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ARTICLE

Association between whey protein, regional fat mass, and strength in resistance-trained men: a cross-sectional study

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Abstract: The purpose of this study was to evaluate the association between whey protein supplementation, body composition, and muscle strength in resistance-trained individuals. Forty-nine healthy males, aged 18 to 35 years and were engaged in resistance training for at least 1 year, were assigned into 2 groups according to whey protein intake (whey – n = 26, age: 30.7 ± 7.4 years, body mass: 75.8 ± 9.0 kg; without whey: n = 23, age: 31.0 ± 7.4 years, body mass: 77.9 ± 9.3 kg). Using a cross-sectional design, a morning assessment of body fat mass (FM) (by dual-energy X-ray absorptiometry) and strength (using 1-repetition maximum for bench press and back squat) was performed. Nutritional assessment was performed by 3-day food records. Regarding nutritional habits, differences between total energy intake (kcal) and estimated energy requirements (kcal) were observed. Results, from raw data or controlling for energy intake, estimated energy requirements, or achieved percentage of energy requirements, showed that whey protein supplementation was inversely correlated with whole-body FM (R = −0.367 (p = 0.010); R = −0.317 (p = 0.049); R = −0.380 (p = 0.011); R = −0.321 (p = 0.047), respectively), trunk FM (R = −0.396 (p = 0.005), R = −0.367 (p = 0.022), R = −0.423 (p = 0.004), R = −0.369 (p = 0.021), respectively) and android FM (R = −0.381 (p = 0.007), R = −0.332 (p = 0.039), R = −0.383 (p = 0.010), R = −0.336 (p = 0.036), respectively). No correlations were found between muscle strength outcomes and whey protein supplementation. The present data suggest that whey protein ingestion has a positive association with whole-body and regional (trunk and android) FM.

Key words: nutrition, protein supplement, fat loss, performance.

Résumé : Cette étude a pour objectif d'évaluer l'association entre la supplémentation en protéine lactosérique, la composition corporelle et la force musculaire chez des individus entraînés à la force. On répartit 49 hommes âgés de 18 à 35 ans, en bonne santé et inscrits dans un programme d'entraînement à la force depuis 1 an au moins dans deux groupes, l'un consommant du lactosérum (n = 26, âge : 30,7 ± 7,4 ans, masse corporelle : 75,8 ± 9,0 kg) et l'autre, n'en consommant pas (n = 23, âge : 31,0 ± 7,4 ans, masse corporelle : 77,9 ± 9,3 kg). Selon un devis transversal, on évalue au matin la masse adipeuse corporelle (« FM ») par absorptiométrie à rayons X en double énergie et la force musculaire, par la charge maximale levée au développé couché et lors d'un accroupissement avec une charge au haut du dos. On évalue l'apport nutritionnel au moyen d'un carnet alimentaire de 3 jours. À propos des habitudes alimentaires, on note des différences entre l'apport énergétique total et les besoins énergétiques estimatifs (kcal). D'après l'analyse des données brutes et la prise en compte de l'apport énergétique, les besoins énergétiques estimatifs et le pourcentage atteint des besoins énergétiques, la supplémentation en protéine lactosérique est inversement corrélée à FM du corps entier (R = −0,367 (p = 0,010); R = −0,317 (p = 0,049); R = −0,380 (p = 0,011); R = −0,321 (p = 0,047),

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respectivement), du tronc (R = −0,396 (p = 0,005), R = −0,367 (p = 0,022), R = −0,423 (p = 0,004), R = −0,369 (p = 0,021), respectivement) et de la région androïde (R = −0,381 (p = 0,007), R = −0,332 (p = 0,039), R = −0,383 (p = 0,010), R = −0,336 (p = 0,036), respectivement). On n'observe pas de corrélations entre les résultats de la force musculaire et la supplémentation en protéine lactosérique. Les présentes données suggèrent que la consommation de protéine lactosérique est associée positivement avec la FM du corps entier et régionale (tronc et androïde). [Traduit par la Rédaction]

Mots-clés : nutrition, supplément en protéine, perte de gras, performance.

