

# Optimized dietary strategies to protect skeletal muscle mass during periods of unavoidable energy deficit

Stefan M. Pasiakos,<sup>1</sup> Lee M. Margolis, and Jeb S. Orr

Military Nutrition Division, U.S. Army Research Institute of Environmental Medicine, Natick, Massachusetts, USA

**ABSTRACT** Interactions between dietary protein and energy balance on the regulation of human skeletal muscle protein turnover are not well described. A dietary protein intake above the recommended dietary allowance during energy balance typically enhances nitrogen retention and up-regulates muscle protein synthesis, which in turn may promote positive protein balance and skeletal muscle accretion. Recent studies show that during energy deficit, muscle protein synthesis is down-regulated with concomitant increases in ubiquitin proteasome-mediated muscle proteolysis and nitrogen excretion, reflecting the loss of skeletal muscle mass. However, consuming high-protein diets (1.6–2.4 g/kg per day), or high-quality, protein-based meals (15–30 g whey) during energy deficit attenuates intracellular proteolysis, restores muscle protein synthesis, and mitigates skeletal muscle loss. These findings are particularly important for physically active, normal-weight individuals because attenuating the extent to which skeletal muscle mass is lost during energy deficit could prevent decrements in performance, reduce injury risk, and facilitate recovery. This article reviews the relationship between energy status, protein intake, and muscle protein turnover, and explores future research directives designed to protect skeletal muscle mass in physically active, normal-weight adults.—Pasiakos, S. M., Margolis, L. M., and Orr, J. S. Optimized dietary strategies to protect skeletal muscle mass during periods of unavoidable energy deficit. *FASEB J.* 29, 1136–1142 (2015). [www.fasebj.org](http://www.fasebj.org)

**Key Words:** leucine • military • mTORC1 • lean body mass

DIETARY ENERGY AND PROTEIN intake are nutritional determinants of skeletal muscle mass. Maintenance of skeletal muscle mass is generally achieved by consuming sufficient energy to meet energy demand and protein at levels consistent with the U.S. recommended dietary allowance (RDA; 0.8 g/kg per day) (1). However, during periods of increased energy demand, protein requirements to sustain protein retention and the maintenance of skeletal muscle mass are increased above the RDA. It is currently recommended that physically active individuals (*e.g.*, aerobic and resistance exercise type athletes) consume 1.2 to 1.7 g

protein/kg per day (2–4). Likewise, it is recommended that military personnel undergoing metabolically challenging training and combat operations consume a diet providing 1.5 to 2.0 g/kg per day of protein to facilitate the repair of damaged proteins, synthesis of new muscle proteins, and maintenance of muscle mass (5). Therefore, it is not surprising that high-protein diets have increased in popularity among physically active, normal-weight adults (3).

In general, overweight and obese individuals adhering to a sustained, moderate energy-deficient diet lose fat and lean body mass (LBM), which approximates 75% fat mass and 25% LBM (6). However, decrements in LBM can be more severe in normal-weight individuals, such as athletes and military personnel, who often undergo unavoidable energy deficits of greater severity (7). The loss of LBM in active populations can lead to degraded performance and increased injury risk (8, 9). Fortunately, protein intake above the RDA is skeletal muscle protective, as studies have consistently shown that consuming protein at twice the RDA spares LBM and that this metabolic advantage is independent of body size (6, 7, 10–12).

Although the independent effects of dietary energy and protein intake on body composition have been studied extensively, and potential energetic, endocrine, and the behavioral mechanisms to account for body composition adaptations to energy and protein manipulations have been explored (13–16), the interaction between energy status and protein intake on skeletal muscle protein turnover and associated regulatory systems remains largely unexplored (7, 17–22). Given that muscle protein turnover, especially muscle protein synthesis (MPS), is a primary regulator of skeletal muscle mass (23) and that the stimulatory effects of dietary protein on MPS are well documented (24–26), the lack of studies in this area of nutritional science is intriguing. Recent studies from our group (7, 20) and others (17) have provided consistent data demonstrating interactive effects of energy and protein on muscle protein turnover. This article highlights the molecular and nutritional regulation of skeletal muscle mass with emphasis given to recent studies establishing a mechanistic link between energy and protein intake on muscle protein turnover, and it proposes new research to

Abbreviations: IGF-1, insulin-like growth factor-1; LBM, lean body mass; MPB, muscle protein breakdown; MPS, muscle protein synthesis; mTORC1, mechanistic target of rapamycin complex 1; RDA, recommended dietary allowance; UPS, ubiquitin proteasome system

<sup>1</sup> Correspondence: Military Nutrition Division, U.S. Army Research Institute of Environmental Medicine, 15 Kansas St., Bldg. 42, Natick, MA 01760, USA. E-mail: [stefan.pasiakos@usarmy.mil](mailto:stefan.pasiakos@usarmy.mil)  
doi: 10.1096/fj.14-266890

identify appropriate nutritional strategies to mitigate skeletal muscle loss during energy deficit.

### **Molecular regulation of human skeletal muscle protein turnover**

Skeletal muscle protein turnover is a dynamic process that encompasses the synthesis of new proteins and breakdown of existing proteins. The rate of muscle protein turnover is dependent on amino acid availability and endogenous protein breakdown (26, 27). Cycling of amino acids between MPS and muscle protein breakdown (MPB) is critical for growth, maintenance, and repair of body tissues, which facilitate adaptation and recovery from physical stressors (25). Dysregulation of muscle protein turnover can contribute to the progression of LBM loss (28–30).

Skeletal muscle protein turnover is regulated by intracellular anabolic (23) and proteolytic systems (31). Cellular regulation of MPS is mediated by the mechanistic target of rapamycin complex 1 (mTORC1) (32, 33). Energy status and protein intake modulate mTORC1 signaling, mRNA translation, and ultimately MPS (32, 33). Specifically, energy deprivation up-regulates AMP-activated protein kinase, thereby diminishing mTORC1 signaling and MPS (34). However, consuming protein either alone or in a mixed-macronutrient meal promotes a robust increase in MPS (35, 36). The anabolic response to protein ingestion occurs as a result of postprandial increases in extracellular amino acid availability (26, 37), insulin secretion (38), and muscle intracellular amino acid transporter expression (39). This sequence leads to increased muscle intracellular amino acid levels, which in turn stimulates mTORC1 and downstream activation of p70 S6 kinase and inactivation of the repressor of mRNA translation, eukaryotic initiation factor 4E-binding protein (40–44). Dietary protein sources that contain high levels of the branched-chain amino acid leucine are particularly effective at increasing MPS. The effectiveness of leucine seems to stem from interactions with the Rag subfamily of Rag small GTPases and subsequent lysosomal translocation of mTORC1 (43, 44).

