Effects of Icelandic yogurt consumption and resistance training in healthy untrained older males

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Effects of Icelandic yogurt consumption and resistance training in healthy untrained older males

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Running title: Resistance training and Icelandic yogurt consumption

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Abstract

Due to the important roles of resistance training and protein consumption in the prevention and treatment of sarcopenia, we assessed the efficacy of post-exercise Icelandic yogurt consumption on lean mass, strength, and skeletal muscle regulatory factors in healthy untrained older males. Thirty healthy untrained older males (age = 68 ± 4 yr) were randomly assigned to Icelandic yogurt (IR; n =15, 18 g of protein) or an iso-energetic placebo (PR; n =15, 0 g protein) immediately following resistance training (3x/week) for eight weeks. Before and after training, lean mass, strength, and skeletal muscle regulatory factors (insulin-like growth factor-1 [IGF-1], transforming growth factor-beta 1 [TGF-β1], growth differentiation factor 15 [GDF15], Activin A, myostatin [MST], and follistatin [FST]) were assessed. There were group x time interactions ($p < 0.05$) for body mass (IR: Δ 1, PR: Δ 0.7 kg), body mass index (IR: Δ 0.3, PR: Δ 0.2 kg·m^{-2}), lean mass (IR: Δ 1.3, PR: Δ 0.6 kg), bench press (IR: Δ 4, PR: 2.3 kg), leg press (IR: Δ 4.2, PR: Δ 2.5 kg), IGF-1 (IR: Δ 0.5, Δ PR: 0.1 ng·mL^{-1}), TGF-β (IR: Δ -0.2, PR: Δ -0.1 ng·mL^{-1}), GDF15 (IR: Δ -10.3, PR: Δ -4.8 pg·mL^{-1}), Activin A (IR: Δ -9.8, PR: Δ -2.9 pg·mL^{-1}), MST (IR: Δ -0.1, PR: Δ -0.04 ng·mL^{-1}), and FST (IR: Δ 0.09, PR: Δ 0.03 ng·mL^{-1}), with Icelandic yogurt consumption resulting in greater changes compared to placebo. The addition of Icelandic yogurt consumption to a resistance training program improved lean mass, strength, and altered skeletal muscle regulatory factors in healthy untrained older males compared to placebo. Therefore, Icelandic yogurt as a nutrient-dense source and cost-effective supplement enhances muscular gains mediated by resistance training and consequently may be used as a strategy for the prevention of sarcopenia.

Keywords: Sarcopenia, Protein, Dairy, Resistance Training, Hypertrophy.

Introduction

Sarcopenia refers to the age-related reduction in muscle quantity and strength (1). The age-related reduction in strength, which is the strongest predictor of health-related outcomes in older adults (2), occurs much more rapidly than the decrease in muscle quantity (3). Although multi-factorial, contributing factors for sarcopenia include physical inactivity (2) and an attenuated anabolic response to dietary protein (i.e., aging anabolic resistance) (4), suggesting that the amount of protein consumed by older adults should be increased to offset sarcopenia. It is well established that resistance training improves aging muscle mass and strength (5; 6; 7). Accumulating research indicates that the addition of protein consumption to a resistance training program can further augment these physiological and neuromuscular adaptations (8). Numerous high-quality complete protein sources such as whey (9), casein (10), egg (11), beef (12), soy (13), and potato (14) acutely elevate the rates of muscle protein synthesis and skeletal muscle regulatory factors, which over time could lead to significant improvements in muscle accretion and strength. Dairy is also a complete protein food source comprised mainly of whey and casein proteins with high essential amino acid content (15). Whey is considered a fast-absorbing protein, while casein is a slow-digesting protein, and their combination appears to be ideal for both initiating and sustaining post-exercise aminoacidemia (16; 17; 18).

Yogurt is a dairy-based probiotic food source and a cost-effective protein source (primarily casein and whey) compared to other marketed protein supplements (19). Research is mixed regarding the efficacy of yogurt consumption during a resistance training program for the improvement of muscular adaptations. One study reported positive outcomes(15), while other investigations reported no significant effects(20; 21). Importantly, previous studies were performed in untrained younger adults; and consequently, the effects of yogurt consumption during a resistance training program in untrained older adults are unknown. While several types of yogurt exist, Icelandic yogurt contains one of the highest concentrations of protein (18 g per 200 g serving), which could serve as an effective adjunct to resistance training for augmenting muscle accretion and strength.

Muscle protein balance is also influenced by various hormones and myokines, which have been suggested to alter the balance between anabolic and catabolic stimuli in muscle, leading to an increase or decrease in muscle mass (22). Briefly, follistatin (FST) stimulates muscle growth while myostatin (MST) is a potent negative regulator of muscle accretion (23). Moreover, Transforming growth factor-beta 1 (TGF-β1) acts as a skeletal muscle regenerator that contributes to extracellular matrix reconstitution and muscle tissue remodeling (24). Activin A is involved in cellular differentiation, remodeling, proliferation, and morphogenesis (25). Growth differentiation factor 15 (GDF15) is a member of the Glial cell-derived neurotrophic factor (GDNF) family, which is bound to GDNF family receptor α-like protein, a transmembrane receptor exclusively expressed in the hindbrain (26). Insulin-like growth factor 1 (IGF-1) is a regulator of the Phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) pathway and widely considered to be required for activating the signal transduction for the initiation of muscle protein synthesis following mechanical loading (27). Two studies have indicated that eight weeks of 3x/week whole-body resistance training increased FST while also decreasing MST concentrations with an enhanced lean mass in middle-aged and sarcopenic elderly males (28; 29). Despite the reported positive impact of resistance training on endocrine markers and myokines in different cohorts, the effects of its combination with yogurt consumption are unknown. Therefore, the primary purpose of this study was to investigate the effects of post-exercise Icelandic yogurt consumption on lean mass and muscle strength in healthy untrained older males. A secondary purpose was to explore the effects of the intervention on skeletal muscle regulatory factors (i.e., FST, MST, TGF-β1, Activin A, GDF15, and IGF-1). We hypothesized that post-exercise Icelandic yogurt consumption, which contains 18 grams of protein, would augment resistance training adaptations (i.e., lean mass and strength) and alter skeletal muscle regulatory factors compared to a placebo consumption.

Experimental methods

Participants

Thirty healthy untrained older males (age = 68 ± 4 yr) volunteered for the study. The Human Subject Committee of the Ferdowsi University of Mashhad approved the study protocol (IR.UM.REC.1399.053). Participants were informed of the benefits, risks, and purpose of the study before their written consent was obtained. Study procedures were in accordance with the Declaration of Helsinki. Inclusion criteria included being ≥ 60 years of age and untrained (performing < 1 h of exercise per week for 12 months prior to the start of the study). Participants were excluded if they were smokers, consumed alcohol regularly, had medical issues that would alter hormonal or muscle biology were lactose intolerant, or consumed dietary supplements containing protein or creatine for 24 weeks beforehand. Participants were also excluded if they were unwilling to comply with the nutritional intervention or resistance training procedures or wanted to engage in additional exercise (independent of the study intervention). Moreover, participants reported no history of smoking or hormonal replacement therapy. Participants were instructed not to alter their lifestyle or habitual dietary intake throughout the study. A physician administered a health and medical questionnaire to determine participant health status. Before the intervention started, participants were familiarized with the testing and experimental procedures.

