Effect of Caloric Restriction and Protein Intake on Metabolism and Anabolic Sensitivity

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1. What is the significance/purpose of the project?

Successful weight loss requires the induction of an energy deficit [1], which can be attained through caloric restriction, exercise, or a combination of both [2]. However, weight loss is not only associated with the favorable loss of adipose tissue, but also with the loss of lean mass [2]. On average, approximately 25% of weight loss consist of lean mass [3], but lean mass loss is even more pronounced in normal-weight individuals [4]. The loss of lean mass during energy deficiency is a result of the degradation of body protein to provide additional energy and substrates to meet the high glucose demand of the brain. Protein degradation takes place primarily in expendable cellular tissues such as the skeletal muscle, which is emphasized by recent findings of reduced muscle protein synthesis [5] and increased muscle protein breakdown [6] during energy deficiency. As a result, energy deficiency and the concomitant loss of lean mass could be associated with undesired health effects such as impaired blood glucose regulation [7] and the loss of functional capacity throughout the life span [8].

Energy deficiency is associated with metabolic and anti-anabolic adaptations. Parallel with the loss of lean mass, resting metabolic rate (RMR) is also suppressed during energy deficiency. Because this reduction in RMR conserves energy, it has been linked to attenuated weight loss and an increased propensity for weight (re)gain [9]. The reduction in RMR is a consequence of the suppression of expendable functions, such as growth and reproduction, in order to conserve energy for life-sustaining functions [10]. As a result, the secretion and bioavailability of anabolic hormones is strongly impaired during energy deficiency [11]. This suppression of anabolic hormones is not only linked to the loss of skeletal muscle, but has also detrimental effects on bone [12-14]. This is particularly concerning because the accrual and maintenance of peak bone mass during adolescence and adulthood is a strong predictor of osteoporosis risk later in life [15].

The profound metabolic consequences of caloric restriction may negate some of the benefits of weight loss. Considering that calorie restriction is the preferred and most-effective strategy for the treatment and prevention of overweight and obesity and their associated comorbidities [2], strategies are needed to preserve the beneficial effects of calorie restriction while attenuating its potentially detrimental effects on metabolism, skeletal muscle, and bone. Lean mass can be preserved through increased dietary protein intake. A high dietary protein intake during energy deficiency attenuates the loss of lean mass and improves protein balance [16, 17].

Further, it is known that a high protein intake, particularly when coupled with exercise, is associated with improved bone strength [18], and effect that is likely modulated by the stimulating effects of dietary protein on the secretion of IGF-1 [15]. These findings lend to the idea that increased protein intake during calorie restriction may preserve the anabolic sensitivity of both skeletal muscle and bone.

However, only little is known about the underlying mechanisms, particularly pertaining to the effects of an increased protein during caloric restriction on circulating concentrations of anabolic hormones and the downstream effects on bone turnover. Therefore, the purpose of our study is to determine whether a strategy of increased dietary protein during energy deficiency can attenuate the suppression of RMR and reductions in hormones with anabolic and anti-catabolic effects on bone. For this purpose, we will conduct a prospective, randomized controlled study involving three experimental conditions: a) calorie restriction with normal protein intake (0.8 g/kg; CR-NP); b) calorie restriction with high protein intake (1.7 g/kg; CR-HP); and c) a control period of balanced caloric intake and matched protein intake (1.7 g/kg; CON).

Aim 1: To determine whether increased protein intake can preserve metabolic function and maintain anabolic sensitivity during calorie restriction

Hypothesis 1A: Weight loss will be similar following CR-HP and CR-LP, but the loss of lean mass and the suppression of resting metabolic rate will be reduced in CR-HP when compared to CR-NP.

Hypothesis 1B: The reduction in circulating concentrations of the anabolic hormones IGF-1, insulin, and testosterone will be attenuated in CR-HP when compared to CR-NP.

Aim 2: To determine whether increased protein intake can attenuate the increase in bone turnover, and particularly the increase in bone resorption, during calorie restriction.

Hypothesis 2A: The increase in markers of bone resorption (NTx, CTx) will be attenuated in CR-HP when compared to CR-NP; whereas, the reduction in markers in bone formation (P1NP, AP) will be similar in CR-HP and CR-NP when compared to CON.

