## 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

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The decrease in resting energy expenditure (REE) and fat oxidation with aging is associated with an increase in fat mass (FM), and both could be prevented by exercise such as resistance training. Dairy consumption has also been shown to promote FM loss in different subpopulations and to be positively associated with fat oxidation. Therefore, we sought to determine whether resistance exercise combined with dairy supplementation could have an additive impact on FM and energy metabolism, especially in individuals with a deficit in muscle mass. Twenty-six older overweight sarcopenic men ( $65 \pm 5$  years old) were recruited for the study. They participated in 4 months of resistance exercise and were randomized into three groups for postexercise shakes (control, dairy, and nondairy isocaloric and isoprotein supplement with 375 ml and ~280 calories per shake). Body composition was measured by dual X-ray absorptiometry and REE by indirect calorimetry. Fasting glucose, insulin, leptin, inflammatory profile, and blood lipid profile were also measured. Significant decreases were observed with FM only in the dairy supplement group; no changes were observed for any other variables. To conclude, FM may decrease without changes in metabolic parameters during resistance training and dairy supplementation with no caloric restriction without having any impact on metabolic properties. More studies are warranted to explain this significant decrease in FM.

Keywords: exercise training, dairy supplementation, protein intake, aging, sarcopenia, body composition

The loss of muscle mass (which is defined as sarcopenia) and a gain in fat mass (FM) is commonly observed with aging. In addition, changes in metabolic parameters, such as a decrease in energy expenditure, can partly explain weight gain in older adults (Corpeleijn et al., 2009; Kelley, 2005; Ravussin et al., 1988). Although the decrease in energy expenditure in aging is mostly explained by decreases in lean mass (Weyer et al., 2000), the decrease in physical activity observed with aging (Colley et al., 2011) also plays a significant role in the reduction of resting energy expenditure (REE; Amati et al., 2009; Crane et al., 2010; Holloszy, 2009). The reduction in REE and physical activity lead to an increase in FM and obesity with aging.

Adipose tissue can secrete adipokines such as leptin that can have an impact on metabolic health in older individuals (Enriori et al., 2006). Leptin, an adipokine that can regulate energy balance by inhibiting hunger, has a decreased sensitivity when obesity is present, which results in an inability to detect satiety despite having high FM (Pan et al., 2014). This leptin resistance contributes to obesity, thus negatively influencing metabolic health in older individuals (Gabriely et al., 2002).

Also, aging is related to an increased inflammatory profile, which can exacerbate the decrease in lean mass. Tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin-6 (IL-6) are two inflammatory markers related to sarcopenia (Pedersen et al., 2003). Aging and obesity are accompanied by inflammation, which can increase muscle degradation because TNF $\alpha$  can inhibit protein synthesis (Dirks & Leeuwenburgh, 2006; Li & Reid, 2000) by stimulating the NF-kB pathway (Li et al., 1998) and can induce alterations in muscle proteins independent of protein loss (Li et al., 2000). Individuals with high levels of inflammatory markers (TNF $\alpha$  and IL-6) seem to have lower muscle mass and strength (Schaap et al., 2006, 2009).

Consuming more dairy products has been shown to promote fat loss in obese individuals (Zemel et al.,

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2004). Studies have indicated that dairy products play a significant role in lipid oxidation and the inhibition of lipid synthesis (Zemel, 2004; Zemel et al., 2005). Moreover, dairy intake intervention studies have shown a positive effect on weight loss in obese individuals (Heaney, 2003; Heaney et al., 2002; Zemel & Miller, 2004). These positive changes are explained by the presence of calcium in dairy products. Many studies have shown that calcium has a direct impact on lipid metabolism. When daily calcium intake is ~1,000 mg/ day, an acceleration of FM loss seems to occur in obese individuals (Shahar et al., 2010; Zemel et al., 2004), and calcium from dairy products could have a more important impact on FM loss (Schrager, 2005). Thus, whereas dairy products are a good source of protein and may be effective in maintaining lean mass (Phillips et al., 2009), they may possibly help improve fat loss in older sarcopenic men, although the exact mechanisms remain unknown.