Design

The study used a randomized, double-blind, placebo-controlled parallel design. Participants were randomly assigned, using a computer program (www.randomizer.org) to consume Icelandic yogurt (IR; n = 15) or placebo (PR; n = 15) during eight weeks of resistance training. Prior to and following the study, measures of lean mass, muscle strength, and skeletal muscle regulatory factors were made. Post-testing measurements occurred 72 hours after the last training session.

Resistance training

Before the initiation of the resistance training program, participants performed three familiarization training sessions (supervised) with the resistance training equipment. After the familiarization phase, participants performed whole-body resistance training (3x/week; supervised) according to guidelines and recommendations established for older individuals (30; 31). Prior to each training session, participants performed a 10-minute warm-up consisting of light stretching. Participants then performed three sets of 8-12 repetitions for eight exercises (leg press, leg extension, lying leg curl, chest press, shoulder press, seated rows, biceps curl, and sit-ups) at 60-80% of 1-repetition maximum (1RM) with 90-second rest intervals between sets (Table 1) (23; 32). All training sessions occurred between 16:00 and 18:00. Training volume was calculated using the following formula (Training volume = [repetitions (n) × sets (n) × load or selected weight (kg)] (33).

Nutritional intervention

Participants in the IR group consumed Icelandic yogurt (200 g serving; 18 g protein, 0 g fat, 4 g carbohydrates, Kalleh Industry, health license number: 49/14305) while participants in the PR group consumed a carbohydrate-based pudding (placebo [contained maltodextrin and water]; 200 g serving: 0 g protein, 0 g fat, 2 g carbohydrates) immediately following each resistance training session (in the presence of an exercise supervisor) and at the same time on non-training days. To monitor compliance on non-training days, supplement packages were returned to the researchers at the next subsequent training session (15) and documented in logs. Compliance was calculated by dividing the number of consumed servings by the expected number of servings. Icelandic yogurt was consumed immediately following each training session because post-exercise protein supplementation is important for augmenting gains in muscle mass in older adults (34). The Icelandic yogurt formulation was verified by independent laboratory testing (ViroMed Laboratory). Icelandic yogurt and placebo were in opaque containers and were very similar in taste (flavored with vanilla) and appearance. All personnel involved in the study were blinded to group allocations.

Body composition

Participants fasted for 12 hours with at least 8 hours of sleep before body composition was assessed. Upon arrival at the laboratory, participants were required to void their bladder. Body mass was measured with a digital scale (Seca, Germany) to the nearest 0.1 kg. Stature was measured with a stadiometer (Race Industrialization, China) to the nearest 0.1 cm. Body mass index (BMI), fat mass, and lean mass were determined by a multi-frequency bioelectrical impedance device (Inbody 770, Seoul, South Korea). The test-retest reliability of the bioelectrical impedance method was r = 0.96 to 0.99.

Strength

Maximal strength (1RM) was determined 24 hours after body composition, and skeletal muscle regulatory factors were measured. Participants were asked to abstain from alcohol for 48 hours, caffeinated drinks for 12 hours, food and drink (water was allowed *ad libitum*) for 2 hours prior to testing. Following a light aerobic warm-up, participants performed two sets of repetitions to volitional fatigue (< 10 repetitions) on the leg press and bench press (Technogym equipment, Italy). Each set was separated by 5 minutes of passive rest (intraclass correlation coefficient [ICC]: 0.96 to 0.98). Maximal strength was estimated using the following formula: $1RM = weight / (1.0278–0.0278 \times repetitions)$ (35). Bench press and leg press exercises were used to measure upper and lower body strength (36), and 1RM's were used to determine individualized resistance training prescriptions.

Skeletal muscle regulatory factors

Fasting blood samples (10 mL) were collected from the antecubital vein using standard procedures. Following blood sampling, the samples were placed at room temperature for 15 minutes to clot. Samples were centrifuged at 3000 rpm for 10 minutes, and serum was stored at -80°C for future analysis. Commercially available ELISA kits were used to determine insulin-like growth factor 1 (IGF-1; CUSABIO, USA; sensitivity: < 1.95 $ng \cdot mL^{-1}$), Activin A (CUSABIO, USA; sensitivity: 3.9 $pg \cdot mL^{-1}$), FST (CUSABIO, USA; sensitivity: 0.025 $ng \cdot mL^{-1}$), MST (CUSABIO, USA;

sensitivity: 0.312 ng·mL^{-1}), TGF-β1 (CUSABIO, USA; sensitivity: 0.747 ng·mL^{-1}), and GDF15 (CUSABIO, USA; sensitivity: 1.95 pg·mL^{-1}). All serum sample concentrations were measured with a microplate reader (GDV, Germany) at a wavelength of 450 nm. The intra and inter-assay coefficient of variation for IGF-1 was <10% and <12%, Activin A, TGF-β1, and GDF15, <8% and 10%, FST and MST <12%, respectively.

Diet

Participants filled out dietary logs (two weekdays and one weekend day) at baseline and immediately after the study (daily dietary habits and supplements' nutrients). Food items were entered and analyzed (Diet Analysis Plus, version 10; Cengage, Boston, MA, USA) to determine changes in total energy (kcal), carbohydrate, fat, and protein over time (5).

Statistical Analyses

The normality of the data was confirmed using the D'Agostino & Pearson test. Based on data from previous studies evaluating muscular outcomes following resistance training combined with different protein supplementation in older adults(36; 37), it was calculated that 12 participants per group would provide 80% power (two-sided α=0.05) to detect 7% between-group changes in lean mass and muscular strength. Unpaired t-tests examined a comparison of baseline descriptive characteristics. The effect of group (placebo, Icelandic yogurt) and time (prior, post) was examined throughout using repeated measures (within [time], between [group]) ANOVA. Significant interactions were followed up using Bonferroni post hoc analyses. Pearson's linear regression was used to examine the relationship between continuous variables with an r^2 value of > 0.02, 0.13, and 0.26 as the threshold for a weak, moderate, and substantial effect (38). A p-value of < 0.05 was considered significant throughout. GraphPad Prism (version 8.4.3) was used for all statistical analysis and figure production.