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2. Describe the data collection procedures and what participants will be asked to complete.

1. Initial Screening Questionnaire

Participants will be asked for information pertaining to inclusion and exclusion criteria in order to establish qualification for study participation. Information provided in the Initial Screening Questionnaire will only be used for screening purposes and not for research purposes.

2. Informed Consent and Screening Visit

Informed Consent: The researcher (or designee) obtaining informed consent will thoroughly review the consent form with each participant and confirm that the participant understands all study procedures and potential risks and benefits of study participation.

Initial Anthropometric Measurements: After obtaining informed consent, participants will be weighed to the nearest 0.1 kg on a digital scale in the laboratory with a standard outfit of t-shirt and gym shorts. Participants also will have their body fat percentage determined using calipermetry. A skinfold caliper will be used to take measurements of skinfold thickness at 7 sites (abdomen, triceps, chest, midaxillary, subscapular, suprailliac, and thigh). The skinfold measurements will be used to calculate body density and body fat percentage (ACSM's Guidelines for Exercise Testing and Prescription, Ninth Edition).

Questionnaires: Participant will then fill out the following questionnaires:

o The Eating Disorder Inventory a self-report questionnaire used to assess the presence of eating disorders (D. M. Garner & Olmstead, 1991; David M. Garner, Olmstead, & Polivy, 1983).

o The Health Exercise and Nutrition Questionnaire, which includes questions regarding demographics, medical history, current and past exercise, bone health, stress fracture history, current medication and supplement use, and dietary habits.

o The Brief Calcium Assessment Tool, a brief questionnaire used to assess habitual dietary calcium intake (Yang, Martin, & Boushey, 2010).

o The Bone-Specific Physical Activity Questionnaire, a questionnaire used to predict. The BPAQ predicted indices of bone strength at clinically relevant sites in both men and women (Weeks & Beck, 2008)

o Absolute and Relative Contraindications to Exercise Testing, a questionnaire pertaining to absolute and relative contraindications to exercise testing to determine if the participant is able to exercise adequately (ACSM's Guidelines for Exercise Testing and Prescription, Ninth Edition).

After admittance into the study, participants will be provided with an over-the-counter calcium and vitamin D supplement. Supplementation of calcium and vitamin D will be initiated at least 2 weeks prior to the start of the first intervention to maintain the participants intake at levels required for optimal bone health.

3. Preliminary testing

Prior to study start, body composition will be assessed with a bioimpedance analyser (Bodystat Quadscan 4000). Peak oxygen uptake (VO2peak) will be assessed using an incremental exercise test on a bicycle ergometer (Monark LC6).

4. Randomization

Each participant will be randomly assigned to one of the six possible permutations of the order of the study conditions (CON/CR-LP/CR-HP). Randomization of study condition order will occur in blocks of 6.

5. Nutritional intervention:

After randomization of study condition order, participants will proceed through each or the following conditions. Each condition will last 5 days with a post-test on day 6:

CR-LP: Participants will consume 30 kcal/kg FFM/day and 0.8 g protein/kg BW/day.

CR-HP: Participants will consume 30 kcal/kg FFM/day and 1.7 g protein/kg BW/day.

CON: Participants will consume 55 kcal/kg FFM/day and 1.7 g protein/kg BW/day.

Dietary energy intake prescriptions will be controlled individually utilizing a combination of two clinical products (Ensure Original and Ensure High Protein, both Therapeutic Nutrition, Abbot) and maltodextrin (Tate & Lyle) such that the target energy intake of 30 kcal/kg FFM/day (CR-LP and CR-HP) or 55 kcal/kg FFM/day (CON) is reached. Participants will be provided clear plastic bottles each day with the prescribed amount of the clinical products and will be asked to consume all of it during the day.

6. Prescribed Exercise

During each conditions, participants will conduct daily supervised exercise on a bicycle ergometer (Monark LC6) at an exercise intensity of 60% VO2peak. Exercise duration will be adjusted individually such that exercise energy expenditure will amount to 15 kcal/kg FFM/day. Exercise will take approximately 60-90 minutes. Additional exercise and intense physical activity will be prohibited. Compliance will be monitored using an activity monitor (Actigraph). Activity monitors will be returned at the end of each study period.

