REVIEW

Measuring Protein Turnover in the Field: Implications for Military Research

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ABSTRACT

Protein turnover reflects the continual synthesis and breakdown of body proteins, and can be measured at a whole-body (i.e. aggregated across all body proteins) or tissue (e.g. skeletal muscle only) level using stable isotope methods. Evaluating protein turnover in free-living environments, such as military training, can help inform protein requirements. We undertook a narrative review of published literature with the aim of reviewing the suitability of, and advancements in, stable isotope methods for measuring protein turnover in field research. The 2 primary approaches for measuring protein turnover are based on precursor- and end-product methods. The precursor method is the gold-standard for measuring *acute* (over several hours) skeletal muscle protein turnover, whereas the end-product method measures *chronic* (over several weeks) skeletal muscle protein turnover and provides the opportunity to monitor free-living activities. Both methods require invasive procedures such as the infusion of amino acid tracers and muscle biopsies to assess the uptake of the tracer into tissue. However, the end-product method can also be used to measure acute (over 9–24 h) whole-body protein turnover noninvasively by ingesting ^{15}N-glycine, or equivalent isotope tracers, and collecting urine samples. The end-product method using ^{15}N-glycine is a practical method for measuring whole-body protein turnover in the field over short (24 h) time frames and has been used effectively in recent military field research. Application of this method may improve our understanding of protein kinetics during conditions of high physiological stress in free-living environments such as military training. *Adv Nutr* 2021;12:887–896.

Keywords: protein metabolism, protein status, ^{15}N-glycine, stable isotope tracer, nutrition, military training, physiological stress

Introduction

Protein turnover is the continual, metabolic process regulating tissue mass and function, and reflects the balance between rates of protein synthesis and breakdown. Exercise training and protein feeding are anabolic stimuli for skeletal muscle and result in a net positive muscle protein balance (1). Conversely, a chronic, negative muscle protein balance results in the loss of skeletal muscle mass, and compromised health and physical performance (2, 3). Military field exercises, training courses, and operations are characterized by prolonged periods of intense physical activity (4–6), activities that engage multiple large muscle groups [e.g. load carriage (7)], restricted feeding (4, 8, 9), and restricted sleep (4). Energy (5, 10) and protein (5, 11, 12) balance are difficult to maintain during military training, likely due to these physiological stressors; consequently, military training and field exercises are often accompanied by a loss of lean body mass (4, 11, 13–15), suppressed circulating anabolic hormones (13, 16–18), and impaired physical performance (5, 13–16, 19, 20). The application of appropriate methods for measuring protein turnover during periods of high physiological stress will improve our understanding of the adaptive, or maladaptive, responses to military training, and help inform the development of strategies to preserve lean mass for physical performance and injury protection.

Current Status of Knowledge

Metabolic isotopes have been used in human metabolism research for several decades (21) to "trace" numerous metabolites, including glucose, lipids, and amino acids. Radioactive isotopes were popular in several early studies (22), but due to their adverse health effects (23), stable isotope tracers are now preferred. Stable isotopes are identical in function to target compounds, but can be distinguished with appropriate analytical techniques due to differences in

Funding: None
Author disclosures: None
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Abbreviations used: BM, body mass; d, 15N oral dose; D_2O, deuterium oxide; E, 24-h urinary nitrogen excretion; En, Enrichment corrected for background abundance of 15N; E_x, Excretion of ammonia; e_x, Amount of 15N in ammonia; FSR, fractional synthetic rate; IAAO, indicator amino acid oxidation; Q, whole-body protein flux.

the number of neutrons within the nucleus, and therefore, atomic mass (21). Typically, carbon, nitrogen, oxygen, or hydrogen isotopes are used as tracers, which are heavier in mass and less abundant than their naturally occurring, native counterparts (21). Stable isotopes can be traced in a tissue of interest to understand their metabolism in vivo (21) and are routinely utilized in laboratory studies using precursor-product and end-product methods for whole-body and muscle amino acid kinetics (22, 24). Logistical challenges with performing field research in free-living individuals mean that the choice of stable isotope tracer method is dependent on access to specialist equipment, the time course of measurement, tissue of interest, and the level of invasiveness that is feasible. The aim of this article is to review the methods available for measuring protein turnover, with a focus on the practical application of each method, to enable advancement of their use within military field research.