MPB provides amino acid precursors to sustain MPS and support hepatic gluconeogenesis (45–47). Four proteolytic systems contribute to MPB, including the ubiquitin proteasome system (UPS), autophagy-lysosomal, calcium-dependent calpains, and the cysteine protease caspase enzymes (48, 49). The UPS is the primary mechanism by which skeletal muscle is degraded (31, 50). The UPS is a highly regulated, irreversible process that involves energy-dependent ubiquitylation of muscle proteins through a discrete series of reactions catalyzed by 3 distinct enzyme complexes. This process begins with calpain and caspase 3–dependent myofibril cleavage, resulting in smaller, more accessible actomyosin fragments (51, 52). These fragments are then marked for degradation by covalent binding of multiple ubiquitin molecules catalyzed by the enzymes E1 (ATP-dependent ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin-ligating enzymes). The polyubiquitin chain is recognized by the 26S proteasome, a large multisubunit proteolytic complex consisting of a central catalytic core (20S proteasome) and 2 terminal regulator complexes (19S complexes). The 19S regulator complex plays a

central role in the recognition and unfolding of the ubiquitylated proteins and guides them to the 20S catalytic core for subsequent protein hydrolysis. Activity and expression of the UPS is up-regulated under conditions of metabolic stress and is thought to be regulated in part through the insulin-mediated mTORC1 intracellular signaling pathway (53).

### **Nutritional regulation of human skeletal muscle protein turnover**

The quantity of dietary protein consumed dictates the activation of MPS. There is a dose-dependent relationship between dietary protein and MPS, and consuming a 20 to 25 g serving (or 0.25 to 0.30 g protein/kg meal or 2.5 g leucine) of high-quality protein maximally stimulates postprandial MPS for approximately 2 hours (54, 55). When higher doses of protein or leucine are consumed, there is an accompanying increase in protein oxidation with no additional anabolic stimulus (56, 57). The mechanisms limiting MPS stimulation remain unclear, but may include reduced amino acid uptake due to negative feedback regulation of amino acid transporter expression (35, 39).

Enriching smaller doses of high-quality protein and essential amino acids with leucine—or, in the case of older adults, consuming more total protein—potentiates postprandial mTORC1 signaling and MPS during conditions of metabolic stress (58), age-related anabolic resistance (59–61), and during recovery from endurance and resistive-type exercise (62–65). Metabolic stressors, such as energy restriction, may circumvent factors limiting MPS during energy balance. Findings from Areta *et al.* (17) provide support for this hypothesis, as increasing total protein (15 to 30 g whey protein) intake after exercise resulted in a progressive increase in postprandial MPS during energy deficit. Recent evidence also suggests that distribution of daily protein intake can modulate MPS (66). Specifically, distributing protein equally across meals, rather than the more traditional, skewed intake of daily protein, extends postprandial MPS (67), which may contribute to long-term regulation of skeletal muscle mass by limiting the duration to which skeletal muscle is in a net catabolic state (68). Although these studies provide valuable insight regarding the quantity and distribution of protein intake on MPS, it is important to reemphasize the importance of protein quality, as lower-quality proteins (*i.e.*, those lower in leucine) may not be as effective in potentiating MPS under such conditions (69).

Kinetic and intracellular assessments of MPB have produced inconsistent, limited data (31). The lack of reproducible data in this area is likely due to methodological limitations (70) and the relative small contribution of MPB to the regulation of skeletal muscle mass in healthy individuals (31). Some studies have shown that protein consumption and the secondary hyperinsulinemia attenuate postresistance exercise MPB (71–73). However, others have shown that provision of exogenous amino acids can up-regulate MPB (74) or may have minimal-to-no impact on MPB, at least when consumed at rest or before or after exercise (57, 75–78). We demonstrated increased post-absorptive MPB and UPS-related gene expression after short-term (10 days) and prolonged (21 days) energy

deficit (18, 19). However, consumption of a protein-containing, mixed-meal attenuated 26S proteolytic activity during energy deficit (18). A better understanding of the intracellular regulatory mechanisms of MPB will be necessary to fully understand the complex pathways modulating skeletal muscle mass in populations susceptible to severe muscle loss resulting from nutritional deprivation.

## RECENT RESULTS

### High-protein diets attenuate decrements in muscle protein synthesis and protect skeletal muscle mass during energy deficit

The extent to which energy deficit modulates protein turnover and LBM is largely due to the degree and duration to which energy intake is deficient. At the whole-body level, acute energy deficiency results in an increase in proteolysis, amino acid oxidation, and nitrogen excretion, which becomes less pronounced and plateaus over time (79–83). This adaptive mechanism to spare protein during prolonged energy deficit was demonstrated by Ancel Keys nearly 70 years ago (84–86). Despite dramatic advances in analytical capabilities, there have been few studies that have examined actual skeletal muscle adaptation to energy deficit (7, 17, 20–22).

Our laboratory has conducted a number of studies where dietary protein was manipulated to better understand the control of muscle protein turnover during energy deficit (7, 18–20). In normal-weight, physically active adults, we demonstrated that short-term (10 days), moderate energy deficit (500 kcal per day) attenuates postabsorptive MPS by 19% with concomitant reductions in mTORC1 signaling (20). Postabsorptive MPB and intracellular markers of muscle proteolysis were also increased in response to short-term energy deficit (19). Although these findings have not been confirmed, increased muscle proteolysis during short-term energy deficit is consistent with studies demonstrating up-regulated whole-body proteolysis in the early stages of energy deficit (80). The observed decrements in MPS were recently confirmed by Areta *et al.* (17), who reported a 27% reduction in postabsorptive MPS in a similar population of physically active, normal-weight adults in response to a 5 day moderate energy deficit.