Results

Compliance, adverse events, diet, and training volume

Compliance with the nutritional interventions and resistance training program was > 90%. One participant from each group withdrew because of personal reasons not related to the study. No adverse events were reported from Icelandic yogurt, placebo, or the resistance training program. There were no significant differences at baseline between groups for any variable (Table 2). There were group x time interactions for total energy (kcal; $p = 0.002$), absolute protein (g/day; $p < 0.001$) and relative protein intake [(g/kg/day; $p < 0.001$), (Table 3)]. Total energy intake was higher at the end of the study (compared to baseline) in the IR group (baseline: 1755.8 ± 46.7 kcal/day; post: 1812 ± 43.7 kcal/day, $p = 0.002$) with no change in the PR group (baseline: 1716.6 ± 50.1 kcal; post: 1729 ± 72 kcal/day, $p = 0.648$). Similarly, absolute protein intake increased in the IR group over time (baseline: 78.7 ± 5.5 g/day; post: 97.2 ± 5.9 g/day, $p < 0.001$) with no change in the PR group (baseline: 78.0 ± 5.1 g/day; post: 79.1 ± 4.1 g/day, $p = 0.809$). Compared to baseline, relative protein intake increased in the IR group over time (pre 1.3 ± 0.9 g/kg/day, post 1.6 ± 0.1 g/kg/day) with no change in the PR group (pre 1.2 ± 0.1 g/kg/day, post 1.3 ± 0.1 g/kg/day). There were no differences between groups for total training volume performed over the eight weeks of resistance training (IR: 106555 ± 7171 kg; PR: 102184 ± 6361 kg; $p = 0.100$).

Body composition and physical performance

There were significant group x time interactions ($p < 0.05$) for body mass (IR: Δ 1 kg, 95% CI: 0.5 to 1.4; PR: Δ 0.7 kg, 95% CI: 1.2 to 0.3), body mass index (IR: Δ 0.3 $kg{\cdot}m^{-2}$, 95% CI: 0.2 to 0.5; PR: Δ 0.2 $kg{\cdot}m^{-2}$, 95% CI: 0.4 to 0.1), lean mass (IR: Δ 1.3 kg, 95% CI: 0.9 to 1.6; PR: Δ 0.6 kg, 95% CI: 0.2 to 1), bench press (IR: Δ 4 kg, 95% CI: 2.8 to 5; PR: Δ 2.3 kg, 95% CI: 1.7 to 2.9), and leg press (IR: Δ 4.2 kg, 95% CI: 3.4 to 5.1; PR: Δ 2.5 kg, 95% CI: 2.1 to 3). Participants in the IR group experienced greater changes in body mass ($p < 0.001$; Figure 1B), body mass index ($p < 0.001$; Figure 1D), lean mass ($p = 0.012$; Figure 1F), bench press ($p = 0.012$; Figure 1H), and leg press ($p = 0.001$; Figure 1J) compared to those in the PR group. However, fat mass remained unchanged in both groups ($p > 0.05$).

Skeletal muscle regulatory factors

There were significant group x time interactions ($p < 0.05$) for IGF-1 (IR: Δ 0.5 $ng{\cdot}mL^{-1}$, 95% CI: 0.3 to 0.6; PR: Δ 0.1 $ng{\cdot}mL^{-1}$, 95% CI: 0.08 to 0.2), TGF-β (IR: Δ -0.2 $ng{\cdot}mL^{-1}$, 95% CI: -0.2 to -0.1; PR: Δ -0.1 $ng{\cdot}mL^{-1}$, 95% CI: -0.1 to -0.05), GDF15 (IR: Δ -10.3 $pg{\cdot}mL^{-1}$, 95% CI: -13.6 to -7; PR: Δ -4.8 $pg{\cdot}mL^{-1}$, 95% CI: -9 to -0.7), Activin A (IR: Δ -9.8 $pg{\cdot}mL^{-1}$, 95% CI: -12.3 to -7.2; PR: Δ -2.9 $pg{\cdot}mL^{-1}$, 95% CI: -5.6 to -0.2), MST (IR: Δ -0.1 $ng{\cdot}mL^{-1}$, 95% CI: -0.1 to -0.08; PR: Δ -0.04 $ng{\cdot}mL^{-1}$, 95% CI: -0.06 to -0.02), and FST (IR: Δ 0.09 $ng{\cdot}mL^{-1}$, 95% CI: 0.06 to 0.1; PR: Δ 0.03 $ng{\cdot}mL^{-1}$, 95% CI: 0.02 to 0.05). The changes in IGF-1 ($p<0.001$; Figure 2B), TGF-β1 ($p = 0.003$; Figure 2D), FST ($p = 0.002$; Figure 2L), GDF15 ($p = 0.034$; Figure 2F), Activin A ($p < 0.001$; Figure 2H), and MST ($p < 0.001$; Figure 2J) were significantly greater in the IR compared to PR group.

Correlations

There was a moderate correlation ($r^2 = 0.160$; $p = 0.035$) between the change in Activin A concentration and change in lean mass over time (Figure 3D), with no other significant correlations ($p > 0.05$).

Discussion

This was the first study to examine the effects of Icelandic yogurt consumption during a supervised whole-body resistance training program in healthy untrained older males who were consuming > 1.2 g/kg/day of dietary protein. Results showed that Icelandic yogurt consumption augmented resistance training gains in lean mass and strength and influenced skeletal muscle regulatory factors compared to placebo. There were no adverse events reported from the nutritional intervention or resistance training program. Icelandic yogurt consumption was also effective at increasing protein and total energy consumption, which is evident by the higher values on these markers in the IR compared to the PR group (Table 3).

The greater increase in lean mass and strength from Icelandic yogurt consumption in healthy untrained older males supports previous findings in untrained younger males. For example, Bridge et al. showed that Greek yogurt consumption (20 g protein/serving, 3 servings on training days [60 g of protein in total] and 2 servings on non-training days [40 g of protein in total]) during a supervised whole-body resistance training program (3 times per week for 12 weeks) significantly increased fat-free mass ($p = 0.046$), elbow flexor muscle thickness ($p = 0.004$) and measures of strength (chest press [$p = 0.026$] and leg extension [$p = 0.004$]) in males (18-25 yrs) compared to those on an isoenergetic placebo (pudding; 0 g protein) (15). Greek yogurt resulted in greater absolute and relative protein intake over time compared to those on placebo ($p < 0.001$) and total energy intake (pre vs. post), but this did not reach statistical significance). Mechanistically, the mechanical stimulus from resistance training increases the rates of muscle protein synthesis, which are further elevated in the presence of dietary proteins (39). Over time, (i.e., a resistance training program) could lead to significant muscle accretion and strength. In addition to its protein content, yogurt also contains calcium and vitamin D. In a systematic review performed by vanDronkelaar et al. (2018), calcium levels were inversely associated with the incidence of sarcopenia, possibly because of altered calcium absorption or homeostasis in aging muscle (40). Furthermore, calcium is dependent on vitamin D for absorption, and vitamin D levels have been shown to be lower in older adults with sarcopenia (4; 40). Further, vitamin D supplementation improved tasks of muscle function in older adults (4).