Precursor-product method

The precursor-product method for measuring protein turnover involves the delivery of stable isotope tracers by a primed, continuous infusion over a number of hours, or by a flooding dose method, to establish rapid equilibrium of the endogenous tracee pool (21). Typically, the amino acids leucine, phenylalanine, lysine, and glycine are used as metabolic tracers, particularly for studying skeletal muscle protein turnover (21, 22). The uptake of amino acid tracers into cells and tissue occurs at the same rate as endogenous amino acids; a net movement away from the arterial pool indicates protein synthesis, and a net appearance in the venous pool indicates protein breakdown (21). The rate of tracer uptake into (skeletal muscle) proteins can be evaluated from the arterio-venous difference or by sampling relevant tissue (e.g. skeletal muscle) using biopsies and analysis with mass spectrometry (21). By measuring the tracer enrichment of the precursor pool and the amount of tracer incorporated into skeletal muscle protein by biopsy, the fractional synthetic rate (FSR) of skeletal muscle proteins can be calculated. The FSR is considered the gold-standard measure of skeletal muscle protein synthesis, since measurement of arterio-venous difference can be affected by amino acid metabolism in other tissues and altered blood flow (21). Measurements of muscle protein synthesis are used to infer changes in lean body mass following an intervention when the time frame of pre-post measurements is too short, or the severity of the metabolic stress is not great enough to detect meaningful changes in body composition [i.e. >1–2% change for gold-standard DXA measurements (25)]. However, although use of the precursor-product method has provided seminal data on the muscle protein synthesis response to feeding and exercise, acute measures of FSR in the early (1–6 h) postexercise period and chronic training adaptations of skeletal muscle do not correlate (26). The ability to translate acute tissue metabolism measured with these techniques to chronic physiological adaptation, appears therefore, to be limited.

End-product method

The disconnect between acute muscle protein metabolic responses and chronic physiological adaptation (26) has generated interest in measuring protein metabolism beyond several hours. Advances in an end-product method for measuring muscle protein synthesis using deuterium oxide (D_2O) has improved our understanding of chronic (several weeks) metabolic responses to protein feeding and exercise (21, 27). D_2O, or, "heavy" water, is consumed as an oral bolus that rapidly (within 2 h) equilibrates with the total body water pool and creates a homogenous, slowly turning over, precursor pool (21). The deuterium from the body water is incorporated into different substrates, and the metabolic flux of these substrate pools can be calculated by measuring the incorporation rate of deuterium into protein. The D_2O method is becoming increasingly popular since it can be used to quantify both acute and longer-term incorporation (weeks and months) (27, 28) of the tracer into substrates and tissues of interest (29–31), and therefore, permits a chronic measurement of muscle protein synthesis. The D_2O method also benefits from reflecting free-living rates of muscle protein synthesis, rather than the laboratory-based precursor method that necessitates participants being largely confined to bed rest. The use of an orally consumed, rather than infused, tracer requires less clinical input and expertise, is less expensive (29, 30), and supports the potential use in field research. However, the requirement for tissue sampling (muscle biopsy) remains challenging in a field research environment.

Whole-body protein turnover

The precursor-product and end-product methods for measuring in vivo amino acid kinetics in specific tissues are robust, valid, and highly informative, but are often impractical for field research due to the requirement for invasive tissue sampling. Moreover, protein breakdown is challenging to measure accurately (32, 33), and protein requirements are often derived from measurements of muscle protein synthesis. Military training involves prolonged periods of whole-body exercise with high-metabolic stress, and the relevance of tissue-specific measurements of protein synthesis in these environments is unclear. Load carriage, for example, is an essential military task that generates physiological and mechanical strains across multiple tissues unlike those seen in typical endurance and resistance-type exercise activities (34). The 3 published studies that have measured protein turnover during military training in the field (**Table 1**) have all measured whole-body protein turnover rather than muscle protein synthesis, likely due to the limitations and challenges of tissue-specific measurements in these environments, the whole-body nature of military activities, and the desire to evaluate protein status rather than simply protein synthesis (5, 6, 35).