We also observed that when total daily protein intake is consistent with the RDA, sustained energy deficit over 21 days blunts postprandial MPS responses to an optimal serving of high-quality protein (20 g milk protein) (7). In contrast, a diet providing 2 and 3 times the RDA for protein spared postprandial MPS responses to the same 20 g serving of protein with simultaneous reductions in UPS-mediated muscle proteolysis (7, 18). Individuals consuming protein at levels 2 and 3 times the RDA also lost 43% less LBM than those consuming RDA levels under identical experimental conditions (7, 18). Areta *et al.* (17) also demonstrated that consuming high-quality, protein-based meals (15 to 30 g whey) in combination with resistance exercise restored MPS above levels observed during energy balance and did so in a dose-dependent manner. These data emphasize the link among protein turnover, total dietary protein intake, and protein consumed at each meal

on the regulation of skeletal muscle mass during energy deficit. Interestingly, a diet providing 3 times the RDA rather than twice the RDA offered no further advantages during moderate energy deficit (1000 kcal per day), as protein oxidation was increased (83), with no additional protection of LBM (7). That said, we suspect that higher levels of dietary protein (more than twice RDA) may be advantageous in some situations, as we recently showed. Soldiers consuming 1.7 g protein/kg per day failed to retain whole-body protein during metabolically challenging, short-term military training that produced extreme energy deficits (~3400 kcal per day) (87).

## FUTURE DIRECTIONS AND CONCLUSION

Our laboratory is currently answering the practical question—would the addition of protein-containing snacks to daily protein intake accelerate recovery of LBM after unavoidable energy deficit? A driver for this work is the unique occupational situations of our target population, who have goals and requirements distinct from the general public. Our nutritional questions are, to an extent, similar to athletic populations, and although overlap exists, military personnel are not competitive athletes who train and compete under optimal conditions. Rather, they operate in austere environments and in situations where the ability to eat ebbs and flows. Moreover, they have to deal with stressors common to combat operations (*e.g.*, energy deficit, sleep deprivation, and sustained load carriage). Studies on students participating in military training courses and sustained operations exercises lasting 3.5 to 64 days have reported weight losses ranging from 3 to 16% of initial body mass (88–97). More importantly, LBM accounted for, on average, over 50% of the total mass lost, which highlights the disparity between military personnel and their civilian counterparts, who experience similar reductions in total body mass with generally no appreciable decrement in LBM (98, 99).

Despite evidence to support an increase in dietary protein intake for LBM preservation during energy deficit (6, 7, 10–12), no studies have established dietary protein requirements during simulated or real-world military operations. We recently observed that soldiers consuming 1.7 g protein/kg per day failed to retain whole-body protein during a 7 day military training exercise (87). Therefore, it is clear that recommendations for 1.2 to 1.7 g protein/kg per day (and perhaps up to 2.0 g protein/kg per day) may be population specific, and there is likely no amount of protein that will completely offset the catabolic state induced by military operational stress and severe energy deficits (100). Thus, the primary goal operational feeding should be to minimize energy deficits and provide adequate levels of protein to spare LBM (5, 7). Identifying the point at which further manipulations in the quantity, type, and pattern of protein intake begins to augment rates of protein oxidation with no discernible impact on protein retention and LBM will be critical for defining how to most effectively use protein in combat rations.

The dramatic reductions in anabolic hormones, such as testosterone and insulin-like growth factor-1 (IGF-1), that occur during severe energy deficit could diminish the

magnitude by which manipulating protein intake spares LBM. However, the contribution of testosterone and IGF-1 in the control of MPS and regulation of LBM remain unclear. In healthy young men, the suppression of endogenous testosterone production has myriad adverse physiologic consequences, including decreased LBM, increased adiposity, and decreased muscle strength (101–104). Finkelstein *et al.* (102) recently demonstrated that decreases in testosterone levels from 530 to 350 ng/dl result in increased adiposity, and further reductions to  $\leq 200$  ng/dl are accompanied by skeletal muscle atrophy and decreased muscle strength. Importantly, numerous studies report that testosterone decreases this magnitude during military training and sustained operations and is associated with concomitant decreases in LBM (90, 105–108). IGF-1 concentrations are sensitive to perturbations in energy balance as well. During various military training courses and studies designed to simulate operational stress, significant decrements in IGF-1 concentrations occur, and there is accompanying loss of LBM (89, 90, 106, 108, 109). Although dietary manipulations have to date proven to be unsuccessful at mitigating the endocrine response to negative energy balance (101, 124–126), pharmacologic interventions that restore anabolic hormone concentrations have been shown to promote nitrogen retention despite energy deficit (110–112). Further research is required to determine whether interventions, such as testosterone replacement, can attenuate decrements in LBM during military operational stress. Additionally, future studies are needed to investigate the interaction between endocrine status and the LBM-sparing effect of increased dietary protein intake.

Relatively little is known about individual responsiveness to dietary protein manipulations and the impact of underfeeding. An important first step will be identifying factors underlying the large interindividual variability with respect to the magnitude of LBM change during energy deficit. Such variability is exemplified by the wide range of changes observed in soldiers during U.S. Army Ranger school, where weight loss ranged from 9 to 23% (6.5 to 20.6 kg) of initial body mass, with a LBM loss of 0.3 to 13.8 kg (113). Interestingly, baseline adiposity accounted for 86% of the variance in fat loss but only 20% of the change in LBM. Studies conducted using monozygotic twins indicate that testosterone may assist with preservation of LBM during energy deficit and lessen adipose tissue accrual during energy surplus (13, 114). More recently, advanced genomic and transcriptomic analyses have been used to elucidate the genetic and molecular basis for individual skeletal muscle responses to resistance and endurance exercise training (115–119). Surprisingly, transcriptional patterns consistent with suppressed mTORC1 activity were observed in individuals displaying the greatest hypertrophic response to resistance training (117). The application of these techniques holds promise for furthering our understanding of the interindividual variability in skeletal muscle responses to operational stress.

Although recent studies provide a mechanistic link between dietary protein, skeletal muscle protein turnover, and the regulation of muscle mass during energy deficit, there are several important questions that remain unanswered. Is there a dose-dependent relationship between the amount of protein consumed and the severity of the

energy deficit imposed? Does MPS become saturated at the same level of protein during energy deficit as energy balance? To what extent does manipulating both the level and pattern of protein intake augment the maintenance of LBM during energy deficit and facilitate the accretion of LBM during recovery? Last, what accounts for the apparently large interindividual difference in LBM loss during energy deficit, and how does this impact dietary recommendations and/or protein requirements for populations susceptible to muscle loss? In addition to providing insight regarding the interactions between energy status and muscle protein turnover, future studies designed to address these questions will play an integral role in developing targeted nutritional interventions to optimize skeletal muscle mass in physically active, normal-weight adults. **FJ**

The authors thank Dr. Scott J. Montain for his support in the development of this article. This work was supported by the U.S. Army Medical Research and Materiel Command. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Army or the U.S. Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official U.S. Department of the Army endorsement of approval of the products or services of these organizations.