The present study involving Icelandic yogurt consumption (18 g of protein) is somewhat comparable to other dairy-based interventions in older adults. Nakayama et al. (2020) showed that six months of milk protein consumption (10 g/day) during body weight and medicine ball exercise training significantly increased lean mass over time in older adults (n = 61; 71 yrs; relative protein intake: 1.28 g/kg/day) compared to no change for those consuming a placebo (n = 61; 70 yrs; relative protein intake: 1.23 g/kg/day) (41). In addition, six months of high-intensity resistance training combined with whey protein improved muscle cross-sectional area and strength in mobility-limited older adults (70-85 yrs) who were consuming 1.2 g/kg/day of protein at the end of the intervention(42). Twelve weeks of whey protein combined with resistance training significantly increased muscle mass, muscular strength, and functional capacity in older women who consumed 1.4 g/kg/day of protein(43). Furthermore, Hevia-Larraín et al. showed that a protein intake of ~ 1.6 g/kg/day (regardless of protein source) had a positive effect on gains in muscle mass and strength(44). In the present study, participants consumed 1.6 g/kg/day of protein (including Icelandic protein), which resulted in lean mass and strength gains. Collectively, findings across studies indicate that older adults may experience some muscle benefits when consuming > 1.2 g/kg/day of protein, including dairy-based protein food sources. Presently, there is a lack of research directly comparing different dairy food sources in conjunction with resistance training on muscle adaptations.

While direct mechanistic actions of muscle protein synthesis and breakdown were not measured in this study, we did measure several skeletal muscle regulatory factors purported to alter muscle accretion. MST is a potent inhibitor of muscle growth and binds to muscle Activin Type II receptors activating the intracellular SMAD protein signaling pathway (45). MST may inhibit muscle hypertrophy by decreasing the mechanistic target of rapamycin complex 1 (mTORC1) and increasing forkhead box protein O1 (FOXO1). FST acts as an antagonist to MST with both paracrine and autocrine effects and is purported to increase muscle accretion (46). Our findings support previous studies showing both reduction in MST and an increase in FST (5; 47; 48; 49; 50) following resistance training. We observed a reduction in MST, TGF-β1, Activin A, and GDF15 and an increase in IGF-1 and FST in both groups. These aforementioned decrements and increments were significantly greater in the Icelandic yogurt consumption group compared to the placebo. The reduction in TGF-β1 may be associated with alterations in MST since there is a coregulatory relationship within skeletal muscle (51). In addition, FST has been indicated to stimulate muscle hypertrophy through the proliferation of satellite cells and MST and Activin A inhibition (52). Further, the expression of IGF-1 within skeletal muscle following resistance training has been suggested to play a critical role in skeletal muscle accretion(53). IGF-1 is a regulator of the PI3K and Akt pathway and is widely considered required for activating the signal transduction for the initiation of muscle protein synthesis following mechanical loading (27). In agreement with our findings, a recent systematic review and meta-analysis reports the positive association of increments of IGF-1 with resistance training (54). Interestingly, despite resistance training clearly altering several known regulators of muscle accretion, we only found one modest correlation ($r^2 = 0.160$; $p = 0.035$) between the change in Activin A and lean mass. Future research is warranted to directly measure acute and chronic alterations following resistance training with muscle protein synthesis.

Many older individuals experience several barriers to exercise(55); therefore, one important strength of this study was the participants' high adherence level to our exercise intervention. This investigation is limited by the absence of measurements of skeletal muscle anabolism (mTORC1 signaling, MPS) which would have assisted in the explanation of our outcomes. However, it has been proposed that promotions in circulating concentrations of signaling molecules increase the likelihood of a receptor interaction, and therefore a biological effect within skeletal muscle (56; 57). Second, we did not include a yogurt-only group. However, the effects of regular yogurt consumption have been previously indicated (20; 21), which showed no further effects on muscular gains after regular resistance training. The lack of positive effects of regular yogurt consumption may be due to the lower amounts of protein (5 g of protein per serving), highlighting the importance of higher amounts of protein to induce significant effects on muscular gains. Given the importance of higher protein intakes in older adults due to the prevalence of anabolic resistance (58; 59) and a lower amount of protein in regular yogurt, we did not incorporate regular yogurt consumption. Additionally, bioelectrical impedance was used to measure body composition, which is not as precise as dual-energy x-ray absorptiometry (the gold standard technique for body composition measurement); however, previous studies have shown that it is a valid and reliable method (60; 61).

In conclusion, post-exercise Icelandic yogurt consumption augmented resistance training gains in lean mass, strength, and altered skeletal muscle regulatory factors in healthy untrained older males. This is critical for older populations as increases in lean mass and strength may prevent sarcopenia as well as improve the risk of falls and enhance independent living (1; 62). Future research should investigate the effects of Icelandic yogurt consumption, with and without resistance training on measures of muscle and bone in younger and older adults.

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Conflict of interest

The authors declare no conflict of interest.

References

1.McKendry J, Currier BS, Lim C *et al.* (2020) Nutritional Supplements to Support Resistance Exercise in Countering the Sarcopenia of Aging. *Nutrients* **12**, 2057.
2.Cruz-Jentoft AJ, Bahat G, Bauer J *et al.* (2019) Sarcopenia: revised European consensus on definition and diagnosis. *Age and ageing* **48**, 16-31.
3.Mitchell WK, Atherton PJ, Williams J *et al.* (2012) Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Frontiers in physiology* **3**, 260.
4.Robinson SM, Reginster J-Y, Rizzoli R *et al.* (2018) Does nutrition play a role in the prevention and management of sarcopenia? *Clinical Nutrition* **37**, 1121-1132.
5.Bagheri R, Rashidlamir A, Motevalli MS *et al.* (2019) Effects of upper-body, lower-body, or combined resistance training on the ratio of follistatin and myostatin in middle-aged men. *European journal of applied physiology*, 1-11.
6.Fragala MS, Cadore EL, Dorgo S *et al.* (2019) Resistance training for older adults: position statement from the national strength and conditioning association. *The Journal of Strength & Conditioning Research* **33**.
7.Bagheri R, Forbes SC, Candow DG *et al.* (2020) Effects of branched-chain amino acid supplementation and resistance training in postmenopausal women. *Experimental Gerontology*, 111185.
8.Finger D, Goltz FR, Umpierre D *et al.* (2015) Effects of protein supplementation in older adults undergoing resistance training: a systematic review and meta-analysis. *Sports medicine* **45**, 245-255.
9.Davies RW, Carson BP, Jakeman PM (2018) The effect of whey protein supplementation on the temporal recovery of muscle function following resistance training: A systematic review and meta-analysis. *Nutrients* **10**, 221.
10.Joy JM, Vogel RM, Broughton KS *et al.* (2018) Daytime and nighttime casein supplements similarly increase muscle size and strength in response to resistance training earlier in the day: a preliminary investigation. *Journal of the International Society of Sports Nutrition* **15**, 24.
11.Bagheri R, Moghadam BH, Jo E *et al.* (2 (020Comparison of whole egg vs. egg white ingestion during 12 weeks of resistance training on skeletal muscle regulatory markers in resistance-trained men. *British Journal of Nutrition*, 1-20.
12.Valenzuela PL, Mata F, Morales JS *et al.* (2019) Does beef protein supplementation improve body composition and exercise performance? A systematic review and meta-analysis of randomized controlled trials. *Nutrients* **11**, 1429.
13.Orsatti FL, Maestá N, de Oliveira EP *et al.* (2018) Adding soy protein to milk enhances the effect of resistance training on muscle strength in postmenopausal women. *Journal of dietary supplements* **15**, 140-152.
14.Oikawa SY, Bahniwal R, Holloway TM *et al.* (2020) Potato Protein Isolate Stimulates Muscle Protein Synthesis at Rest and with Resistance Exercise in Young Women. *Nutrients* **12**, 1235.
15.Bridge A, Brown J, Snider H *et al.* (2019) Greek yogurt and 12 weeks of exercise training on strength, muscle thickness and body composition in lean, untrained, university-aged males. *Frontiers in nutrition* **6**, 55.
16.Dangin M, Boirie Y, Garcia-Rodenas C *et al.* (2001) The digestion rate of protein is an independent regulating factor of postprandial protein retention. *American Journal of Physiology-Endocrinology And Metabolism* **280**, E340-E348.
17.Josse AR ,Phillips SM (2012) Impact of milk consumption and resistance training on body composition of female athletes. In *Acute Topics in Sport Nutrition*, vol. 59, pp. 94-103: Karger Publishers.