Energy deficit (through aerobic exercise or caloric restriction) has been shown to effectively decrease FM in overweight populations (Larson-Meyer et al., 2006). However, energy restriction is deleterious to older adults (Weinheimer et al., 2010), especially in a context of sarcopenia. Although resistance training has long been known to efficiently maintain muscle mass and function in older adults (Hunter, McCarthy, & Bamman, 2004), adding a dairy supplementation after a resistance training session has been shown to significantly reduce FM (Josse et al., 2011) and increase muscle mass (Wilkinson et al., 2007) in overweight postmenopausal women. This study proposes a new insight into weight loss in older individuals by adding a postexercise shake made from dairy products (which are rich in calcium) after a resistance training session, but without interfering with daily food intake.

We speculate that combining resistance exercise with dairy products could have a beneficial impact on FM, REE, inflammation, and blood lipid profile in healthy but overweight sarcopenic older adults. The objective of this study was to evaluate whether 4 months of resistance training combined with postexercise dairy milk supplementation could improve metabolic parameters in older sarcopenic men to a greater extent than a nondairy protein and calcium supplement (commercial protein powder) or a control beverage.

### **Methods**

Twenty-six older sarcopenic men (60–75 years old) were recruited in this study. Sarcopenia was determined as an appendicular lean mass index lower than 10.75 kg/m<sup>2</sup> (Janssen et al., 2004). Inclusion criteria were as follows: nonsmokers, sedentary for at least 5 years (structured exercise less than 3 times per week), body mass index less than 30 kg/m<sup>2</sup>, no major physical disabilities, no medical treatment influencing metabolism, light drinkers (<15 g ethanol/day = 1 alcoholic beverage), weight stable (±2 kg) for 6 months, no resistance exercise for the past 3 years, and controlled blood pressure (for at least 6 months, range = 120-130/80-90). In addition, participants had no diagnosis or any sign of kidney disease.

#### Study Protocol

The experimental design was approved by the Ethics Committee of the Geriatric Institute of the University of Sherbrooke at the Research Center on Aging (RCA). All participants gave their written informed consent to participate in the study during their first visit to the RCA. After a phone screening, participants were invited for a baseline visit at the RCA. The first visit consisted of three tests: body composition measurements, physical activity level, and dietary intake assessment.

#### Anthropometric and Body Composition Measurements

Body weight (±0.2 kg) was determined with an electronic scale (SECA707; SECA, Hamburg, Germany) and standing height (in meters) was measured using a wall stadiometer (Takei, Tokyo, Japan). Body mass index was calculated as body weight in kilograms divided by height in meters squared. FM and lean mass (in kilograms) were measured using the dual-energy x-ray absorptiometry (GE Prodigy Lunar; General Electric Healthcare, Little Chalfont, United Kingdom).

#### **Physical Activity Level**

Physical activity level was measured using the Physical Activity Scale for the Elderly (PASE; Washburn & Ficker, 1999) such as in past studies from our group (Lord et al., 2007). Participants reported their leisure time, household, and work-related activities during the past week. Daily activity was scored according to the intensity and time of reported activities. The data were summed by the investigator to produce a global score representing physical activity energy expenditure (score range = 0-793).

### **Dietary Intake**

Despite its recognized limitations, the 3-day food record remains the best tool to assess dietary intake in the context of the proposed study (free-living condition, healthy participants). As such, a 3-day food record has been shown to be valid and reliable (representative of energy and nutrient intakes) in an older population with no cognitive impairments (Lührmann et al., 1999). Participants were instructed to maintain normal dietary habits throughout the dietary record. They were provided with a 5-kg (11-lb) food scale and were instructed on how to complete a 3-day dietary record. Foods and liquids were recorded (detailed and weighted) during 2 weekdays and 1 weekend day. A dietary analysis was completed using the Nutrifiq software (Laval University, Quebec City, Québec, Canada). During a second visit (12 hr fasted), the completed 3-day food record was returned and REE (kcal/day) was measured, followed by a blood draw to measure fasting glucose (mmol/L), insulin (pmol/L), triglycerides (mmol/L), low-density lipoproteins (mmol/L), high-density lipoproteins (mmol/L), cholesterol (mmol/L), free fatty acids (mmol/L), leptin (ng/mL), creatinine ( $\mu$ mol/L), inflammatory markers (IL-6; pg/mL), TNF $\alpha$  (pg/mL), and C-reactive protein (CRP; ng/mL).