## REFERENCES

1. National Academy of Sciences, Institute of Medicine, and Food and Nutrition Board. (2005) *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, National Academy of Sciences, Washington, DC
2. American Dietetic Association; Dietitians of Canada; American College of Sports Medicine; Rodriguez, N. R., Di Marco, N. M., and Langley, S. (2009) American College of Sports Medicine position stand. Nutrition and athletic performance. *Med. Sci. Sports Exerc.* **41**, 709–731
3. Phillips, S. M. (2004) Protein requirements and supplementation in strength sports. *Nutrition* **20**, 689–695
4. Phillips, S. M. (2006) Dietary protein for athletes: from requirements to metabolic advantage. *Appl. Physiol. Nutr. Metab.* **31**, 647–654
5. Pasiakos, S. M., Austin, K. G., Lieberman, H. R., and Askew, E. W. (2013) Efficacy and safety of protein supplements for U.S. Armed Forces personnel: consensus statement. *J. Nutr.* **143**, 1811S–1814S
6. Wycherley, T. P., Moran, L. J., Clifton, P. M., Noakes, M., and Brinkworth, G. D. (2012) Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* **96**, 1281–1298
7. Pasiakos, S. M., Cao, J. J., Margolis, L. M., Sauter, E. R., Whigham, L. D., McClung, J. P., Rood, J. C., Carbone, J. W., Combs, G. F., Jr., and Young, A. J. (2013) Effects of high-protein diets on fat-free mass and muscle protein synthesis following weight loss: a randomized controlled trial. *FASEB J.* **27**, 3837–3847
8. Artioli, G. G., Gualano, B., Franchini, E., Scagliusi, F. B., Takesian, M., Fuchs, M., and Lancha, A. H., Jr. (2010) Prevalence, magnitude, and methods of rapid weight loss among judo competitors. *Med. Sci. Sports Exerc.* **42**, 436–442
9. Johnson, M. J., Friedl, K. E., Frykman, P. N., and Moore, R. J. (1994) Loss of muscle mass is poorly reflected in grip strength performance in healthy young men. *Med. Sci. Sports Exerc.* **26**, 235–240
10. Mettler, S., Mitchell, N., and Tipton, K. D. (2010) Increased protein intake reduces lean body mass loss during weight loss in athletes. *Med. Sci. Sports Exerc.* **42**, 326–337