Introduction

At least 85% of athletes and regular exercise practitioners consume dietary supplements daily (Stohs and Kitchens 2013). Among these supplements, whey is commonly used (Maughan et al. 2007; Williams 2005).

Whey protein (WP) supplements display unique characteristics such as rapid digestion and a very high concentration of essential amino acids (45–55 g/100 g of protein) (Hayes and Cribb 2008), which makes whey a high-quality protein (Kerksick et al. 2006). Additionally, whey is the highest dietary source of branch chain amino acids, in particular, leucine (up to 14 g/100 g of protein) (Hayes and Cribb 2008). These amino acids are fundamental to trigger skeletal muscle protein synthesis (Volek et al. 2013). This bears particular interest for resistance-trained athletes' aiming to increase muscle strength and hypertrophy gains (Cribb et al. 2007; Hayes and Cribb 2008). Concerning resistance-trained individuals, although several studies showed increases in muscle strength (Burke et al. 2001; Cribb et al. 2006, 2007; Lockwood et al. 2017), only 2 studies suggested WP as responsible for these positive outcomes (Cribb et al. 2006, 2007). Two recent meta-analysis concluded that resistance training (RT) combined with WP supplementation is associated with extra increases in maximal strength (Morton et al. 2018), particularly in trained individuals (Naclerio and Larumbe-Zabala 2016).

On the other hand, WP supplementation seems to display both an anti-adipogenic and a muscle-protective role (Pal and Radavelli-Bagatini 2013). When aiming for increases in performance, health, and physical and mental wellness, many athletes and coaches focus on weight management, specifically fat mass (FM) reduction (Haraguchi et al. 2006; Malina 2007). The effect of WP supplementation during RT on FM has been equivocal (Miller et al. 2014). Two studies did not find significant effects (Burke et al. 2001; Cribb et al. 2007); conversely, 2 other studies showed decreases in FM (Cribb et al. 2006; Lockwood et al. 2017). Despite of these equivocal results, a well-designed meta-analysis concluded that WP combined with RT may be an effective supplement strategy to improve body composition, including FM reduction (Miller et al. 2014).

Nevertheless, it should be noted that FM is not equally distributed through the body. Only 2 studies analyzed the effects of WP supplementation on regional FM: 1 was performed in overweight/obese individuals (Arciero et al. 2014) while another was performed in active individuals, without previous experience in RT (Hulmi et al. 2015). Arciero et al. (2014) showed that WP alone and in combination with RT reduced abdominal FM while Hulmi et al. (2015) observed a reduction in trunk and android FM with WP supplementation after an RT program. Research studies investigating this topic are lacking, and because abdominal fat is one of the major concerns regarding body fat in athletes, we strongly believe further research is mandatory. Athlete's concerns are not exclusively related to health and performance issues, but also with aesthetic reasons. Therefore, the purpose of this study was to examine the association between WP supplementation, body composition (total and regional FM), and performance (muscle strength) in a sample of resistance-trained men.

Materials and methods

Participants

This study used a cross-sectional design, in which participants were assigned into 2 groups, according to WP intake. Male participants had to meet the study's requirements before enrolling. Inclusion criteria included (*i*) age between 18 and 45 years, and (*ii*) engaged in RT consistently (≥3 training sessions per week) for at least 1 year. Participants were excluded if they were smokers or had consumed any type of drug (including anabolic steroids), medicines, or supplements that may enhance body composition or performance for ≤3 months before the trial (including creatine, β-hydroxy-β-methylbutyrate, β-alanine, and phosphatidic acid). According to these criteria, 49 resistance-trained male volunteers were recruited to participate in the study. Participants were informed of the purpose of the investigation and experimental procedures prior to signing an informed consent document. All procedures were approved by the Ethics Committee of the Faculty of Human Kinetics, University of Lisbon, and were conducted in accordance with the *Declaration of Helsinki* for human studies (World Medical Association 2013). Participants were allocated into 2 groups according to WP ingestion (whey: n = 26; without whey: n = 23). In the whey group, 4 participants reported using multivitamins and 3 participants reported using fish oil in addition to WP, and in the without-whey group, 1 participant reported using multivitamins and 2 participants reported using fish oil. All measurements were assessed in the morning, at rest, following an 8-h fast. No alcohol or stimulant beverages were consumed in the previous day, and participants refrained from exercise within the 12 h prior to evaluation.