### Resting Energy Expenditure and Respiratory Exchange Ratio

REE and respiratory quotient were determined using indirect calorimetry for a 30-min period (15-min rest and 15-min measurement). During the rest and measurement periods, participants were laying down on a bed in a comfortable room with minimal light and noise. They were asked to remain still, silent, yet awake. REE (kcal/ day) was calculated using the Weir equation (Weir, 1990).

#### **Metabolic Health**

Blood samples were collected after a 12-hr overnight fast by an experienced nurse while participants were in a sitting position after a 15-min rest. Venous blood (35 ml) was withdrawn and placed in 3-ml and 4-ml Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ). Plasma lipid profile, that is, total cholesterol, high-density lipoproteins, low-density lipoproteins, and triacylglycerol, along with plasma glucose levels, were immediately analyzed at the Sherbrooke University Hospital Center. Blood was stored in a -80 °C freezer for future analyses. Free fatty acids were measured with an automated clinical chemistry analyzer (Dimension Xpand Plus; Siemens, Deerfield, IL) using a commercially available kit (Wako Diagnostics, Richmond, VA) at the Geriatric Institute. Leptin values were measured (spectrophotometry; Victor V; Perkin-Elmer, Woodbridge, Ontario, Canada) using enzymelinked immunosorbent assay (ELISA) kits specific for human leptin (ALPCO, Salem, NH). The minimum detectable doses were 1 ng/ml.

Serum IL-6, TNF $\alpha$ , and CRP were measured (spectrophotometry; Victor V; Perkin-Elmer, Woodbridge, Ontario, Canada) using ELISA kits specific for humans (IL-6 and TNF $\alpha$ , R&D Systems, Inc., Minneapolis, MN; CRP, EMD Millipore Corporation, Billerica, MA). The minimum detectable doses were 0.7 pg/mL (IL-6), 0.106 pg/mL (TNF $\alpha$ ), and 0.20 ng/mL (CRP).

### **Postexercise Supplements**

Supplements were provided as postexercise shakes. All participants were asked to drink their shake immediately after the exercise session while still at the RCA. The two experimental groups received approximately 400 mg of calcium. The three types of shakes were as follows:

• Control: Rice milk was used as a control supplement (395 ml, containing 0.6 g protein, 0 g essential

amino acids [EAA], 59.5 g carbohydrate, 3.75 g fat, 0 mg calcium; 280 calories). This beverage was used to mimic the conditions of the two other experimental groups (taking a postexercise beverage). Chocolate syrup was added so that all mixtures tasted similar.

- Dairy shake: To attain the amount of protein, milk powder was added to 1% chocolate cow milk (375 ml, containing 13.53 g protein, 7 g EAA, 37.5 g carbohydrate, 3.8 g fat, 375 mg calcium; 270 calories).
- Nondairy protein shake: This shake consisted of chocolate soy beverage to which commercial EAA powder (Nutricia North America) was added (379 ml, containing 12 g protein, 7 g EAA, 39 g carbohydrate, 5.3 g fat, 450 mg calcium; 252 calories).

Because this study was a double-blind randomized controlled trial, all cups containing protein shakes were opaque, had the same appearance and the same volume, and were all chocolate flavored to ensure that both participants and investigators were blinded to the supplementation group.

### **Exercise Program**

Three weekly 1 hr-sessions, including a 10-min warm-up, were held on 3 nonconsecutive days for 16 weeks. Resistance exercises included free weight lifting (shoulder press, sit ups, and biceps curls) and resistance equipment for leg press, bench press, leg extension, rowing extensions, and leg curls (three sets  $\times$  eight repetitions). One-repetition maximum (1 RM) was used to determine maximum weight lifted for each exercise. Resting periods of 1 min were held between series. A total number of eight repetitions at 80% of 1 RM has been selected because it has been shown to be optimal to induce muscle hypertrophy in older adults (Evans, 1999). Participants had to attend a minimum of 85% of all exercise sessions, missing a maximum of six consecutive sessions.