11. Westerterp-Plantenga, M. S., Nieuwenhuizen, A., Tomé, D., Soenen, S., and Westerterp, K. R. (2009) Dietary protein, weight loss, and weight maintenance. *Annu. Rev. Nutr.* **29**, 21–41
12. Wycherley, T. P., Brinkworth, G. D., Clifton, P. M., and Noakes, M. (2012) Comparison of the effects of 52 weeks weight loss with either a high-protein or high-carbohydrate diet on body composition and cardiometabolic risk factors in overweight and obese males. *Nutr. Diabetes* **2**, e40
13. Bouchard, C., Tchernof, A., and Tremblay, A. (2014) Predictors of body composition and body energy changes in response to chronic overfeeding. *Int. J. Obes. (Lond.)* **38**, 236–242
14. Ochner, C. N., Barrios, D. M., Lee, C. D., and Pi-Sunyer, F. X. (2013) Biological mechanisms that promote weight regain following weight loss in obese humans. *Physiol. Behav.* **120**, 106–113
15. Stock, M. J. (1999) Gluttony and thermogenesis revisited. *Int. J. Obes. Relat. Metab. Disord.* **23**, 1105–1117
16. Bray, G. A., Smith, S. R., de Jonge, L., Xie, H., Rood, J., Martin, C. K., Most, M., Brock, C., Mancuso, S., and Redman, L. M. (2012) Effect of dietary protein content on weight gain, energy expenditure, and body composition during overeating: a randomized controlled trial. *JAMA* **307**, 47–55
17. Areta, J. L., Burke, L. M., Camera, D. M., West, D. W., Crawshaw, S., Moore, D. R., Stellingwerff, T., Phillips, S. M., Hawley, J. A., and Coffey, V. G. (2014) Reduced resting skeletal muscle protein synthesis is rescued by resistance exercise and protein ingestion following short-term energy deficit. *Am. J. Physiol. Endocrinol. Metab.* **306**, E989–E997
18. Carbone, J. W., Margolis, L. M., McClung, J. P., Cao, J. J., Murphy, N. E., Sauter, E. R., Combs, G. F., Jr., Young, A. J., and Pasiakos, S. M. (2013) Effects of energy deficit, dietary protein, and feeding on intracellular regulators of skeletal muscle proteolysis. *FASEB J.* **27**, 5104–5111
19. Carbone, J. W., Pasiakos, S. M., Vislocky, L. M., Anderson, J. M., and Rodriguez, N. R. (2014) Effects of short-term energy deficit on muscle protein breakdown and intramuscular proteolysis in normal-weight young adults. *Appl. Physiol. Nutr. Metab.* **39**, 960–968
20. Pasiakos, S. M., Vislocky, L. M., Carbone, J. W., Altieri, N., Konopelski, K., Freake, H. C., Anderson, J. M., Ferrando, A. A., Wolfe, R. R., and Rodriguez, N. R. (2010) Acute energy deprivation affects skeletal muscle protein synthesis and associated intracellular signaling proteins in physically active adults. *J. Nutr.* **140**, 745–751
21. Campbell, W. W., Haub, M. D., Wolfe, R. R., Ferrando, A. A., Sullivan, D. H., Apolzan, J. W., and Iglay, H. B. (2009) Resistance training preserves fat-free mass without impacting changes in protein metabolism after weight loss in older women. *Obesity (Silver Spring)* **17**, 1332–1339
22. Villareal, D. T., Smith, G. I., Shah, K., and Mittendorfer, B. (2012) Effect of weight loss on the rate of muscle protein synthesis during fasted and fed conditions in obese older adults. *Obesity (Silver Spring)* **20**, 1780–1786
23. Pasiakos, S. M. (2012) Exercise and amino acid anabolic cell signaling and the regulation of skeletal muscle mass. *Nutrients* **4**, 740–758
24. Pasiakos, S. M., and McClung, J. P. (2011) Supplemental dietary leucine and the skeletal muscle anabolic response to essential amino acids. *Nutr. Rev.* **69**, 550–557
25. Walker, D. K., Dickinson, J. M., Timmerman, K. L., Drummond, M. J., Reidy, P. T., Fry, C. S., Gundersmann, D. M., and Rasmussen, B. B. (2011) Exercise, amino acids, and aging in the control of human muscle protein synthesis. *Med. Sci. Sports Exerc.* **43**, 2249–2258
26. Wolfe, R. R., and Miller, S. L. (1999) Amino acid availability controls muscle protein metabolism. *Diabetes Nutr. Metab.* **12**, 322–328
27. Wolfe, R. R. (2002) Regulation of muscle protein by amino acids. *J. Nutr.* **132**, 3219S–3224S
28. Evans, W. J. (2010) Skeletal muscle loss: cachexia, sarcopenia, and inactivity. *Am. J. Clin. Nutr.* **91**, 1123S–1127S
29. Evans, W. J., Morley, J. E., Argilés, J., Bales, C., Baracos, V., Guttridge, D., Jatoi, A., Kalantar-Zadeh, K., Lochs, H., Mantovani, G., Marks, D., Mitch, W. E., Muscaritoli, M., Najand, A., Ponikowski, P., Rossi Fanelli, F., Schambelan, M., Schols, A., Schuster, M., Thomas, D., Wolfe, R., and Anker, S. D. (2008) Cachexia: a new definition. *Clin. Nutr.* **27**, 793–799
30. Phillips, S. M., Glover, E. I., and Rennie, M. J. (2009) Alterations of protein turnover underlying disuse atrophy in human skeletal muscle. *J. Appl. Physiol.* **107**, 645–654
31. Pasiakos, S. M., and Carbone, J. W. (2014) Assessment of skeletal muscle proteolysis and the regulatory response to nutrition and exercise. *IUBMB Life* **66**, 478–484
32. Proud, C. G. (2007) Cell signaling. mTOR, unleashed. *Science* **318**, 926–927
33. Proud, C. G. (2007) Amino acids and mTOR signalling in anabolic function. *Biochem. Soc. Trans.* **35**, 1187–1190
34. Bolster, D. R., Crozier, S. J., Kimball, S. R., and Jefferson, L. S. (2002) AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. *J. Biol. Chem.* **277**, 23977–23980
35. Atherton, P. J., Etheridge, T., Watt, P. W., Wilkinson, D., Selby, A., Rankin, D., Smith, K., and Rennie, M. J. (2010) Muscle full effect after oral protein: time-dependent concordance and discordance between human muscle protein synthesis and mTORC1 signaling. *Am. J. Clin. Nutr.* **92**, 1080–1088
36. Bohé, J., Low, J. F., Wolfe, R. R., and Rennie, M. J. (2001) Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J. Physiol.* **532**, 575–579
37. Bohé, J., Low, A., Wolfe, R. R., and Rennie, M. J. (2003) Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose–response study. *J. Physiol.* **552**, 315–324
38. Floyd, J. C., Jr., Fajans, S. S., Pek, S., Thiffault, C. A., Knopf, R. F., and Conn, J. W. (1970) Synergistic effect of essential amino acids and glucose upon insulin secretion in man. *Diabetes* **19**, 109–115
39. Drummond, M. J., Glynn, E. L., Fry, C. S., Timmerman, K. L., Volpi, E., and Rasmussen, B. B. (2010) An increase in essential amino acid availability upregulates amino acid transporter expression in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **298**, E1011–E1018
40. Dickinson, J. M., and Rasmussen, B. B. (2011) Essential amino acid sensing, signaling, and transport in the regulation of human muscle protein metabolism. *Curr. Opin. Clin. Nutr. Metab. Care* **14**, 83–88
41. Gulati, P., Gaspers, L. D., Dann, S. G., Joaquin, M., Nobukuni, T., Natt, F., Kozma, S. C., Thomas, A. P., and Thomas, G. (2008) Amino acids activate mTOR complex 1 via Ca<sup>2+</sup>/CaM signaling to hVps34. *Cell Metab.* **7**, 456–465
42. Nobukuni, T., Joaquin, M., Rocco, M., Dann, S. G., Kim, S. Y., Gulati, P., Byfield, M. P., Backer, J. M., Natt, F., Bos, J. L., Zwartkruis, F. J., and Thomas, G. (2005) Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. *Proc. Natl. Acad. Sci. USA* **102**, 14238–14243
43. Han, J. M., Jeong, S. J., Park, M. C., Kim, G., Kwon, N. H., Kim, H. K., Ha, S. H., Ryu, S. H., and Kim, S. (2012) Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. *Cell* **149**, 410–424
44. Kim, E., Goraksha-Hicks, P., Li, L., Neufeld, T. P., and Guan, K. L. (2008) Regulation of TORC1 by Rag GTPases in nutrient response. *Nat. Cell Biol.* **10**, 935–945
45. Felig, P., Marliss, E., Owen, O. E., and Cahill, G. F., Jr. (1969) Role of substrate in the regulation of hepatic gluconeogenesis in fasting man. *Adv. Enzyme Regul.* **7**, 41–46
46. Felig, P., Owen, O. E., Wahren, J., and Cahill, G. F., Jr. (1969) Amino acid metabolism during prolonged starvation. *J. Clin. Invest.* **48**, 584–594
47. Rasmussen, B. B., and Phillips, S. M. (2003) Contractile and nutritional regulation of human muscle growth. *Exerc. Sport Sci. Rev.* **31**, 127–131
48. Béchet, D., Tassa, A., Combaret, L., Taillandier, D., and Attaix, D. (2005) Regulation of skeletal muscle proteolysis by amino acids. *J. Ren. Nutr.* **15**, 18–22
49. Sandri, M. (2013) Protein breakdown in muscle wasting: role of autophagy–lysosome and ubiquitin–proteasome. *Int. J. Biochem. Cell Biol.* **45**, 2121–2129

