British Journal of Nutrition (2022), 127, 1334–1342 © The Author(s), 2021. Published by Cambridge University Press on behalf of The Nutrition Society

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

Reza Bagheri¹, Babak Hooshmand Moghadam², Darren G. Candow³, Bradley T. Elliott⁴, Alexei Wong⁵, Damoon Ashtary-Larky⁶, Scott C. Forbes⁷ and Amir Rashidlamir²*

(Submitted 23 January 2021 - Final revision received 12 May 2021 - Accepted 9 June 2021 - First published online 14 June 2021)

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 years) 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 (3×/week) for 8 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- β 1), growth differentiation factor 15 (GDF15), Activin A, myostatin (MST) and follistatin (FST)) were assessed. There were group × time interactions (P < 0.05) for body mass (IR: Δ 1, PR: Δ 0.7 kg), BMI (IR: Δ 0.3, PR: Δ 0.2 kg/m²), 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), TGF- β (IR: Δ 0.0, PR: Δ 0.01 ng/ml), GDF15 (IR: Δ 0.03 ng/ml), with Icelandic yogurt consumption resulting in greater changes compared with placebo. The addition of Icelandic yogurt consumption to a resistance training programme improved lean mass, strength and altered skeletal muscle regulatory factors in healthy untrained older males compared with 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.

Key words: Sarcopenia: Protein: Dairy products: Resistance Training: Hypertrophy

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., ageing 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 ageing muscle mass and strength^(5–7). Accumulating research indicates that the addition of protein consumption to a resistance training programme

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⁾, soya⁽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 product 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 amino-acidemia⁽¹⁶⁻¹⁸⁾.

Abbreviations: FST, follistatin; GDF15, growth differentiation factor 15; IGF-1, insulin-like growth factor-1; MST, myostatin; TGF-β1, transforming growth factor-beta 1.

* Corresponding author: Amir Rashidlamir, email rashidlamir@um.ac.ir



¹Department of Exercise Physiology, University of Isfahan, Isfahan, Iran

²Department of Exercise Physiology, Ferdowsi University of Mashhad, Mashhad, Iran

³Faculty of Kinesiology and Health Studies, University of Regina, Regina, SK, Canada

⁴Translational Physiology Research Group, School of Life Sciences, University of Westminster, London, UK

 $^{^5}$ Department of Health and Human Performance, Marymount University, Arlington, VA, USA

 $^{^6}$ Nutrition and Metabolic Diseases Research Center, Abvaz Jundishapur University of Medical Sciences, Abvaz, Iran

 $^{^{7}}$ Department of Physical Education Studies, Faculty of Education, Brandon University, Brando, MB, Canada

Yogurt is a dairy-based probiotic food source and a cost-effective protein source (primarily casein and whey) compared with other marketed protein supplements⁽¹⁹⁾. Research is mixed regarding the efficacy of yogurt consumption during a resistance training programme 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 programme 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- β 1) acts as a skeletal muscle regenerator that contributes to extracellular matrix reconstitution and muscle tissue remodelling⁽²⁴⁾. Activin A is involved in cellular differentiation, remodelling, proliferation and morphogenesis⁽²⁵⁾. Growth differentiation factor 15 (GDF15) is a member of the Glial cell-derived neurotrophic factor family, which is bound to Glial cell-derived neurotrophic factor 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 and protein kinase B 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 8 weeks of 3x/week wholebody 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 hypothesised that post-exercise Icelandic yogurt consumption, which contains 18 g of protein, would augment resistance training adaptations (i.e., lean mass and strength) and alter skeletal muscle regulatory factors compared with a placebo consumption.

Experimental methods

Participants

Thirty healthy untrained older males (age = 68 ± 4 years) 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 \geq 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 familiarised with the testing and experimental procedures.

Design

The study used a randomised, double-blind, placebo-controlled parallel design. Participants were randomly assigned, using a computer programme (www.randomizer.org) to consume Icelandic yogurt (IR; n 15) or placebo (PR; n 15) during 8 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 h after the last training session.