Anthropometry

Weight and stature were assessed to the nearest 0.1 kg and 0.1 cm (Seca, Hamburg, Germany) according to standardized procedures. Body mass index (BMI) was calculated as weight (kg) divided by the square of the stature (m) (Gordon et al. 1988).

Body composition

Body composition, namely FM and fat-free mass (FFM) were determined by dual-energy X-ray absorptiometry (DXA) (Hologic Explorer-W, software QDR for Windows version 12.4, Hologic, Waltham, Mass., USA). Following the protocol for DXA described by the manufacturer (Hologic, Waltham, Mass., USA), comprising 6 fields of acrylic and aluminum of varying thickness and known absorptive properties was scanned to serve as an external standard for the analysis of different tissue components.

The same technician positioned the participants, performed the scans, and executed the analysis according to the operator's manual using the standard analysis protocol. Analyses provided total FFM and FM from the whole body and different regions. Although software automatically generates regions (i.e., arms, legs, and trunk), those were adjusted manually by the technician. Specifically, trunk region was separated from the legs by a horizontal line right above the iliac crest (lower boundary) and from the head by neck cut (upper boundary) (Kang et al. 2011). The trunk region includes the neck, chest, abdominal, and pelvic areas except the gluteal area that was included into legs, whereas the android region is the area from the pelvis (lower boundary) to 20% of the distance between the pelvic horizontal line and the

Table 1. Participant characteristics, body composition, muscle strength, and nutritional intakes.

	Whey protein supplementation (n = 26)	Without whey protein supplementation (n = 23)	p
Age (y)	30.7±7.4	31.0±7.4	NS
Weight (kg)	75.8±9.0	77.9±9.3	NS
Height (cm)	174.4±6.2	173.8±4.7	NS
BMI (kg/m²)	24.9±1.7	25.8±2.7	NS
WB FFM (kg)	64.1±8.2	63.1±6.8	NS
WB FM (kg)	10.8±2.9	13.8±4.8	0.013
WB FM (%)	14.4±3.3	17.7±4.9	0.008
Trunk mass (kg)	35.2±4.4	36.7±4.7	NS
Trunk FFM (kg)	30.4±4.0	30.0±3.6	NS
Trunk FM (kg)	4.9±1.6	6.7±2.6	0.007
Trunk FM (%)	13.8±3.8	17.9±5.9	0.006
Android mass (kg)	5.2±0.7	5.5±0.9	NS
Android FFM (kg)	4.4±0.6	4.4±0.6	NS
Android FM (kg)	0.8±0.3	1.1±0.5	0.010
Android FM (%)	14.5±4.1	19.6±7.1	0.005
1RM back squat (kg)	130.5±25.9	126.4±26.0	NS
1RM bench press (kg)	91.2±26.5	86.7±22.8	NS
Total energy intake (kcal)	2734.2±669.2	2618.6±605.2	NS
Total energy intake (kcal/kg BW)	36.5±9.9	34.3±9.7	NS
Estimated energy requirement (kcal)	3546.5±241.5	3589.6±246.5	NS
Achieved percentage of energy requirements (%)	77.1±19.5	73.5±18.8	NS
Protein intake (g)	172.1±38.5	133.9±26.8	0.001
Protein intake (g/(kg BW·d))	2.3±0.5	1.8±0.4	0.001
Protein intake (kcal)	688.3±154.1	535.7±107.2	0.001
CHO intake (g)	309.6±124.7	283.1±92.9	NS
Fat intake (g)	95.3±24.0	100.1±25.2	NS

Note: Values are means ± SD. 1RM, 1-repetition maximum; BMI, body mass index; BW, body weight; CHO, carbohydrates; FFM, fat-free mass; FM, fat mass; ns, not significant; WB, whole body.

neck line (upper boundary), delimited laterally by the arm lines (Hologic 2014).