### **Statistical Methods**

Results are reported as  $M \pm SD$  (standard errors in figures). Baseline similarities between groups were ensured by using the Kruskal–Wallis test. To verify the effect of 4 months of exercise in each group separately, we used nonparametric Wilcoxon signed-rank test. The amplitude of change was compared among groups using Kruskal-Wallis applied to delta of values. All analysis were performed using SPSS Version 20.0 for Windows (SPSS Inc., Chicago, IL). Statistical significance was set at  $p \le .05$ .

### Results

Eleven participants were excluded because they were not sarcopenic and were not able to be enrolled in the study. Hence, of the 41 participants recruited, 26 completed the 4-month intervention (dairy shakes, n = 8; nondairy shakes, n = 8; controls, n = 10). The average compliance for all participants was higher than 90%. Four of the 41 participants withdrew from the study because of a lack of interest.

Resistance training significantly increased LM in all groups (all  $ps \le .05$ ) independently of supplementation (1.9 kg, nondairy shake; 1.7 kg, dairy shake; 1.4 kg, control). However, only the dairy shake group significantly decreased FM after the intervention (0.1 kg, nondairy shake; -1.1 kg, dairy shake; -0.9 kg, control;  $p \le .05$ ). In addition, we observed a significant increase in muscle mass to FM ratio in the dairy shake group only (0.6 kgLM/kgFM,  $p \le .05$ ). Body weight significantly increased in the nondairy shake group only (1.9 kg,  $p \le .05$ ). No changes were observed for body mass index.

Furthermore, we found no significant differences between energy intake and calcium intake in the study. Fat, carbohydrates, and protein intake did not change before and after the intervention.

No changes were observed for REE nor for respiratory exchange ratio after the 4-month resistance training program combined with protein supplementation. In addition, no significant changes were observed for any of the cardiometabolic parameters, such as triacylglycerol, low-density lipoproteins, high-density lipoproteins, cholesterol, free fatty acids, leptin, fasting glucose and insulin, and inflammatory profile. All results are shown in Table 1.

### Discussion

The results of this study demonstrate positive adaptations of body composition with regard to resistance training and milk protein supplementation (a significant decrease in FM and a significant increase in LM). Significant decreases in FM were observed only in the dairy shake group. Although we could not explain these findings from variables that were measured, this study generates interesting hypotheses.

Our results are consistent with previous research examining changes in LM after a resistance training program and milk consumption intervention (Hartman et al., 2007; Phillips et al., 2009). In addition, other studies have indicated a positive effect of an increased consumption of milk on FM (Anderson & Hoie, 2005; Lin et al., 2000).

It is interesting that although we did not include a caloric restriction intervention, we observed a loss in FM in the dairy shake group only. A few studies have demonstrated that dairy products are associated with greater losses in FM during caloric restriction (Josse et al., 2011; Zemel et al., 2005). Our study demonstrates that fat loss is possible with postexercise dairy supplementation combined with resistance training in overweight sarcopenic men. This is an interesting avenue because caloric restriction was not present in this study.

The lack of change in REE is consistent with two studies that found similar results after a resistance training program (Broeder et al., 1992; Van Etten et al., 1997). Even if LM is significantly related to REE, it does not coincide with increases in REE when LM is increased with resistance training because of small gains of LM during resistance training.

No changes were also found for inflammation profile. These results are in disagreement with studies that have observed significant changes in these variables after a resistance training program (Calle & Fernandez, 2010; Fahlman et al., 2002). Nonetheless, it could have been difficult to induce significant changes in these parameters because our participants were healthy.

Participants in the dairy shake group did not decrease their energy intake or increase their physical activity differently than other groups. Thus, the decrease in FM may be explained by other potential mechanisms related to milk consumption caused by the presence of calcium found in dairy products that is capable of increasing lipolysis and lipid oxidation (Gonzalez et al., 2012). Dairy milk has also been suggested to contain an important bioactive compound such as angiotensin-converting enzyme (ACE) inhibitory activity. ACE inhibitory activity can significantly attenuate obesity in rodents (Causey & Zemel, 2003; Moustaïd-Moussa & Berdanier, 2001). These were not measured in the current study and deserve further investigation.