Resistance training

Before the initiation of the resistance training programme, participants performed three familiarisation training sessions (supervised) with the resistance training equipment. After the familiarisation 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-min 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 with 90-s rest intervals between sets (Table 1)(23,32). All training sessions occurred between 16.00 and 18.00 hours. Training volume was calculated using the following formula (Training volume = (repetitions $(n) \times sets(n) \times load$ 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



1336 R. Bagheri et al.

Table 1. Resistance training programme

		Resistance training					
Week	Exercises	Set	Rest interval (s)	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		

1RM, one-repetition maximum.

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 (flavoured with vanilla) and appearance. All personnel involved in the study were blinded to group allocations.

Body composition

Participants fasted for 12 h with at least 8 h 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) to the nearest 0.1 cm. BMI, fat mass and lean mass were determined by a multi-frequency bioelectrical impedance device (Inbody 770). The test-retest reliability of the bioelectrical impedance method was r = 0.96 to 0.99.

Strength

Maximal strength (1-repetition maximum) was determined 24 h after body composition, and skeletal muscle regulatory factors were measured. Participants were asked to abstain from alcohol for 48 h, caffeinated drinks for 12 h, food and drink (water was allowed ad libitum) for 2 h 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). Each set was separated by 5 min of passive rest (intraclass correlation coefficient (ICC): 0.96 to 0.98). Maximal strength was estimated using the following formula: 1-repetition maximum = weight/ $(1.0278-0.0278 \times repeti$ tions)(35). Bench press and leg press exercises were used to measure upper and lower body strength⁽³⁶⁾, and 1-repetition

Table 2. Descriptive characteristics of participants' values represent mean and standard errors. P-values indicate unpaired sample t test (IR v. PR)

(Mean values and standard errors, n 14 per group)

	IR		PR		
	Mean	SE	Mean	SE	<i>P</i> -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)	10	0.4	10.3	0.5	0.672
TGF- β 1 (ng/ml)	21.5	0.3	21	0.5	0.417
GDF15 (pg/ml)	196⋅5	4.1	204.7	3.7	0.156
Activin A (pg/ml)	228.4	6.6	213.4	5	0.084
Myostatin (ng/ml)	8.1	0.1	8.1	0.2	0.817
Follistatin (ng/ml)	2	0.06	2	0.07	0.571

IGF-1, Insulin-like growth factor 1; TGF-β1, Transforming growth factor-beta 1; GDF15, Growth differentiation factor 15; IR, Icelandic yogurt; PR, placebo.

maximums were used to determine individualised 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 min to clot. Samples were centrifuged at 3000 rpm for 10 min, and serum was stored at -80°C for future analysis. Commercially available ELISA kits were used to determine IGF-1 (CUSABIO; sensitivity: < 1.95 ng/ml), Activin A (CUSABIO; sensitivity: 3.9 pg/ml), FST (CUSABIO; sensitivity: 0.025 ng/ml), MST (CUSABIO; sensitivity: 0-312 ng/ml), TGF-β1 (CUSABIO; sensitivity: 0-747 ng/ml) and GDF15 (CUSABIO; sensitivity: 1.95 pg/ml). All serum sample concentrations were measured with a microplate reader (GDV, Germany) at a wavelength of 450 nm. The intra- and inter-assay CV for IGF-1 was < 10% and < 12%, Activin A, TGF- β 1, and GDF15, < 8 % and 10 %, FST and MST < 12 %, respectively.

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 analysed (Diet Analysis Plus, version 10; Cengage) 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 supplementations in older adults^(36,37), it was calculated that twelve participants per group would provide 80 % power (two-sided $\alpha = 0.05$) to detect 7 % between-group changes in lean mass and muscular strength. Unpaired t tests examined a comparison of baseline descriptive characteristics.

Table 3. Energy and macronutrients (mean values and standard deviations)

	Group	Pre-training		Post-training		
Variables		Mean	SD	Mean	SD	<i>P</i> -value
Energy (kcal/d)	IR	1755-8	46.7	1812 [*]	43.7	P = 0.045
	PR	1716-6	50.1	1729	72	
Protein (g/d)	IR	78.7	5.5	97·2*	5.9	P < 0.001
	PR	78.0	5.1	79.1	4.1	
Fat-(g/d)	IR	51.9	2.3	50.3	3.8	P = 0.354
,	PR	49.5	4.2	49.9	5.7	
Carbohydrate (g/d)	IR	243.5	8.2	242.6	6.1	P = 0.589
,,	PR	239.7	8.3	241	6.6	

IR, Icelandic yogurt; PR, placebo. 'P value' column indicates condition × time interaction.