Based on data from 10 young active adults (5 males and 5 females), the coefficient of variation in our laboratory for FM and FFM is 1.7% and 0.8%, respectively. The technical errors of measurement are 0.21 kg for FM and 0.34 kg for FFM (Santos et al. 2013).

Muscle strength

Muscle strength was assessed through 1-repetition maximum (1RM) of the back squat and bench press exercises. These 2 exercises were selected since they involve major muscle groups in both the lower and upper body. A Multipower Base Model machine (Technogym, Cesena, Forli-Cesena, Italy) was used to measure 1RM. The 1RM testing protocols followed the National Strength and Conditioning Association guidelines (McGuigan 2016). Participants were previously familiarized with the test procedures and performed warm-up. The warm-up session comprised 5 min in a cycle ergometer and 5–10 repetitions for each movement (back squat and bench press) using a weight that was comfortable for each participant (light to moderate load). Then, 2 heavier warm-up sets of 2 to 5 repetitions each was completed before the first actual 1RM attempt. To measure 1RM, weight was progressively increased for each subsequent attempt, with a 2-min rest interval. Participants' maximal lift was determined within 3 to 5 attempts after warm-up.

Dietary assessment

Participants completed dietary records for 3 days (2 weekdays and 1 weekend day) during the week in which assessments were made. The participants were asked to record everything they consumed, including supplements, with as much detail as possible, such as time of consumption, ingredients, preparation/confection methods, and quantities. To quantify the portion of foods and fluids, participants were instructed to use the provided photographic album with food pictures of several portions, standardized household measures, weight/volume, or even photographs. After the 3-day period participants were interviewed by a qualified nutritionist to confirm these records. The Food Processor version 11.2.274 dietary analysis program (ESHA Research, Salem, Ore., USA) was used to analyze records. Each food item was entered with the information about the amount and the program provided a 3-day average of energy, protein, carbohydrates, and fat intake. Energy requirements were calculated using the Harris Benedict formula for the resting metabolic rate (Jagim et al. 2018) and multiplied by the physical activity level factor (FAO/WHO/UNU Expert Consultation 2011).

Statistical analysis

Data were analyzed with IBM SPSS Statistics for Windows (version 24.0; IBM Corp., Armonk, N.Y. USA) and statistical significance was set at $p < 0.05$. Normality was verified using the Shapiro–Wilk test and data were presented as means ± SD. Comparison between group means was performed using independent-sample t tests. One-sample t test was used to assess the difference for reference values. Bivariate correlations were performed to investigate the association between WP supplementation (dichotomous variable) and FM (total and regional) and maximal strength (1RM). Partial correlation analysis was additionally conducted controlling for energy intake, estimated energy requirements, and achieved percentage of energy requirements (calculated as total energy intake divided by estimated energy requirements, multiplied by 100).

Results

All participant's descriptive characteristics are presented in Table 1. No differences were observed between the 2 groups in all parameters, except for FM (whole-body absolute and relative, trunk and android region) and for protein intake (expressed in g, g/(kg BW·day), or kcal). Regarding nutritional habits, in both groups, differences between total energy intake (kcal) and estimated energy requirements (kcal) were observed, while total energy intake was lower than the estimated energy requirements.

Table 2. Associations between whey protein supplementation, body composition, and strength variables with unadjusted analysis and controlling for energy intake, energy requirements, and achieved percentage of energy requirements.