This study has some limitations. First, our cohort included healthy older adult individuals, which may explain in part why we did not observe any changes in metabolic profile. Second, this study did not explore potential mechanisms that could explain fat loss, such as excess postexercise oxygen consumption and the complete analysis of our dairy supplement. Because of the small number of participants included per group, statistical power to investigate any potential underlying mechanisms is limited and may explain the nonsignificant changes in some variables. However, given the small sample size, we still observed a significant decrease in the dairy shake group that warrants further examination in other studies.

In conclusion, resistance training combined with a milk-based postexercise supplementation significantly reduced FM and increased LM in overweight sarcopenic older men. However, we could not explain these results by changes in energy metabolism, energy intake, or physical activity. More studies are obviously needed to elucidate the mechanisms underlying the decrease in FM with milk and resistance exercise in older overweight sarcopenic men and potentially promote this intervention in the context of aging and improvements in metabolic health. In addition, from a clinical standpoint, there is a reluctance to induce caloric restriction in older individuals because it can cause a decrease in bone mineral density (Villareal et al., 2006). Therefore, promoting FM loss through resistance exercise and dairy supplementation can be an interesting avenue to pursue with overweight to obese older individuals.

	Nondairy Shake (n = 8)		Dairy Shake (n = 8)		Controls (n = 10)	
Characteristic	T1	T2	T1	T2	T1	T2
Age (years)	$64 \pm 4.9$		$68 \pm 5.1$		$64 \pm 4.5$	
Weight (kg)	$80.6 \pm 13.5$	82.5 ± 13.4*	$76.7 \pm 9.0$	$77.3 \pm 9.9$	$79.5 \pm 11.8$	$79.8 \pm 11.9$
Body mass index (kg/m <sup>2</sup> )	$27.0\pm2.7$	$27.8 \pm 2.7$	$25.8 \pm 3.0$	$25.9 \pm 3.2$	$25.9 \pm 3.1$	$26.2 \pm 3.1$
FM (kg)	$20.9 \pm 7.1$	$20.8\pm6.9$	$19.5 \pm 7.1$	$18.4 \pm 7.8^{*}$	$20.2 \pm 7.8$	$19.3 \pm 7.2$
LM (kg)	$56.3 \pm 8.1$	$58.2 \pm 7.9^{*}$	$54.5 \pm 5.3$	$56.2 \pm 4.5^{*}$	$56.2 \pm 6.3$	$57.6 \pm 6.8*$
ALMI (kg/m <sup>2</sup> )	$9.34 \pm 0.86$	$9.73 \pm 0.90^{*}$	$8.90 \pm 0.94$	$9.17 \pm 0.92$	$8.94 \pm 0.94$	9.31 ± 1.13*
LM/FM	$2.9 \pm 0.9$	$3.0 \pm 0.9$	$3.1 \pm 1.2$	$3.7 \pm 1.8^{*}$	$2.9 \pm 0.9$	$3.0 \pm 0.9$
Glucose (mmol/L)	$5.4 \pm 0.5$	$5.3 \pm 0.8$	$5.0 \pm 0.4$	$5.1 \pm 0.4$	$5.2 \pm 0.7$	$5.3 \pm 0.6$