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 programme 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 programme. 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/d; P < 0.001) and relative protein intake ((g/kg per d; P < 0.001), (Table 3)). Total energy intake was higher at the end of the study (compared with baseline) in the IR group (baseline: 1755.8 ± 46.7 kcal/d; post: 1812 ± 43.7 kcal/d, P = 0.002) with no change in the PR group (baseline: 1716.6 ± 50.1 kcal; post: 1729 ± 72 kcal/d, P = 0.648). Similarly, absolute protein intake increased in the IR group over time (baseline: 78.7 ± 5.5 g/d; post: 97.2 ± 5.9 g/d, P < 0.001) with no change in the PR group (baseline: 78.0 ± 5.1 g/d; post: 79.1 ± 4.1 g/d, P = 0.809). Compared with baseline, relative protein intake increased in the IR group over time (pre 1.3 ± 0.9 g/kg per d, post 1.6 ± 0.1 g/kg per d) with no change in the PR group (pre 1.2 ± 0.1 g/kg per d, post 1.3 ± 0.1 g/kg per d). There were no differences between groups for total training volume performed over the eight weeks of resistance training (IR: 106 555 ± 7171 kg; PR: 102 184 ± 6361 kg; P = 0.100).

Body composition and physical performance

There were significant group \times time interactions (P < 0.05) for body mass (IR: Δ 1 kg, 95 % CI: 0·5, 1·4; PR: Δ 0·7 kg, 95 % CI: 1·2, 0·3), BMI (IR: Δ 0.3 kg/m², 95 % CI: 0.2, 0.5; PR: Δ 0.2 kg/m², 95 % CI: 0.4, 0·1), lean mass (IR: Δ 1·3 kg, 95 % CI: 0·9, 1·6; PR: Δ 0·6 kg, 95 % CI: 0.2, 1), bench press (IR: \triangle 4 kg, 95 % CI: 2.8, 5; PR: \triangle 2.3 kg, 95 % CI: 1.7, 2.9) and leg press (IR: Δ 4.2 kg, 95 % CI: 3.4, 5.1; PR: Δ 2.5 kg, 95 % CI: 2·1, 3). Participants in the IR group experienced greater changes in body mass (P < 0.001; Fig. 1(b)), BMI (P < 0.001; Fig. 1(b))1(d)), lean mass (P = 0.012; Fig. 1(f)), bench press (P = 0.012; Fig. 1(f))Fig. 1(h)) and leg press (P = 0.001; Fig. 1(j)) compared with those in the PR group. However, fat mass remained unchanged in both groups (P > 0.05).

Skeletal muscle regulatory factors

There were significant group \times time interactions (P < 0.05) for IGF-1 (IR: Δ 0.5 ng/ml, 95% CI: 0.3, 0.6; PR: Δ 0.1 ng/ml, 95 % CI: 0.08, 0.2), TGF- β (IR: Δ -0.2 ng/ml, 95 % CI: -0.2, -0.1; PR: Δ -0.1 ng/ml, 95% CI: -0.1, -0.05), GDF15 (IR: Δ -10·3 pg/ml, 95 % CI: -13·6, -7; PR: Δ -4·8 pg/ml, 95 % CI: -9, -0.7), Activin A (IR: $\Delta -9.8$ pg/ml, 95 % CI: -12.3, -7.2; PR: Δ -2.9 pg/ml, 95 % CI: -5.6, -0.2), MST (IR: Δ -0.1 ng/ml, 95 % CI: -0·1, -0·08; PR: Δ -0·04 ng/ml, 95 % CI: -0·06, -0·02) and FST (IR: Δ 0.09 ng/ml, 95 % CI: 0.06, 0.1; PR: Δ 0.03 ng/ml, 95 % CI: 0.02, 0.05). The changes in IGF-1 (P < 0.001; Fig. 2(b)), TGF- β 1 (P = 0.003; Fig. 2(d)), FST (P = 0.002; Fig. 2(l)), GDF15 (P=0.034; Fig. 2(f)), Activin A (P<0.001; Fig. 2(h)) and MST(P < 0.001; Fig. 2(j)) were significantly greater in the IR compared with 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 (Fig. 3(d)), 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 programme in healthy untrained older males who were consuming > 1.2 g/kg per d of dietary protein. Results showed that Icelandic yogurt consumption augmented resistance training gains in lean mass and strength and influenced skeletal muscle



^{*} Indicates difference between time points within group (P < 0.05).