18.Granic A, Hurst C, Dismore L *et al.* (2019) Milk and resistance exercise intervention to improve muscle function in community-dwelling older adults at risk of sarcopenia (MIlkMAN): protocol for a pilot study. *BMJ open* **9**, e031048.

19.El-Abbadi NH, Dao MC, Meydani SN (2014) Yogurt: role in healthy and active aging. *The American journal of clinical nutrition* **99**, 1263S-1270S.

20.White KM, Bauer SJ, Hartz KK *et al.* (2009) Changes in body composition with yogurt consumption during resistance training in women. *International journal of sport nutrition and exercise metabolism* **1**.18-33 ,9

21.Thomas DT, Wideman L, Lovelady CA (2011) Effects of a dairy supplement and resistance training on lean mass and insulin-like growth factor in women. *International journal of sport nutrition and exercise metabolism* **21**, 181-188.

22.Solomon A, Bouloux P (2006) Modifying muscle mass–the endocrine perspective. *Journal of Endocrinology* **191**, 349-360.

23.Bagheri R, Moghadam BH, Church DD *et al.* (2020) The effects of concurrent training order on body composition and serum concentrations of follistatin ,myostatin and GDF11 in sarcopenic elderly men. *Experimental Gerontology* **133**, 110869.

24.Touvra A-M, Volaklis KA, Spassis AT *et al.* (2011) Combined strength and aerobic training increases transforming growth factor-β1 in patients with type 2 diabetes. *Hormones* **10**, 125-130.

25.Silva R, Bueno P, Avó L *et al.* (2014) Effect of physical training on liver expression of activin A and follistatin in a nonalcoholic fatty liver disease model in rats. *Brazilian Journal of Medical and Biological Research* **47**, 746-752.

26.Baek SJ, Eling T (2019) Growth differentiation factor 15 (GDF15): A survival protein with therapeutic potential in metabolic diseases. *Pharmacology & therapeutics* **198**, 46-58.

27.Spangenburg EE (2009) Changes in muscle mass with mechanical load: possible cellular mechanisms. *Applied Physiology, Nutrition, and Metabolism* **34**, 328-335.

28.Bagheri R, Rashidlamir A, Motevalli MS *et al.* (2019) Effects of upper-body, lower-body, or combined resistance training on the ratio of follistatin and myostatin in middle-aged men. *European journal of applied physiology* **119**, 1921-1931.

29.Negaresh R, Ranjbar R, Baker JS *et al.* (2019) Skeletal muscle hypertrophy, insulin-like growth factor 1, myostatin and follistatin in healthy and sarcopenic elderly men: the effect of whole-body resistance training. *International journal of preventive medicine* **10**.

30.Chodzko-Zajko WJ, Proctor DN, Singh MAF *et al.* (2009) Exercise and physical activity for older adults. *Medicine & science in sports & exercise* **41**, 1510-1530.

31.Medicine ACoS (2013) *ACSM's guidelines for exercise testing and prescription*: Lippincott Williams & Wilkins.

32.Mayer F, Scharhag-Rosenberger F, Carlsohn A *et al.* (2011) The intensity and effects of strength training in the elderly. *Deutsches Ärzteblatt International* **108**, 359.

33.Cunha PM, Nunes JP, Tomeleri CM *et al.* (2020) Resistance training performed with single and multiple sets induces similar improvements in muscular strength, muscle mass, muscle quality, and IGF-1 in older women: A randomized controlled trial. *The Journal of Strength & Conditioning Research* **34**, 1008-1016.

34.Esmarck B, Andersen J, Olsen S *et al.* (2001) Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *The Journal of physiology* **535**, 301-311.

35.Murach KA, Bagley JR (2016) Skeletal muscle hypertrophy with concurrent exercise training: contrary evidence for an interference effect. *Sports medicine* **46**, 1029-1039.

36.Krause M, Crognale D, Cogan K *et al.* (2019) The effects of a combined bodyweight-based and elastic bands resistance training, with or without protein supplementation, on muscle mass, signaling and heat shock response in healthy older people. *Experimental gerontology* **115**, 104-113.

37.Maltais ML, Perreault K, Courchesne-Loyer A *et al.* (2016) Effect of resistance training and various sources of protein supplementation on body fat mass and metabolic profile in sarcopenic overweight older adult men: A pilot study. *International journal of sport nutrition and exercise metabolism* **26**, 71-77.

38.Cohen J (1988) Statistical power. *Analysis for the behavioral sciences*, 273-406.

39.Damas F, Phillips S, Vechin FC *et al.* (2015) A review of resistance training-induced changes in skeletal muscle protein synthesis and their contribution to hypertrophy. *Sports medicine* **45**, 801-807.

40.van Dronkelaar C, van Velzen A, Abdelrazek M *et al.* (2018) Minerals and sarcopenia; the role of calcium, iron, magnesium, phosphorus, potassium, selenium, sodium, and zinc on muscle mass, muscle strength ,and physical performance in older adults: a systematic review. *Journal of the American Medical Directors Association* **19**, 6-11. e13.

41.Nakayama K, Saito Y, Sanbongi C *et al.* (2020) Effects of low-dose milk protein supplementation following low-to-moderate intensity exercise training on muscle mass in healthy older adults: a randomized placebo-controlled trial. *European Journal of Nutrition*, 1-12.