The precursor-product method is the gold standard for measuring whole-body protein turnover but involves a continuous tracer infusion and frequent blood and breath

TABLE 1 Studies using stable isotope tracers to measure whole-body protein turnover in military personnel in the field

Study	Participants	Protocol	Measurement of protein turnover	Results and conclusions
Berryman et al. (6)	• 63 male US marines assigned to 1 of 3 groups: control (CON), moderate (MOD), and high (HIGH) protein feeding groups.	• 18 d of survival, evasion, resistance, and escape (SERE) training followed by 27 d of refeeding by supplement.	• End-product method: single dose of ^{15}N-alanine (4 mg·kg^{-1}) after evening meal before 10 to 12 h urine collection during overnight fast.	• ↓ Protein synthesis (8%) and ↑ protein breakdown (19%) post-SERE resulting in ↓ (net negative) protein balance, with no difference between feeding groups. Energy balance was −4203 ± 1686 kcal·d^{-1} during SERE.
		• Daily supplemental provision during the refeeding period (consumed in addition to a self-selected ad libitum diet): CON: 1042 kcal, 7 g protein; MOD: 974 kcal, 84 g protein; HIGH: 1103 kcal, 133 g protein.	• Whole-body protein turnover measured on days 2 (baseline), 18 (post-SERE), and 45 (post-refeed).	• ↑ Protein synthesis (34%) resulting in ↑ (net positive) protein balance following refeeding, with no difference between feeding groups. Energy balance was 977 ± 435 kcal·d^{-1} during refeeding. • ↓ Lean body mass post-SERE, which recovered with refeeding, with no difference between feeding groups.
Margolis et al. (5)	• 21 male Norwegian soldiers.	• 4 d of military task training (MTT) followed by a 3-d ski march [20 km·d^{-1} carrying ∼45 kg (SKI)].	• End-product method: single dose of ^{15}N-glycine (2 mg·kg^{-1}) before 10 h urine collection period during fasted rest. • Whole-body protein turnover measured on days 1 (baseline), 4 (post-MTT), and 7 (post-SKI).	• ↔ Protein synthesis and protein breakdown during MTT compared with baseline. Energy balance was −2382 ± 499 kcal·d^{-1} during MTT. • ↑ Protein synthesis (18%) and protein breakdown (27%) during SKI, compared with baseline. Energy balance was −3461 ± 586 kcal·d^{-1} during SKI. • ↔ Protein balance during MTT but ↓ (net negative) during SKI, compared with baseline.
Margolis et al. (35)	• 71 male and 2 female Norwegian soldiers assigned to 1 of 3 groups: control (CON), protein (PRO), and carbohydrate (CHO) feeding groups.	• 4 d of Arctic military training [51 km ski march carrying 45 kg, (AMT)]. • Daily supplemental provision, provided from 4 snack bars (consumed in addition to rations): PRO: 1062 kcal, 102 g carbohydrate, 85 g protein, 35 g fat; CHO: 1058 kcal, 189 g carbohydrate, 11 g protein, 29 g fat.	• End-product method: single dose of ^{15}N-glycine (4 mg·kg^{-1}) before 24-h urine collection period. • Whole-body protein turnover measured pre and post AMT.	• ↓ Protein synthesis (by 1.62 ± 2.41 g·kg^{-1}.d^{-1}) and protein breakdown (by 1.47 ± 2.49 g·kg^{-1}·d^{-1}) resulting in ↓ (net negative) protein balance during AMT, with no difference between groups. Average energy balance across all groups was −3313 ± 776 kcal·d^{-1}.

samples (12, 36). This infusion protocol is unsuitable for military field studies where participants often operate in remote and hostile environments. The precursor-product method of measuring whole-body protein turnover is typically used in tightly controlled laboratory exercise studies (12, 37, 38), whereas the end-product method is more widely used in field studies (4, 32, 33) (Table 1). Although the noninvasive nature of the end-product method is favorable for field-based research, it is important to note that studies using this method are unable to determine how tissue-specific changes drive changes in whole-body protein turnover. Nonetheless, the end-product method remains an attractive proposition for military field research; offering practical methodology and enabling the metabolic impact of military activity on all body systems to be more completely understood.