50. Carbone, J. W., McClung, J. P., and Pasiakos, S. M. (2012) Skeletal muscle responses to negative energy balance: effects of dietary protein. *Adv. Nutr.* **3**, 119–126
51. Goll, D. E., Thompson, V. F., Li, H., Wei, W., and Cong, J. (2003) The calpain system. *Physiol. Rev.* **83**, 731–801
52. Du, J., Wang, X., Miereles, C., Bailey, J. L., Debigare, R., Zheng, B., Price, S. R., and Mitch, W. E. (2004) Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. *J. Clin. Invest.* **113**, 115–123
53. Kandarian, S. C., and Jackman, R. W. (2006) Intracellular signaling during skeletal muscle atrophy. *Muscle Nerve* **33**, 155–165
54. Moore, D. R., Robinson, M. J., Fry, J. L., Tang, J. E., Glover, E. I., Wilkinson, S. B., Prior, T., Tarnopolsky, M. A., and Phillips, S. M. (2009) Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am. J. Clin. Nutr.* **89**, 161–168
55. Witard, O. C., Jackman, S. R., Breen, L., Smith, K., Selby, A., and Tipton, K. D. (2014) Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am. J. Clin. Nutr.* **99**, 86–95
56. Cuthbertson, D., Smith, K., Babraj, J., Leese, G., Waddell, T., Atherton, P., Wackerhage, H., Taylor, P. M., and Rennie, M. J. (2005) Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J.* **19**, 422–424
57. Glynn, E. L., Fry, C. S., Drummond, M. J., Timmerman, K. L., Dhanani, S., Volpi, E., and Rasmussen, B. B. (2010) Excess leucine intake enhances muscle anabolic signaling but not net protein anabolism in young men and women. *J. Nutr.* **140**, 1970–1976
58. Xia, X., Wang, X., Li, Q., Li, N., and Li, J. (2013) Essential amino acid enriched high-protein enteral nutrition modulates insulin-like growth factor-I system function in a rat model of trauma-hemorrhagic shock. *PLoS ONE* **8**, e77823
59. Luiking, Y. C., Deutz, N. E., Memelink, R. G., Verlaan, S., and Wolfe, R. R. (2014) Postprandial muscle protein synthesis is higher after a high whey protein, leucine-enriched supplement than after a dairy-like product in healthy older people: a randomized controlled trial. *Nutr. J.* **13**, 9
60. Katsanos, C. S., Kobayashi, H., Sheffield-Moore, M., Aarsland, A., and Wolfe, R. R. (2006) A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am. J. Physiol. Endocrinol. Metab.* **291**, E381–E387
61. Moore, D. R., Churchward-Venne, T. A., Witard, O., Breen, L., Burd, N. A., Tipton, K. D., and Phillips, S. M. (2015) Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *J. Gerontol. A Biol. Sci. Med. Sci.* **70**, 57–62
62. Pasiakos, S. M., McClung, H. L., McClung, J. P., Margolis, L. M., Andersen, N. E., Cloutier, G. J., Pikosky, M. A., Rood, J. C., Fielding, R. A., and Young, A. J. (2011) Leucine-enriched essential amino acid supplementation during moderate steady state exercise enhances postexercise muscle protein synthesis. *Am. J. Clin. Nutr.* **94**, 809–818
63. Rowlands, D. S., Nelson, A. R., Phillips, S. M., Faulkner, J. A., Clarke, J., Burd, N. A., Moore, D., and Stellingwerff, T. (2015) Protein-leucine fed dose effects on muscle protein synthesis after endurance exercise. *Med. Sci. Sports Exerc.* **47**, 547–555
64. Churchward-Venne, T. A., Breen, L., Di Donato, D. M., Hector, A. J., Mitchell, C. J., Moore, D. R., Stellingwerff, T., Breuille, D., Offord, E. A., Baker, S. K., and Phillips, S. M. (2014) Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial. *Am. J. Clin. Nutr.* **99**, 276–286
65. Churchward-Venne, T. A., Burd, N. A., Mitchell, C. J., West, D. W., Philp, A., Marcotte, G. R., Baker, S. K., Baar, K., and Phillips, S. M. (2012) Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *J. Physiol.* **590**, 2751–2765
66. Paddon-Jones, D., and Rasmussen, B. B. (2009) Dietary protein recommendations and the prevention of sarcopenia. *Curr. Opin. Clin. Nutr. Metab. Care* **12**, 86–90
67. Mamerow, M. M., Mettler, J. A., English, K. L., Casperson, S. L., Arentson-Lantz, E., Sheffield-Moore, M., Layman, D. K., and Paddon-Jones, D. (2014) Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults. *J. Nutr.* **144**, 876–880
68. Rodriguez, N. R. (2014) Protein-centric meals for optimal protein utilization: can it be that simple? *J. Nutr.* **144**, 797–798
69. Norton, L. E., Wilson, G. J., Layman, D. K., Moulton, C. J., and Garlick, P. J. (2012) Leucine content of dietary proteins is a determinant of postprandial skeletal muscle protein synthesis in adult rats. *Nutr. Metab. (Lond)* **9**, 67
70. Holm, L., and Kjaer, M. (2010) Measuring protein breakdown rate in individual proteins in vivo. *Curr. Opin. Clin. Nutr. Metab. Care* **13**, 526–531
71. Biolo, G., Declan Fleming, R. Y., and Wolfe, R. R. (1995) Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J. Clin. Invest.* **95**, 811–819
72. Biolo, G., Tipton, K. D., Klein, S., and Wolfe, R. R. (1997) An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am. J. Physiol.* **273**, E122–E129
73. Biolo, G., Williams, B. D., Fleming, R. Y., and Wolfe, R. R. (1999) Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes* **48**, 949–957
74. Tipton, K. D., Borsheim, E., Wolf, S. E., Sanford, A. P., and Wolfe, R. R. (2003) Acute response of net muscle protein balance reflects 24-h balance after exercise and amino acid ingestion. *Am. J. Physiol. Endocrinol. Metab.* **284**, E76–E89
75. Glynn, E. L., Fry, C. S., Drummond, M. J., Dreyer, H. C., Dhanani, S., Volpi, E., and Rasmussen, B. B. (2010) Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **299**, R533–R540
76. Glynn, E. L., Fry, C. S., Timmerman, K. L., Drummond, M. J., Volpi, E., and Rasmussen, B. B. (2013) Addition of carbohydrate or alanine to an essential amino acid mixture does not enhance human skeletal muscle protein anabolism. *J. Nutr.* **143**, 307–314
77. Levenhagen, D. K., Gresham, J. D., Carlson, M. G., Maron, D. J., Borel, M. J., and Flakoll, P. J. (2001) Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. *Am. J. Physiol. Endocrinol. Metab.* **280**, E982–E993
78. Tipton, K. D., Rasmussen, B. B., Miller, S. L., Wolf, S. E., Owens-Stovall, S. K., Petrini, B. E., and Wolfe, R. R. (2001) Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *Am. J. Physiol. Endocrinol. Metab.* **281**, E197–E206
79. Hoffer, L. J., and Forse, R. A. (1990) Protein metabolic effects of a prolonged fast and hypocaloric refeeding. *Am. J. Physiol.* **258**, E832–E840
80. Knapik, J., Meredith, C., Jones, B., Fielding, R., Young, V., and Evans, W. (1991) Leucine metabolism during fasting and exercise. *J. Appl. Physiol.* **70**, 43–47
81. Stein, T. P., Rumlper, W. V., Leskiw, M. J., Schluter, M. D., Staples, R., and Bodwell, C. E. (1991) Effect of reduced dietary intake on energy expenditure, protein turnover, and glucose cycling in man. *Metabolism* **40**, 478–483
82. Yang, R. D., Matthews, D. E., Bier, D. M., Wen, Z. M., and Young, V. R. (1986) Response of alanine metabolism in humans to manipulation of dietary protein and energy intakes. *Am. J. Physiol.* **250**, E39–E46
83. Pasiakos, S. M., Margolis, L. M., McClung, J. P., Cao, J. J., Whigham, L. D., Combs, G. F., and Young, A. J. (2014) Whole-body protein turnover response to short-term high-protein diets during weight loss: a randomized controlled trial. *Int. J. Obes. (Lond.)* **38**, 1015–1018
84. Keys, A. (1946) Experimental studies of starvation on men. *Bull. Chic. Med. Soc.* **49**, 42–46
85. Keys, A. (1946) Human starvation and its consequences. *J. Am. Diet. Assoc.* **22**, 582–587
86. Keys, A. (1946) Experimental human starvation; general and metabolic results of a loss of one fourth the body weight in six months. *Fed. Proc.* **5**, 55