42.Chalé A, Cloutier GJ, Hau C *et al.* (2013) Efficacy of whey protein supplementation on resistance exercise–induced changes in lean mass, muscle strength, and physical function in mobility-limited older adults. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences* **68**, 682-690.

43.Nabuco HC, Tomeleri CM, Sugihara Junior P *et al.* (2018) Effects of whey protein supplementation pre-or post-resistance training on muscle mass, muscular strength, and functional capacity in pre-conditioned older women: a randomized clinical trial. *Nutrients* **10**, 563.

44.Hevia-Larraín V, Gualano B, Longobardi I *et al.* (2021) High-Protein Plant-Based Diet Versus a Protein-Matched Omnivorous Diet to Support Resistance Training Adaptations: A Comparison Between Habitual Vegans and Omnivores. *Sports Medicine*, 1-14.

45.Elliott B, Renshaw D, Getting S *et al.* (2012) The central role of myostatin in skeletal muscle and whole body homeostasis. *Acta Physiologica* **205**, 324-340.

46.Tortoriello DV, Sidis Y, Holtzman DA *et al.* (2001) Human follistatin-related protein: a structural homologue of follistatin with nuclear localization .*Endocrinology* **142**, 3426-3434.

47.Pourabbas M, Bagheri R, Hooshmand Moghadam B *et al.* (2021) Strategic Ingestion of High-Protein Dairy Milk during a Resistance Training Program Increases Lean Mass, Strength, and Power in Trained Young Males. *Nutrients* **13**.948 ,

48.Bagheri R, Rashidlamir A, Attarzadeh Hosseini SR (2018) Effect of resistance training with blood flow restriction on follistatin to myostatin ratio, body composition and anaerobic power of trained-volleyball players. *Medical Laboratory Journal* **1**.28-33 ,2

49.Bagheri R, Moghadam BH, Jo E *et al.* (2020) Comparison of whole egg v. egg white ingestion during 12 weeks of resistance training on skeletal muscle regulatory markers in resistance-trained men. *British Journal of Nutrition* **124**, 1035-1043.

50.Bagheri R, Forbes SC, Candow DG *et al.* (2021) Effects of branched-chain amino acid supplementation and resistance training in postmenopausal women. *Experimental Gerontology* **144**, 111185.

51.Zhu J, Li Y, Shen W *et al.* (2007) Relationships between transforming growth factor-β1, myostatin, and decorin implications for skeletal muscle fibrosis. *Journal of Biological Chemistry* **282**, 25852-25863.

52.Gilson H, Schakman O, Kalista S *et al.* (2009) Follistatin induces muscle hypertrophy through satellite cell proliferation and inhibition of both myostatin and activin. *American Journal of Physiology-Endocrinology and Metabolism* **297**, E157-E164.

53.Barbé C, Kalista S, Loumaye A *et al.* (2015) Role of IGF-I in follistatin-induced skeletal muscle hypertrophy. *American Journal of Physiology-Endocrinology and Metabolism* **309**, E557-E567.

54.Jiang Q, Lou K, Hou L *et al.* (2020) The effect of resistance training on serum insulin-like growth factor 1 (IGF-1): a systematic review and meta-analysis. *Complementary Therapies in Medicine*, 102360.

55.Hickey ME, Mason SE (2017) Age and gender differences in particpation rates, motivators for, and barriers to exercise. *Modern Psychological Studies* **22**, 3.

56.Kraemer WJ, Marchitelli L, Gordon SE *et al.* (1990) Hormonal and growth factor responses to heavy resistance exercise protocols. *Journal of Applied Physiology* **69**, 1442-1450.

57.Ahtiainen JP, Pakarinen A, Alen M *et al.* (2003) Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. *European journal of applied physiology* **89**, 555-563.

58.Bauer J, Biolo G, Cederholm T *et al.* (2013) Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *Journal of the american Medical Directors association* **14**, 542-559.

59.Deutz NE, Bauer JM, Barazzoni R *et al.* (2014) Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group. *Clinical nutrition* **33**, 929-936.

60.Ling CH, de Craen AJ, Slagboom PE *et al.* (2011) Accuracy of direct segmental multi-frequency bioimpedance analysis in the assessment of total body and segmental body composition in middle-aged adult population. *Clinical nutrition* **30**, 610-615.

61.Jackson A, Pollock ML, Graves JE *et al.* (1988) Reliability and validity of bioelectrical impedance in determining body composition. *Journal of Applied Physiology* **64**, 529-534.

62.Talar K, Hernández-Belmonte A, Vetrovsky T *et al.* (2021) Benefits of Resistance Training in Early and Late Stages of Frailty and Sarcopenia: A Systematic Review and Meta-Analysis of Randomized Controlled Studies. *Journal of Clinical Medicine* **10**, 1630.