	Unadjusted analysis (n = 49)	Controlling for energy intake (n = 44)	Controlling for energy requirements (n = 49)	Controlling for achieved percentage of energy requirements (n = 44)
WB FFM	R = 0.066	R = 0.065	R = 0.278	R = 0.086
WB FM	R = −0.367 (p = 0.010)	R = −0.317 (p = 0.049)	R = −0.380 (p = 0.011)	R = −0.321 (p = 0.047)
WB FM %	R = −0.376 (p = 0.008)	R = −0.322 (p = 0.046)	R = −0.376 (p = 0.012)	R = −0.329 (p = 0.041)
Trunk mass	R = −0.161	R = −0.113	R = −0.268	R = −0.101
Trunk FFM	R = 0.049	R = 0.056	R = 0.223	R = 0.077
Trunk FM	R = −0.396 (p = 0.005)	R = −0.367 (p = 0.022)	R = −0.423 (p = 0.004)	R = −0.369 (p = 0.021)
Trunk FM %	R = −0.401 (p = 0.004)	R = −0.369 (p = 0.021)	R = −0.422 (p = 0.004)	R = −0.374 (p = 0.019)
Android mass	R = −0.198	R = −0.122	R = −0.229	R = −0.116
Android FFM	R = 0.019	R = 0.040	R = 0.074	R = 0.059
Android FM	R = −0.381 (p = 0.007)	R = −0.332 (p = 0.039)	R = −0.383 (p = 0.010)	R = −0.336 (p = 0.036)
Android FM %	R = −0.411 (p = 0.003)	R = −0.368 (p = 0.021)	R = −0.413 (p = 0.005)	R = −0.374 (p = 0.019)
1RM back squat	R = 0.080	R = 0.058	R = 0.120	R = 0.069
1RM bench press	R = 0.093	R = 0.092	R = 0.138	R = 0.103

Note: R values without p values were not significant. 1RM, one repetition maximum; FFM, fat free mass; FM, fat mass; WB, whole body.

Participants in the whey group ingested an average of 41 g per day (mean ± SD, 41.1 ± 25.0 g (data not shown)) of WP.

Bivariate correlations were explored (Table 2) and an inverse correlation between WP intake and (*i*) whole-body FM (absolute and relative), (*ii*) trunk FM (absolute and relative), and (*iii*) android FM (absolute and relative) was detected.

When controlling separately for energy intake, estimated energy requirements, or achieved percentage of energy requirements, all correlations endured for statistical significance (Table 2). No correlations were observed between WP supplementation and any strength outcomes.

Additionally, to verify the effect of dairy proteins on satiety, we further explored if total energy intake was correlated with protein intake (kcal) and a direct correlation was observed in both groups.

Discussion

Using 2 groups of resistance-trained males, this study indicated that WP consumption is associated with lower levels of total FM. Although the effect of WP supplementation during RT on FM is equivocal (Miller et al. 2014), these results are consistent with previous studies from Cribb et al. (2006) and Lockwood et al. (2017), also performed in resistance-trained individuals. Cribb et al. (2006) reported a significant decrease in FM, when comparing with a casein supplementation group, during a 10-week RT program, in previously trained men. Additionally, Lockwood et al. (2017) reported that FM decreased in the group consuming hydrolyzed WP, when comparing with placebo, also performing a RT protocol.

To our knowledge, this is the first study to investigate the association between WP supplementation and regional FM (trunk and android FM) in resistance-trained men. Only 2 studies (Arciero et al. 2014; Hulmi et al, 2015) analyzed the effects of WP on regional FM; however, none used the same population as the present study. Arciero et al. (2014), in a sample of inactive, overweight, and obese (BMI >25 kg/m²) individuals, observed that WP alone and combined with RT reduced total, abdominal, and visceral FM. In another study, performed in active men but without previous systematic RT background, Hulmi et al. (2015) reported a decrease of total, trunk, and android FM with WP supplementation after 12 weeks of an RT protocol. These results are in agreement with our data, where WP supplementation showed to be associated with lower levels of whole-body, trunk, and android FM. Taken together, the results of these studies suggest that WP supplementation may decrease FM, including regional FM, in combination with an RT program.