87. Margolis, L. M., Murphy, N. E., Martini, S., Spitz, M. G., Thrane, I., McGraw, S. M., Blatny, J. M., Castellani, J. W., Rood, J. C., Young, A. J., Montain, S. J., Gundersen, Y., and Pasiakos, S. M. (2014) Effects of winter military training on energy balance, whole-body protein balance, muscle damage, soreness, and physical performance. *Appl. Physiol. Nutr. Metab.* **39**, 1395–1401
88. Christensen, P. A., Jacobsen, O., Thorlund, J. B., Madsen, T., Møller, C., Jensen, C., Suetta, C., and Aagaard, P. (2008) Changes in maximum muscle strength and rapid muscle force characteristics after long-term special support and reconnaissance missions: a preliminary report. *Mil. Med.* **173**, 889–894
89. Alemany, J. A., Nindl, B. C., Kellogg, M. D., Tharion, W. J., Young, A. J., and Montain, S. J. (2008) Effects of dietary protein content on IGF-I, testosterone, and body composition during 8 days of severe energy deficit and arduous physical activity. *J. Appl. Physiol.* **105**, 58–64
90. Friedl, K. E., Moore, R. J., Hoyt, R. W., Marchitelli, L. J., Martinez-Lopez, L. E., and Askew, E. W. (2000) Endocrine markers of semistarvation in healthy lean men in a multistressor environment. *J. Appl. Physiol.* **88**, 1820–1830
91. Hoyt, R. W., Opstad, P. K., Haugen, A. H., DeLany, J. P., Cymerman, A., and Friedl, K. E. (2006) Negative energy balance in male and female rangers: effects of 7 d of sustained exercise and food deprivation. *Am. J. Clin. Nutr.* **83**, 1068–1075
92. Margolis, L. M., Rood, J., Champagne, C., Young, A. J., and Castellani, J. W. (2013) Energy balance and body composition during U.S. Army Special Forces training. *Appl. Physiol. Nutr. Metab.* **38**, 396–400
93. Nindl, B. C., Barnes, B. R., Alemany, J. A., Frykman, P. N., Shippee, R. L., and Friedl, K. E. (2007) Physiological consequences of U.S. Army Ranger training. *Med. Sci. Sports Exerc.* **39**, 1380–1387
94. Nindl, B. C., Rarick, K. R., Castellani, J. W., Tuckow, A. P., Patton, J. F., Young, A. J., and Montain, S. J. (2006) Altered secretion of growth hormone and luteinizing hormone after 84 h of sustained physical exertion superimposed on caloric and sleep restriction. *J. Appl. Physiol.* **100**, 120–128
95. Nindl, B. C., Leone, C. D., Tharion, W. J., Johnson, R. F., Castellani, J. W., Patton, J. F., and Montain, S. J. (2002) Physical performance responses during 72 h of military operational stress. *Med. Sci. Sports Exerc.* **34**, 1814–1822
96. Welsh, T. T., Alemany, J. A., Montain, S. J., Frykman, P. N., Tuckow, A. P., Young, A. J., and Nindl, B. C. (2008) Effects of intensified military field training on jumping performance. *Int. J. Sports Med.* **29**, 45–52
97. Thorlund, J. B., Jakobsen, O., Madsen, T., Christensen, P. A., Nedergaard, A., Andersen, J. L., Suetta, C., and Aagaard, P. (2011) Changes in muscle strength and morphology after muscle unloading in Special Forces missions. *Scand. J. Med. Sci. Sports* **21**, e56–e63
98. Varady, K. A., Bhutani, S., Klempel, M. C., Kroeger, C. M., Trepanowski, J. F., Haus, J. M., Hoddy, K. K., and Calvo, Y. (2013) Alternate day fasting for weight loss in normal weight and overweight subjects: a randomized controlled trial. *Nutr. J.* **12**, 146
99. Raben, A., Jensen, N. D., Marckmann, P., Sandström, B., and Astrup, A. (1995) Spontaneous weight loss during 11 weeks' ad libitum intake of a low fat/high fiber diet in young, normal weight subjects. *Int. J. Obes. Relat. Metab. Disord.* **19**, 916–923
100. Ferrando, A. A. (2013) Increased protein intake in military special operations. *J. Nutr.* **143**, 1852S–1856S
101. Bhasin, S., Woodhouse, L., Casaburi, R., Singh, A. B., Bhasin, D., Berman, N., Chen, X., Yarasheski, K. E., Magliano, L., Dzekov, C., Dzekov, J., Bross, R., Phillips, J., Sinha-Hikim, I., Shen, R., and Storer, T. W. (2001) Testosterone dose-response relationships in healthy young men. *Am. J. Physiol. Endocrinol. Metab.* **281**, E1172–E1181
102. Finkelstein, J. S., Lee, H., Burnett-Bowie, S. A., Pallais, J. C., Yu, E. W., Borges, L. F., Jones, B. F., Barry, C. V., Wulczyn, K. E., Thomas, B. J., and Leder, B. Z. (2013) Gonadal steroids and body composition, strength, and sexual function in men. *N. Engl. J. Med.* **369**, 1011–1022