7. Washout

Once a participant completes a study condition, they will be allowed a washout period before beginning the next study condition. Washout periods between conditions will be 14 days to allow protein balance to return to baseline (Hoffer & Forse, 1990). During this time, participants will resume their regular diet and physical activity. Calcium and vitamin D supplementation will continue to maintain their intake at levels required for optimal bone health.

8. Assessments

All assessments will be identical during each study condition, which lasts for 5 days and includes post testing on day 6. On day 1 and 6, participants will arrive at the lab in the morning following an overnight fast and undergo the following assessments:

o Bioimpedance: Body composition is assessed with a bioimpedance analyser (Bodystat Quadscan 4000). While lying in the supine position (with no parts of the body touching one another), four electrodes are attached to the wrist, hand, ankle and foot on the right side of the body. The cables from the bioimpedance analyzer are then attached to the electrodes and the device is turned on, running an electrical current through the body to estimate body composition.

o Resting Metabolic Rate: Resting Metabolic Rate (RMR) will be measured following an overnight fast (no food or drink, except water, 12 hours before the scheduled test) between 6-10:00 a.m. and before exercise. Participants will be asked to lie supine at rest for 30 minutes in order to achieve a steady state prior to measurement. Oxygen consumption and carbon dioxide production will then be measured with a ventilated hood system (COSMED Quark CPET Metabolic Testing System) for 30-45 minutes. For the determination of RMR, only data during steady state will be used, which is defined as variations in oxygen uptake, carbon dioxide production of less than 10%, and in respiratory quotient by less than 5% for at least 10 minutes. RMR will be calculated from oxygen uptake and carbon dioxide production using the Weir equation (Weir, 1949).

o Fasting Blood Collection: A fasting blood sample will be collected from a forearm vein by trained personnel.

o VO2peak: Participants will be asked to complete a graded cycle ergometer test to determine aerobic fitness (VO2 peak). Participants will begin cycling on the bicycle ergometer at 60 W for 3 minutes. The work rate will be increased by 35 W every 3 minutes until exhaustion (Achten & Jeukendrup, 2003). Exhaustion is operationally defined when at least two of the following are met: 1) 90% age predicted heart rate max [220-age], 2) respiratory exchange ratio 1.1, 3) a rate of perceived exertion 19, 4) a plateau in VO2 despite an increase in workload, 5) and/or unable to maintain a pedal rate of 60 rpm. During the test, expired gases will be measured through indirect calorimetry (COSMED Quark CPET Metabolic Testing System), and heart rate will be monitored through telemetry (Garmin).

o Questionnaires: The Three-Factor Eating Questionnaire, a questionnaire that measures three dimensions of human eating behavior (cognitive dietary restraint, disinihibition, hunger) (Stunkard & Messick, 1985), The Profile of Mood States (POMS), a questionnaire used to assess overall mood. This

profile describes six sub-components of the overall mood construct: anger, confusion, depression, fatigue, tension and vigor (McNair et al., 1971); an abbreviated version of the Eating Disorder Inventory, a self-report questionnaire used to assess the presence of eating disorders (D. M. Garner & Olmstead, 1991; David M. Garner, Olmstead, & Polivy, 1983); Satiety/Hunger Questionnaire: Participants will complete a visual analog scale (VAS) which quantifies 1) appetite, 2) fullness, 3) perception of quantity of food which can be eaten, 4) nausea, 5) thirst, and 6) stress

3. Describe how long, in terms of time, the procedures will take the participant to complete. The description should include the duration of a session, the number of sessions, over what period of time and the total time required to complete the procedures.

Following screening, the study will take at least 9 weeks to complete:

- 2 week pre-study period for preliminary testing and calcium/vitamin D equilibration

- 6 days per study condition (3x)

- Minimum 2 weeks between study conditions (2x)

4. Statistical Analysis Plan

Paired one-sided t tests will be used to assess whether changes from baseline are significantly different from 0. Where significant differences from 0 exist and differences between groups would be expected, additional t tests will be used to compare change scores between CR-LP and CR-HP or CR-HP and CON. Differences between CR-LP and CON will not be evaluated due to the existence of two manipulated variables (protein and kcal) causing the difference in those groups. If exploratory analyses are conducted, multiple comparisons will be corrected for using Holm's sequential version of the Bonferroni correction (Holm S 1979).