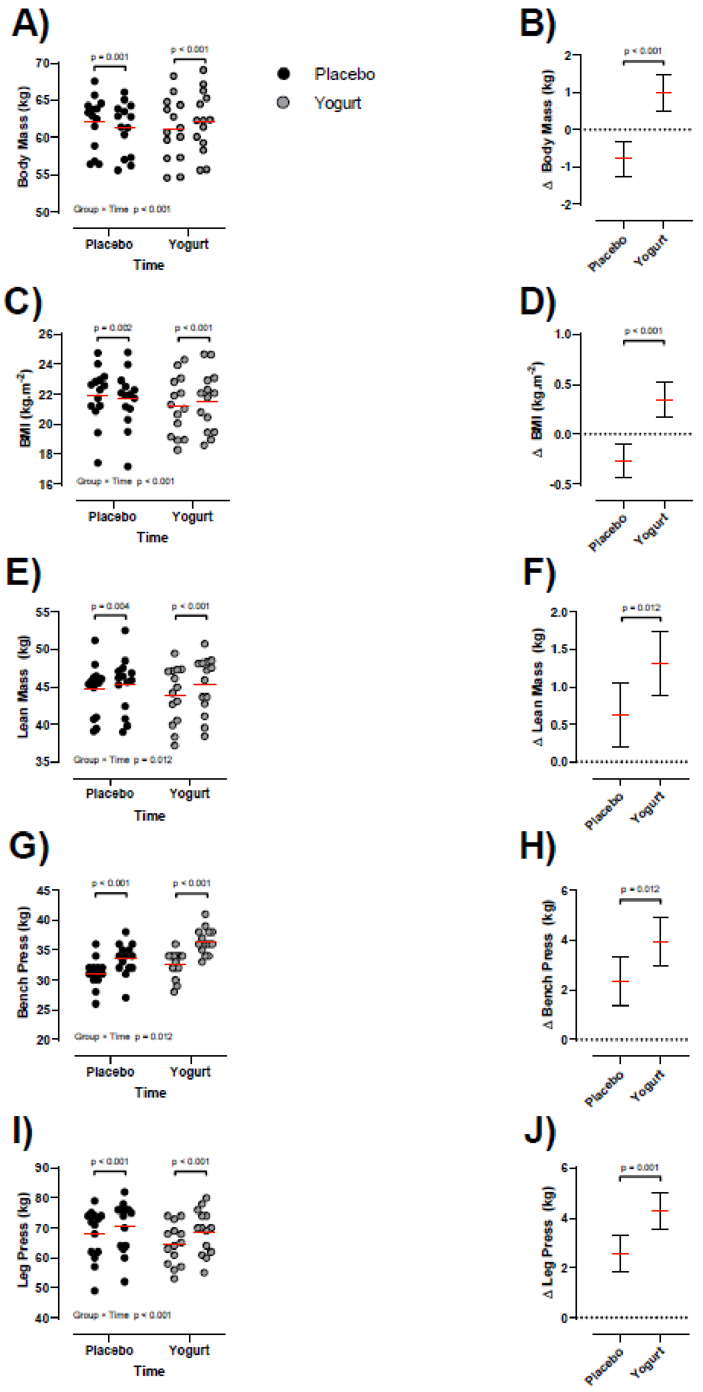

Figure 1: Effect of training time (pre, post) and experimental group (Icelandic yogurt, placebo) on body composition and strength. Red horizontal lines indicate group means; error bars indicate 95% confidence intervals. N = 14 per group, placebo indicated by black circles, yogurt indicated by grey circles. **A)** Body Mass and **B)** Δ Body Mass, **C)** BMI and **D)** Δ BMI (kg.m^{-2}), **E)** Lean Mass and **F)** Δ Lean Mass (kg), **G)** Bench Press and **H)** Δ Bench Press (kg), and **I)** Leg Press and **J)** Δ Leg Press (kg).

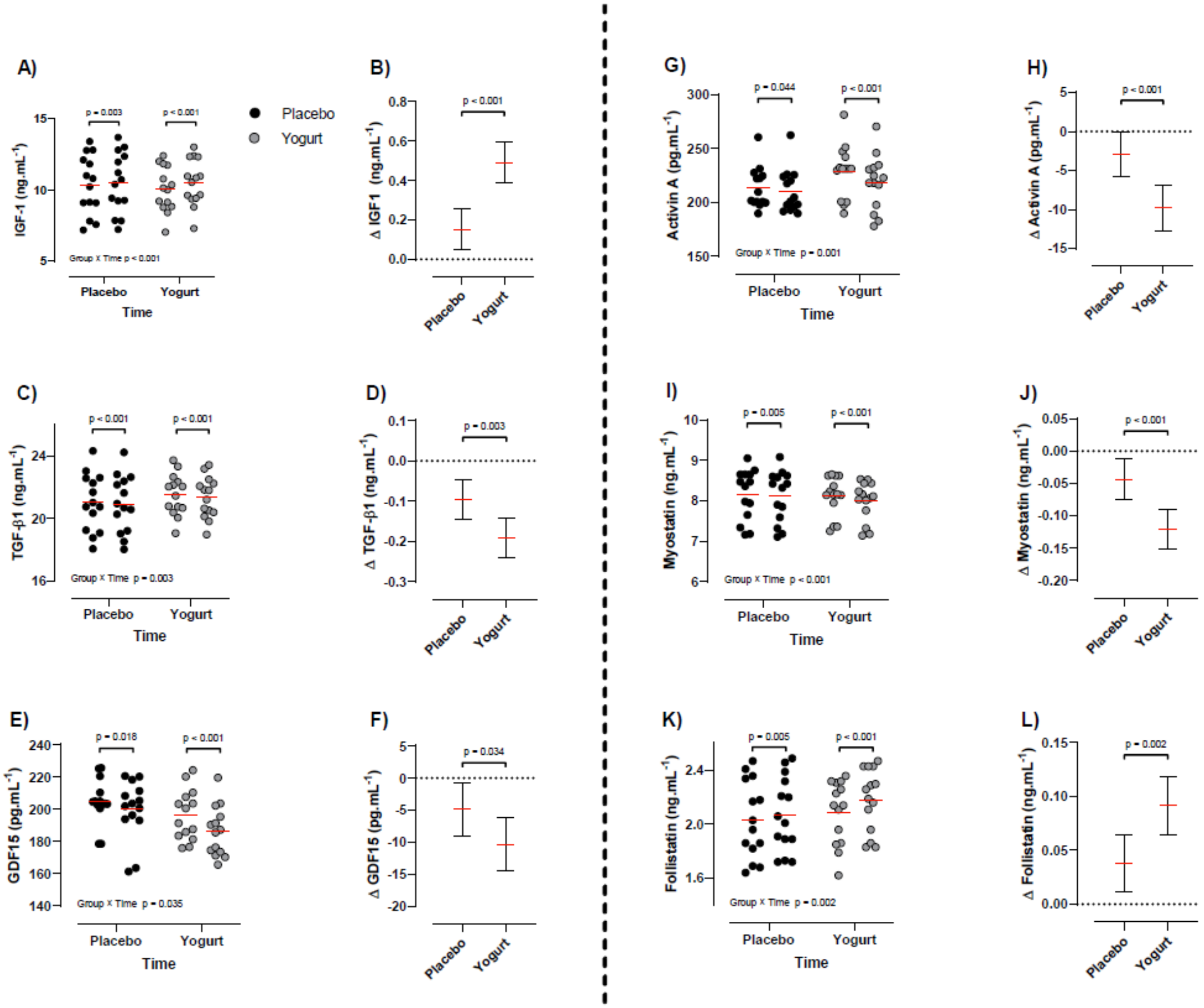

Figure 2: Effect of training time (pre, post) and experimental group (Icelandic yogurt, placebo) on skeletal muscle regulatory factors. Red horizontal lines indicate group means; error bars indicate 95% confidence intervals. N = 14 per group, placebo indicated by black circles, yogurt indicated by grey circles. **A)** IGF-1 and **B)** Δ IGF-1 (ng·mL^{-1}), **C)** TGF-β1 and **D)** Δ TGF-β1 (ng·mL^{-1}), **E)** GDF15 and **F)** Δ GDF15 (pg·mL^{-1}), **G)** Activin and **H)** Δ Activin A (pg·mL^{-}1), **I)** Myostatin and **J)** Δ Myostatin (pg·mL^{-1}), and **K)** Follistatin and **L)** Δ Follistatin (ng·mL^{-1}).