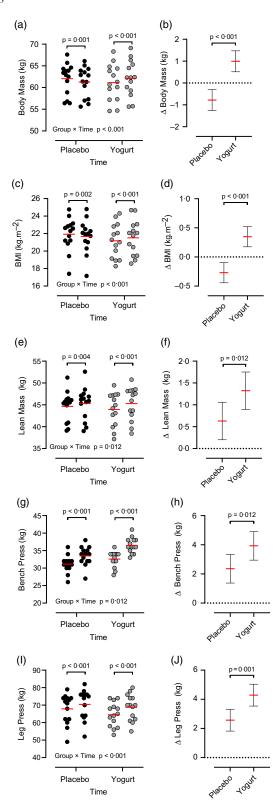


Fig. 1 Effect of training time (pre, post) and experimental group (Icelandic yogurt, placebo) on body composition and strength. Red horizontal lines indicate group means; error bars indicate 95 % CI. n 14 per group, placebo indicated by black circles, yogurt indicated by grey circles. (a) Body Mass and (b) Δ Body Mass, (c) BMI and (d) Δ BMI (kg/m²), (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). ●, Placebo; ⊚, Yogurt.

regulatory factors compared with placebo. There were no adverse events reported from the nutritional intervention or resistance training programme. 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 with 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 programme (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 years) compared with those on an isoenergetic placebo (pudding; 0 g protein)(15). Greek yogurt resulted in greater absolute and relative protein intake over time compared with those on placebo (P < 0.001) and total energy intake (pre v. 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 programme) could lead to significant muscle accretion and strength. In addition to its protein content, yogurt also contains Ca and vitamin D. In a systematic review performed by vanDronkelaar et al. (2018), Ca levels were inversely associated with the incidence of sarcopenia, possibly because of altered Ca absorption or homoeostasis in ageing muscle (40). Furthermore, Ca 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 6 months of milk protein consumption (10 g/d) during body weight and medicine ball exercise training significantly increased lean mass over time in older adults (n 61; 71 years; relative protein intake: 1.28 g/kg per d) compared with no change for those consuming a placebo (n 61; 70 years; relative protein intake: 1.23 g/kg per d)(41). In addition, 6 months of high-intensity resistance training combined with whey protein improved muscle cross-sectional area and strength in mobility-limited older adults (70-85 years) who were consuming 1.2 g/kg per d 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 per d of protein (43). Furthermore, Hevia-Larraín et al. showed that a protein intake of about 1.6 g/kg per d (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 per d 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

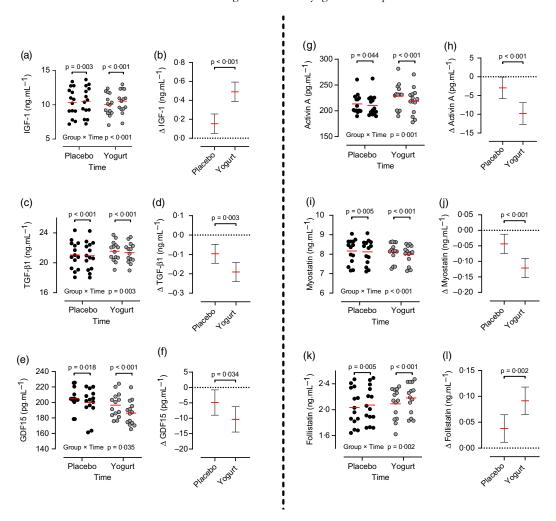


Fig. 2 Effect of training time (pre, post) and experimental group (Icelandic yogurt, placebo) on skeletal muscle regulatory factors. Red horizontal lines indicate group means; error bars indicate 95 % CI. n14 per group, placebo indicated by black circles, yogurt indicated by grey circles. (a) IGF-1 and (b) Δ IGF-1 (ng/ml), (c) TGF-β1 and (d) Δ TGF-β1 (ng/ml), (e) GDF15 and (f) Δ GDF15 (pg/ml), (g) Activin and (h) Δ Activin A (pg·ml1), (i) Myostatin and (j) Δ Myostatin (pg/ml), and (k) Follistatin and (I) ∆ Follistatin (ng/ml). ●, Placebo; ⊚, Yogurt.