Reasons and mechanisms that may explain how WP may lead to body fat loss are not clear at this point; however, some possibilities have been proposed. The effect of dairy proteins on satiety and decreased energy intake could, at least partially, account for the reduction of body fat (Hulmi et al. 2015). This action seems related to a swifter elevation of amino acids in plasma that would influence gut hormones, stimulating anorexigenic hormones such as glucose-dependent insulinotropic polypeptide, glucagon-like peptide-1, leptin, and cholecystokinin, reducing orexigenic hormones such as ghrelin (Pal and Radavelli-Bagatini 2013; Pesta and Samuel 2014), neuropeptide orexigenic, and conversely increasing neuropeptide anorexigenic in the hypothalamus (Sousa et al. 2012). Protein amounts of 20 g are deemed sufficient to impact subjective appetite sensations and further decrease food intake (MacKenzie-Shalders et al. 2015). In the present study, participants consumed an average of 41 g per day of WP; however, an association between higher protein intakes and lower total energy intake was not found. Thus, satiety does not seem to support our findings regarding an inverse association between FM levels and WP protein intake. Another possible mechanism is related to increased thermogenesis with WP. It seems WP further increases thermogenesis (Pal and Radavelli-Bagatini 2013), displaying a greater thermogenic effect than either casein or soy (Jakubowicz and Froy 2013). The higher leucine content, and the faster absorption kinetics, seems to further enhance muscle protein synthesis. It has been proposed that this mechanism might be related to an augmented postprandial thermogenesis with this type of protein (Jakubowicz and Froy 2013). In brief, the rapid absorption and higher concentration of leucine may account for the higher thermogenic effect with WP, after digestion, when comparing with other protein sources (Jakubowicz and Froy 2013). Leucine may, in fact, trigger downstream metabolites of the mammalian target of rapamycin pool of kinases, further enhancing muscle protein synthesis (Appuhamy et al. 2012; Li et al. 2011). Additionally, in vitro research also proposes an important action of leucine by inhibiting the AMP-activated protein kinase (AMPK), which would ultimately hinder translation elongation processes and prevent muscle protein synthesis (Du et al. 2007). We propose that both the inhibition of AMPK and the direct effect of this amino acid on muscle protein synthesis are accountable for the purported superior thermogenic effect of WP (Boirie et al. 1997). Further, WP has also displayed increased lipolysis (Hector et al. 2015) and fat oxidation (Acheson et al. 2011) when compared with carbohydrates, possibly because of enhanced use of body fat for fuel (Cribb et al. 2006). An in-depth mechanistic analysis suggests that a higher lipolysis rate with protein consumption might be the result of an increase in energy expenditure to support muscle protein synthesis, since polypeptide assembling from the elongation phase of messenger RNA translation requires a substantial amount of energy (Browne and Proud 2002). In vitro research also points towards a possible beneficial effect of leucine in both inhibiting fatty acid synthase (FAS) expression in adipocytes while increasing fatty acid oxidation in muscle cells. Involved biochemical pathways seem related to the inhibition of FAS and peroxisome proliferator-activated receptor gamma expression and an increase in mitochondrial uncoupling protein 3, respectively (Sun and Zemel 2007). Bearing in mind the limited evidence and uncertainty regarding this topic, we suggest that future studies may explore and clarify which mechanisms are involved regarding the effects of WP consumption and fat mass.

Similarly to other authors (Burke et al. 2001; Lockwood et al. 2017), no effects of WP supplementation were observed on muscle strength. These studies (Burke et al. 2001; Lockwood et al. 2017) also employed 1RM assessments to evaluate muscle strength. Lockwood et al. (2017) proposed that trained individuals may require daily protein intakes superior to 2.0 g/kg of body weight to enhance muscle mass and strength. In the present study, participants ingested higher amounts of protein (2.3 g/kg of body weight). Thus, protein intake does not seem related to the observed muscle strength outcome (absence of correlation between protein intake and strength). Although being out of our primary research goals, as far as FFM and WP intake, no association was found in our sample of resistance-trained men.

In conclusion, WP supplementation in resistance-trained men is associated with a lower amount of FM. Consequently, if the main goal is to promote FM loss, especially from trunk and android regions, either because of aesthetic, health, or performance reasons, WP supplements may be an effective dietary strategy. Further research should clarify which amount and type of WP, as well as timing of intake (throughout the day, before or after training), has more beneficial effects on whole-body and regional FM.

Conflict of interest statement

There are no conflicts of interest to disclose.

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