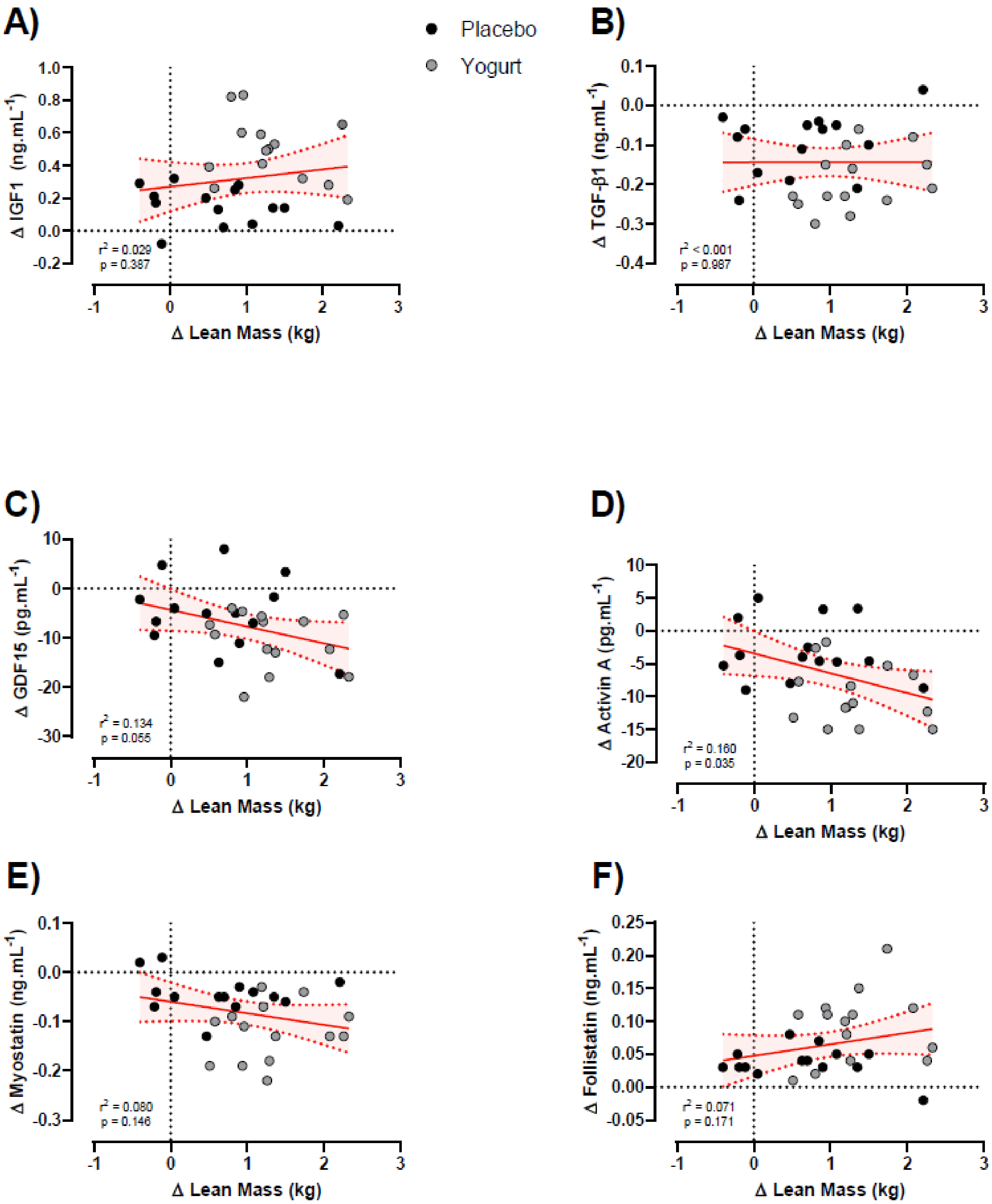

Figure 3: Relationship between Δ lean mass (kg) and Δ skeletal muscle regulatory factors. Solid red line indicates linear regression; red shaded area indicates 95% confidence intervals. N = 14 per group, placebo indicated by black circles, yogurt indicated by grey circles. **A)** Δ IGF-1 (ng·mL^{-1}), **B)** TGF-β1 (ng·mL^{-1}), **C)** GDF15 (pg·mL^{-1}), **D)** Δ Activin A (pg·mL^{-1}), **E)** myostatin (ng·mL^{-1}), and **F)** follistatin (ng·mL^{-1}).

Table 1. Resistance training program.

Week	Exercises	Resistance training			
		Set	Rest interval (seconds)	Repetition	Intensity (%1RM)
1	Leg press	3	60	12	60
2	Leg extension	3	60	12	60
3	Lying leg curl	3	70	10	65
4	Chest press	3	70	10	65
5	Shoulder press	3	80	8	70
6	Seated rows	3	80	8	70
7	Biceps curl	3	90	8	80
8	Sit-ups	3	90	8	80

Abbreviation. 1RM, one-repetition maximum.

Table 2. Descriptive characteristics of participants' values represent mean, standard error in brackets. P-values indicate unpaired sample t-test (IR vs PR), n = 14 per group.

	IR	**PR**	**P-value**
Age (years)	67.3 (1.1)	68.4(1.2)	0.489
Stature (cm)	170.1 (1.4)	168.4 (1.5)	0.414
Body mass (kg)	61.1 (1.1)	62.1 (1.0)	0.540
BMI (kg.m^{-2})	21.2 (0.5)	21.9 (0.5)	0.300
Lean mass (kg)	44.0 (1.0)	44.7 (0.9)	0.615
Bench press (kg)	32.5 (0.5)	31.1 (0.6)	0.114
Leg press (kg)	64.5 (1.8)	67.8 (2.2)	0.262
IGF-1 (ng.mL^{-1})	10 (0.4)	10.3 (0.5)	0.672
TGF-β1 (ng.mL^{-1})	21.5 (0.3)	21 (0.5)	0.417
GDF15 (pg.mL^{-1})	196.5 (4.1)	204.7 (3.7)	0.156
Activin A (pg.mL^{-1})	228.4 (6.6)	213.4 (5)	0.084
Myostatin (ng.mL^{-1})	8.1 (0.1)	8.1 (0.2)	0.817
Follistatin (ng.mL^{-1})	2 (0.06)	2 (0.07)	0.571

Abbreviations: BMI, body mass index; IGF-1, Insulin-like growth factor 1; TGF-β1, Transforming growth factor-beta 1; GDF15, Growth differentiation factor 15; ng.mL^{-1}, nanogram per milliliter; pg.mL^{-1}, picogram per milliliter; IR, Icelandic yogurt; PR, placebo.

Table 3. Energy and macronutrients (mean ± SD).

Variables	Group	Pre-training	Post-training	p-value
Energy (kcal/day)	IR	1755.8 ± 46.7	1812 ± 43.7*	p = 0.045
	PR	1716.6 50.1	1729 ± 72	
Protein (g/day)	IR	78.7 ± 5.5	97.2 ± 5.9*	p < 0.001
	PR	78.0 ± 5.1	79.1 ± 4.1	
Fat (g/day)	IR	51.9 ± 2.3	50.3 ± 3.8	p = 0.354
	PR	49.5 ± 4.2	49.9 ± 5.7	

Abbreviations. kcal/day, kilocalorie/day; g/day; gram/day; IR, Icelandic yogurt; PR, placebo. ‘p value’ column indicates condition x time interaction, * indicates difference between time-points within group ($p < 0.05$).