https://doi.org/10.1249/MSS.0000000000000320

4 Sherk, V. D. *et al.* Calcium Supplementation Attenuates Disruptions in Calcium Homeostasis during Exercise. *Med Sci Sports Exerc* **49**, 1437–1442 (2017). https://doi.org/10.1249/MSS.0000000000001239

5 Kohrt, W. M. *et al.* Maintenance of Serum Ionized Calcium During Exercise Attenuates Parathyroid Hormone and Bone Resorption Responses. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* **33**, 1326–1334 (2018). https://doi.org/10.1002/jbmr.3428

6 Kohrt, W. M. *et al.* Dermal Calcium Loss Is Not the Primary Determinant of Parathyroid Hormone Secretion during Exercise. *Med. Sci. Sports Exerc.* **51**, 2117–2124 (2019). https://doi.org/10.1249/Mss.0000000000002017

7 Wherry, S. J. *et al.* Bone Biomarker Response to Walking under Different Thermal Conditions in Older Adults. *Med Sci Sports Exerc* **51**, 1599–1605 (2019). https://doi.org/10.1249/MSS.0000000000001967

8 Guillemant, J., Accarie, C., Peres, G. & Guillemant, S. Acute effects of an oral calcium load on markers of bone metabolism during endurance cycling exercise in male athletes. *Calcif. Tissue Int.* **74**, 407–414 (2004).

9 Scott, J. P. *et al.* The role of exercise intensity in the bone metabolic response to an acute bout of weight-bearing exercise. *J. Appl. Physiol.* **110**, 423–432 (2011).

10 Scott, J. P. *et al.* The effect of training status on the metabolic response of bone to an acute bout of exhaustive treadmill running. *J. Clin. Endocrinol. Metab.* **95**, 3918–3925 (2010). https://doi.org/10.1210/jc.2009-2516

11 Townsend, R. *et al.* Parathyroid Hormone Secretion Is Controlled by Both Ionized Calcium and Phosphate During Exercise and Recovery in Men. *J Clin Endocrinol Metab* **101**, 3231–3239 (2016). https://doi.org/10.1210/jc.2016-1848

12 Haakonssen, E. C. *et al.* The effects of a calcium-rich pre-exercise meal on biomarkers of calcium homeostasis in competitive female cyclists: a randomised crossover trial. *PloS one* **10**, e0123302 (2015). https://doi.org/10.1371/journal.pone.0123302

13 Miyauchi, A. *et al.* Effect of teriparatide on bone mineral density and biochemical markers in Japanese women with postmenopausal osteoporosis: a 6-month dose-response study. *J Bone Miner Metab* **26**, 624–634 (2008). https://doi.org/10.1007/s00774-008-0871-3

14 Henriksen, K. *et al.* Evaluation of the efficacy, safety and pharmacokinetic profile of oral recombinant human parathyroid hormone [rhPTH(1-31)NH(2)] in postmenopausal women with osteoporosis. *Bone* **53**, 160–166 (2013). https://doi.org/10.1016/j.bone.2012.11.045

15 Eastell, R. *et al.* Bone turnover markers to explain changes in lumbar spine BMD with abaloparatide and teriparatide: results from ACTIVE. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* **30**, 667–673 (2019). https://doi.org/10.1007/s00198-018-04819-1

16 Fitzpatrick, L. A. *et al.* The effects of ronacaleret, a calcium-sensing receptor antagonist, on bone mineral density and biochemical markers of bone turnover in postmenopausal women with low bone mineral density. *J Clin Endocrinol Metab* **96**, 2441–2449 (2011). https://doi.org/10.1210/jc.2010-2855

17 Cohen, A. *et al.* Teriparatide for idiopathic osteoporosis in premenopausal women: a pilot study. *J Clin Endocrinol Metab* **98**, 1971–1981 (2013). https://doi.org/10.1210/jc.2013-1172

18 Glover, S. J. *et al.* Rapid and robust response of biochemical markers of bone formation to teriparatide therapy. *Bone* **45**, 1053–1058 (2009). https://doi.org/10.1016/j.bone.2009.07.091

19 Almirol, E. A. *et al.* Short-term effects of teriparatide versus placebo on bone biomarkers, structure, and fracture healing in women with lower-extremity stress fractures: A pilot study. *J Clin Transl Endocrinol* **5**, 7–14 (2016). https://doi.org/10.1016/j.jcte.2016.05.004
20 Hogan, J. W., Roy, J. & Korkontzelou, C. Handling drop-out in longitudinal studies. *Stat. Med.* **23**, 1455–1497 (2004). https://doi.org/10.1002/sim.1728
21 Fairclough, D. L. *Design and Analysis of Quality of Life Studies in Clinical Trials*. (CRC Press, 2010).
22 Diggle, P. K., M.G. Informative Drop-out in Longitudinal Data Analysis. *Applied Statistics* **43** (1994). https://doi.org/10.2307/2986113
23 Hedeker, D. G., R.D. Application of random-effects pattern-mixture models for missing data in longitudinal studies. *Psych Methods* **2**, 64–78 (1997).