consuming > 1.2 g/kg per d 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 signalling pathway⁽⁴⁵⁾. MST may inhibit muscle hypertrophy by decreasing the mechanistic target of rapamycin complex 1 and increasing forkhead box protein O1. 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^(5,47-50) following resistance training. We observed a reduction in MST, TGF- β 1, 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 with the placebo. The reduction in TGF- β 1 may be associated with alterations in MST since there is a co-regulatory 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 phosphoinositide 3-kinase and protein kinase B 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.



R. Bagheri et al.

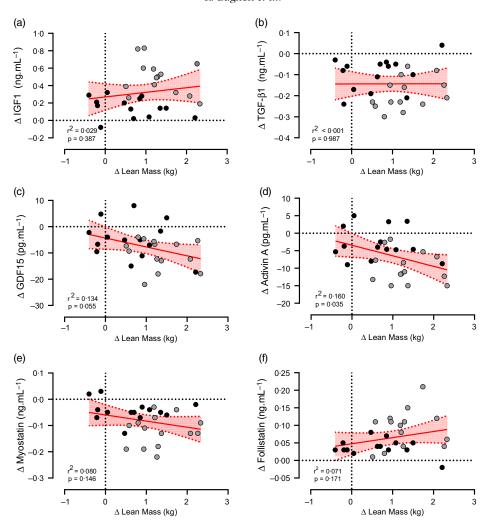


Fig. 3 Relationship between Δ lean mass (kg) and Δ skeletal muscle regulatory factors. Solid red line indicates linear regression; red-shaded area indicates 95 % Cl. n 14 per group, placebo indicated by black circles, yogurt indicated by grey circles. (a) Δ IGF-1 (ng/ml), (b) TGF-β1 (ng/ml), (c) GDF15 (pg/ml), (d) Δ Activin A (pg/ml), (e) myostatin (ng/ml), and (f) follistatin (ng/ml). ●, Placebo; ○, Yogurt.

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 (mechanistic target of rapamycin complex 1 signalling, MPS) which would have assisted in the explanation of our outcomes. However, it has been proposed that promotions in circulating concentrations of signalling 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 vogurt 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.

Acknowledgements

The authors wish to thank all the participants in this research project.



This work was financially supported by a grant (50916/2) from the Vice Chancellor for Research Affairs, Ferdowsi University of Mashhad.

A. R. and B. H. M. conceived and designed research. B. H. M. and A. R. conducted experiments. A. R. and R. B. contributed new reagents or analytical tools. B. T. E. analysed data. R. B. wrote the manuscript. A. W., D. G. C., S. C. F., and D. A. L. revised the manuscript. All authors read and approved the manuscript.