Insulin (pmol/L)	$58.1 \pm 39.0$	$46.0 \pm 39.0$	$48.8 \pm 23.1$	$51.9 \pm 30.7$	$40.6 \pm 17.5$	$44.8 \pm 17.4$
Total cholesterol	$4.6 \pm 0.4$	$4.4 \pm 0.4$	$4.4 \pm 1.1$	$4.2 \pm 1.0$	$5.4 \pm 1.0$	$5.2 \pm 1.1$
TG (mmol/L)	$1.1 \pm 0.4$	$1.2 \pm 0.4$	$0.9 \pm 0.4$	$0.9 \pm 0.4$	$1.2 \pm 0.3$	$1.1 \pm 0.3$
HDL (mmol/L)	$1.4 \pm 0.3$	$1.3 \pm 0.2$	$1.3 \pm 0.3$	$1.3 \pm 0.2$	$1.3 \pm 0.3$	$1.3 \pm 0.3$
LDL (mmol/L)	$2.7 \pm 0.3$	$2.6 \pm 0.4$	$2.6 \pm 1.0$	$2.5 \pm 0.9$	$3.5 \pm 0.9$	$3.4 \pm 0.9$
FFA (mmol/L)	$0.44 \pm 0.11$	$0.37 \pm 0.13$	$0.50 \pm 0.21$	$0.40\pm0.07$	$0.36 \pm 0.1$	$0.47\pm0.20$
Leptin (ng/ml)	$8.3 \pm 8.2$	$6.1 \pm 5.5$	$5.7 \pm 5.6$	$5.8 \pm 6.6$	$5.6 \pm 6.5$	$4.1 \pm 4.3$
TNFα (pg/mL)	$1.69 \pm 0.71$	$1.76 \pm 0.63$	$1.41 \pm 0.28$	$1.45 \pm 0.17$	$1.82 \pm 0.34$	$1.69 \pm 0.32$
IL-6 (pg/ml)	$1.86 \pm 1.09$	$1.36 \pm 0.42$	$1.37 \pm 0.95$	$1.13 \pm 0.67$	$1.38 \pm 0.29$	$1.43 \pm 0.58$
CRP (ng/ml)	$1.37 \pm 0.95$	$1.13 \pm 0.67$	$2.56 \pm 2.29$	$1.44 \pm 1.25$	$2.10\pm2.04$	$1.81 \pm 2.01$
REE (kcal/day)	1,596 ± 251	$1,604 \pm 444$	$1,378 \pm 334$	$1,460 \pm 254$	$1,412 \pm 280$	$1,610 \pm 242$
RER	$0.83 \pm 0.05$	$0.85 \pm 0.05$	$0.79 \pm 0.30$	$0.82 \pm 0.04$	$0.84 \pm 0.03$	$0.83 \pm 0.07$
PASE	$165 \pm 34$	$160 \pm 35$	$122 \pm 47$	$118 \pm 41$	$139 \pm 72$	$150 \pm 77$
Energy intake (kcal/day)	$2,296 \pm 345$	$2,476 \pm 466$	$2,326 \pm 319$	$2,328 \pm 868$	$2,353 \pm 546$	$2,162 \pm 558$
Carbohydrates intake (g/day)	$277 \pm 58$	$283 \pm 50$	$297 \pm 50$	$317 \pm 161$	$303 \pm 100$	$257 \pm 7$
Fat intake (g/day)	$124 \pm 88$	$133 \pm 94$	$137 \pm 87$	$104 \pm 78$	$79 \pm 17$	$73 \pm 37$
Protein intake (g/day)	$92 \pm 18$	$104 \pm 32$	$97 \pm 79$	$164 \pm 89$	$100 \pm 26$	$85 \pm 16$
Calcium intake (mg)	$774 \pm 220$	$1,005 \pm 358$	$965 \pm 342$	$943 \pm 400$	$955 \pm 407$	$972 \pm 439$

 Table 1
 Effect of Resistance Training and Protein Supplementation on Body Composition,

 Cardiometabolic Parameters, Lipid Profile, Inflammation, and Energy Intake, *M* ± SD

*Note.* ALMI = appendicular lean mass index; ; CRP = C-reactive protein; FFA = free fatty acid; FM = fat mass; HDL = high-density lipoprotein; IL-6 = interleukin-6; LDL = low-density lipoprotein; LM = lean mass; PASE = Physical Activity Scale for the Elderly; REE = resting metabolic rate; RER = respiratory exchange ratio; TG = triglycerides; TNF $\alpha$  = tumor necrosis factor-alpha; T1 = Time 1 baseline results; T2 = Time 2, after 16 weeks of resistance training. \*Significant difference between T1 and T2 ( $p \le .05$ ).

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