103. Kvorning, T., Andersen, M., Brixen, K., and Madsen, K. (2006) Suppression of endogenous testosterone production attenuates the response to strength training: a randomized, placebo-controlled, and blinded intervention study. *Am. J. Physiol. Endocrinol. Metab.* **291**, E1325–E1332
104. Mauras, N., Hayes, V., Welch, S., Rini, A., Helgeson, K., Dokler, M., Veldhuis, J. D., and Urban, R. J. (1998) Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. *J. Clin. Endocrinol. Metab.* **83**, 1886–1892
105. Opstad, P. K. (1992) The hypothalamo-pituitary regulation of androgen secretion in young men after prolonged physical stress combined with energy and sleep deprivation. *Acta Endocrinol. (Copenh.)* **127**, 231–236
106. Henning, P. C., Scofield, D. E., Spiering, B. A., Staab, J. S., Matheny, R. W., Jr., Smith, M. A., Bhasin, S., and Nindl, B. C. (2014) Recovery of endocrine and inflammatory mediators following an extended energy deficit. *J. Clin. Endocrinol. Metab.* **99**, 956–964
107. Nindl, B. C., Friedl, K. E., Frykman, P. N., Marchitelli, L. J., Shippee, R. L., and Patton, J. F. (1997) Physical performance and metabolic recovery among lean, healthy men following a prolonged energy deficit. *Int. J. Sports Med.* **18**, 317–324
108. Nindl, B. C., Alemany, J. A., Kellogg, M. D., Rood, J., Allison, S. A., Young, A. J., and Montain, S. J. (2007) Utility of circulating IGF-I as a biomarker for assessing body composition changes in men during periods of high physical activity superimposed upon energy and sleep restriction. *J. Appl. Physiol.* **103**, 340–346
109. Gomez-Merino, D., Drogou, C., Chennaoui, M., Tiollier, E., Mathieu, J., and Guezennec, C. Y. (2005) Effects of combined stress during intense training on cellular immunity, hormones and respiratory infections. *Neuroimmunomodulation* **12**, 164–172
110. Murphy, M. G., Plunkett, L. M., Gertz, B. J., He, W., Wittreich, J., Polvino, W. M., and Clemmons, D. R. (1998) MK-677, an orally active growth hormone secretagogue, reverses diet-induced catabolism. *J. Clin. Endocrinol. Metab.* **83**, 320–325
111. Clemmons, D. R., Smith-Banks, A., and Underwood, L. E. (1992) Reversal of diet-induced catabolism by infusion of recombinant insulin-like growth factor-I in humans. *J. Clin. Endocrinol. Metab.* **75**, 234–238
112. Kupfer, S. R., Underwood, L. E., Baxter, R. C., and Clemmons, D. R. (1993) Enhancement of the anabolic effects of growth hormone and insulin-like growth factor I by use of both agents simultaneously. *J. Clin. Invest.* **91**, 391–396
113. Friedl, K. E., Moore, R. J., Martínez-Lopez, L. E., Vogel, J. A., Askew, E. W., Marchitelli, L. J., Hoyt, R. W., and Gordon, C. C. (1994) Lower limit of body fat in healthy active men. *J. Appl. Physiol.* **77**, 933–940
114. Pritchard, J., Després, J. P., Gagnon, J., Tchernof, A., Nadeau, A., Tremblay, A., and Bouchard, C. (1999) Plasma adrenal, gonadal, and conjugated steroids following long-term exercise-induced negative energy balance in identical twins. *Metabolism* **48**, 1120–1127
115. Timmons, J. A. (2011) Variability in training-induced skeletal muscle adaptation. *J. Appl. Physiol.* **110**, 846–853
116. Timmons, J. A., Knudsen, S., Rankinen, T., Koch, L. G., Sarzynski, M., Jensen, T., Keller, P., Scheele, C., Vollaard, N. B., Nielsen, S., Akerstrom, T., MacDougald, O. A., Jansson, E., Greenhaff, P. L., Tarnopolsky, M. A., van Loon, L. J., Pedersen, B. K., Sundberg, C. J., Wahlestedt, C., Britton, S. L., and Bouchard, C. (2010) Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. *J. Appl. Physiol.* **108**, 1487–1496
117. Phillips, B. E., Williams, J. P., Gustafsson, T., Bouchard, C., Rankinen, T., Knudsen, S., Smith, K., Timmons, J. A., and Atherton, P. J. (2013) Molecular networks of human muscle adaptation to exercise and age. *PLoS One* **8**, e1003389
118. Keller, P., Vollaard, N. B., Gustafsson, T., Gallagher, I. J., Sundberg, C. J., Rankinen, T., Britton, S. L., Bouchard, C., Koch, L. G., and Timmons, J. A. (2011) A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. *J. Appl. Physiol.* **110**, 46–59
119. Ghosh, S., Vivar, J. C., Sarzynski, M. A., Sung, Y. J., Timmons, J. A., Bouchard, C., and Rankinen, T. (2013) Integrative pathway analysis of a genome-wide association study of (V)O<sub>2</sub>(max) response to exercise training. *J. Appl. Physiol.* **115**, 1343–1359

Received for publication October 24, 2014.  
Accepted for publication December 1, 2014.