The authors declare no conflicts 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
- Cruz-Jentoft AJ, Bahat G, Bauer J, et al. (2019) Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing **48**, 16–31.
- 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. Front Physiol 3,
- Robinson SM, Reginster J-Y, Rizzoli R, et al. (2018) Does nutrition play a role in the prevention and management of sarcopenia? Clin Nutr 37, 1121-1132.
- 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. Eur J Appl Physiol **119**, 1–11.
- Fragala MS, Cadore EL, Dorgo S, et al. (2019) Resistance training for older adults: position statement from the national strength and conditioning association. J Strength Conditioning Res 33,
- 7. Bagheri R, Forbes SC, Candow DG, et al. (2020) Effects of branched-chain amino acid supplementation and resistance training in postmenopausal women. Exp Gerontol 144, 111185.
- 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 Med 45, 245–255.
- 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. J Int Soc Sports Nutr 15, 24.
- 11. 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 resistancetrained men. Br J Nutr 124, 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.
- 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. J Diet Supplement 15, 140–152.
- 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. Front Nutr 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. Am J Physiol-Endocrinol Metab 280, E340-E348.
- 17. Josse AR & Phillips SM (2012) Impact of Milk Consumption and Resistance Training on Body Composition of Female Athletes. Acute Topics in Sport Nutrition. Basel, Switzerland: 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. Am J Clin Nutr 99, 1263S-1270S.
- White KM, Bauer SJ, Hartz KK, et al. (2009) Changes in body composition with yogurt consumption during resistance training in women. Int J Sport Nutr Exercise Metab 19, 18-33.
- 21. Thomas DT, Wideman L & Lovelady CA (2011) Effects of a dairy supplement and resistance training on lean mass and insulinlike growth factor in women. Int J Sport Nutr Exercise Metab **21**, 181–188.
- 22. Solomon A & Bouloux P (2006) Modifying muscle mass-the endocrine perspective. J Endocrinol 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. Exp Gerontol 133, 110869.
- Touvra A-M, Volaklis KA, Spassis AT, et al. (2011) Combined strength and aerobic training increases transforming growth factor- β 1 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. Braz J Med Biol Res 47, 746-752.
- 26. Baek SJ & Eling T (2019) Growth differentiation factor 15 (GDF15): a survival protein with therapeutic potential in metabolic diseases. Pharmacol Ther 198, 46-58.
- 27. Spangenburg EE (2009) Changes in muscle mass with mechanical load: possible cellular mechanisms. Appl Physiol Nutr Metab **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. Eur J Appl Physiol **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. Int J Prev Med 10, 29.
- 30. Chodzko-Zajko WJ, Proctor DN, Singh MAF, et al. (2009) Exercise and physical activity for older adults. Med Sci Sports Exerc 41, 1510-1530.
- 31. Medicine ACoS (2013) ACSM's Guidelines for Exercise Testing and Prescription. Philadelphia: 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 Int 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. J Strength Conditioning Res 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. J Physiol 535, 301–311.
- Murach KA & Bagley JR (2016) Skeletal muscle hypertrophy with concurrent exercise training: contrary evidence for an interference effect. Sports Med 46, 1029-1039.
- 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. Exp Gerontol 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. Int J Sport Nutr Exerc Metab 26, 71-77.
- Cohen J (1988) Statistical power. Anal Behav Sci 2, 273–406.
- 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 Med 45,
- 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. J Am Med Director Assoc 19, 6-11.
- Nakayama K, Saito Y, Sanbongi C, et al. (2020) Effects of lowdose milk protein supplementation following low-to-moderate intensity exercise training on muscle mass in healthy older adults: a randomized placebo-controlled trial. Eur J Nutr 60, 1-12.
- 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. J Gerontol Ser A: Biomed Sci Med Sci 68, 682-690.
- 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.
- 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 Med 51, 1-14.
- Elliott B, Renshaw D, Getting S, et al. (2012) The central role of myostatin in skeletal muscle and whole body homeostasis. Acta Physiol 205, 324-340.
- 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.
- 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. Med Lab J 12, 28-33.
- 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 resistancetrained men. Br J Nutr 124, 1035-1043.
- Bagheri R, Forbes SC, Candow DG, et al. (2021) Effects of branched-chain amino acid supplementation and resistance training in postmenopausal women. Exp Gerontol 144,
- 51. Zhu J, Li Y, Shen W, et al. (2007) Relationships between transforming growth factor- β 1, myostatin, and decorin implications for skeletal muscle fibrosis. J Biol Chem 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. Am J Physiol-Endocrinol Metab 297, E157-E164.
- 53. Barbé C, Kalista S, Loumaye A, et al. (2015) Role of IGF-I in follistatin-induced skeletal muscle hypertrophy. Am J Physiol-Endocrinol Metab 309, E557-E567.
- 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 Ther Med 50, 102360.
- 55. Hickey ME & Mason SE (2017) Age and gender differences in participation rates, motivators for, and barriers to exercise. Mod Psychol Stud 22, 3.
- 56. Kraemer WJ, Marchitelli L, Gordon SE, et al. (1990) Hormonal and growth factor responses to heavy resistance exercise protocols. J Appl Physiol 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. Eur J Appl Physiol 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. J Am Med Director Assoc 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. Clin Nutr 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. Clin Nutr 30, 610-615.
- 61. Jackson A, Pollock ML, Graves JE, et al. (1988) Reliability and validity of bioelectrical impedance in determining body composition. J Appl Physiol 64, 529-534.
- 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. J Clin Med 10, 1630.