End-product methods of measuring whole-body protein turnover include stable isotopes (e.g. ^{15}N-glycine), nitrogen balance, and the indicator amino acid oxidation (IAAO) technique. Nitrogen balance, determined from nitrogen loss in urine and nitrogen intake from diet records, has traditionally been used to measure protein requirements and, given the nitrogen component of protein, is based on the premise that nitrogen gains and losses represent protein gains and losses (39). However, the measurement of nitrogen balance can underestimate true protein requirements (40), lacks sensitivity (41), and simply derives a value for the protein intake needed to preserve a net neutral nitrogen balance. The nitrogen balance method, may therefore, lead to inaccurate guidelines for protein requirements, particularly for active individuals aiming to achieve a net positive protein balance and increase muscle mass (41). The IAAO method is minimally invasive, quicker to administer than the nitrogen balance method (40), and enables calculation of protein requirements in exercising individuals in specific, contextually relevant scenarios (42). However, this approach does not take into account protein breakdown and, therefore, cannot assess protein balance, and requires the collection of regular urine and breath samples, which may prohibit its use in field research. A full evaluation of these methods is beyond the scope of this review, for which the reader is directed to Elango et al. (40). Given the limitations of the nitrogen balance and IAAO methods, the oral administration of ^{15}N-glycine, or equivalent tracer, is a favored method for measuring whole-body protein turnover in field environments (5, 6, 35). The noninvasive nature of an oral tracer and urine collection methodology makes this technique attractive for field and military research.

Advancing understanding of the whole-body protein requirements of military personnel using ^{15}N-glycine

The end-product method using ^{15}N-glycine was first developed in 1949 (43), but was not used widely in research until the late 1960s (44). The principle of this whole-body method of measuring protein turnover is based on the transfer of the nitrogen isotope from amino acids (e.g. ^{15}N-glycine, ^{15}N-alanine) to an end-product of nitrogen metabolism (i.e. nitrogen turnover) measured in urine (urea excretion) (43, 44). It was originally assumed that there was a single metabolic pool of nitrogen in the body and the same precursor pool provided the nitrogen that combined into the end-product and protein (43). The nitrogen turnover rate, however, is slow because of the large urea pool, and the time taken to achieve a plateau in labeled nitrogen in adults is typically 24–48 h, and sometimes $\leq$72 h in individuals with low protein intake (45). Therefore, the use of this method is not suitable for measuring acute changes in protein turnover (46). A second end-product (ammonia) technique was developed (45) to simplify the approach and reduce the measurement time, which estimated the rate of nitrogen turnover from the excretion of ^{15}N-ammonia in urine (46). Waterlow et al. (45) recommended the use of ammonia as an end-product due to its rapid turnover, but this rapid turnover means that the measurement of excreted, labeled ammonia does not produce the same flux estimates as excreted, labeled urea (44, 47). Urea is generated in the liver and its flux is more dependent on the viscera (internal organs), whereas ammonia flux is more dependent on peripheral tissues where ammonia is metabolized from glutamine, produced in muscle (44). It is now acknowledged that 2 separate nitrogen pools exist (44) and the end-product method, therefore, reflects the rate of whole-body nitrogen turnover as predicted by the sum of these 2 individual fluxes. The relationship between ammonia and urea flux appears to be inverse, implying that the best estimate of the "real" flux is probably the arithmetic average of the 2 measures (44, 46), and studies should aim to measure both urea and ammonia end-products, where possible.

Choice of tracer

Estimates of absolute protein turnover rates differ between ^{15}N-labeled tracers. Fern et al. (48) measured the rate of whole-body protein synthesis in a human case-study with nine different ^{15}N-labeled amino acids and protein mixtures as tracers (aspartic acid, glutamic acid, glutamine, lysine, glycine, leucine, alanine, uniformly labeled wheat and uniformly labeled yeast). Measurements of ammonia and urea as end-products rarely produced similar estimated fluxes, and only glycine and alanine produced fluxes of the 2 end-products within 10% of each other when oral doses were administered. Protein synthesis rates obtained from [L-^{13}C] leucine via the precursor method differed considerably between the tracers. Lysine resulted in protein synthesis rates 4–10 times higher, glutamine and alanine resulted in protein synthesis rates 2–4 times lower, and glutamate, glycine, leucine, wheat, and yeast produced protein synthesis rates within the mean range determined from [L-^{13}C] leucine (2.8–6.3 g·kg^{-1}·d^{-1}). Glycine demonstrated the strongest agreement between oral and intravenous delivery methods, and the smallest difference between ammonia and urea end-product values, and therefore, was deemed the most suitable tracer (48). Glycine may be more representative of liver metabolism than alanine, however, alanine is considered more representative of the entire body nitrogen pool (49).

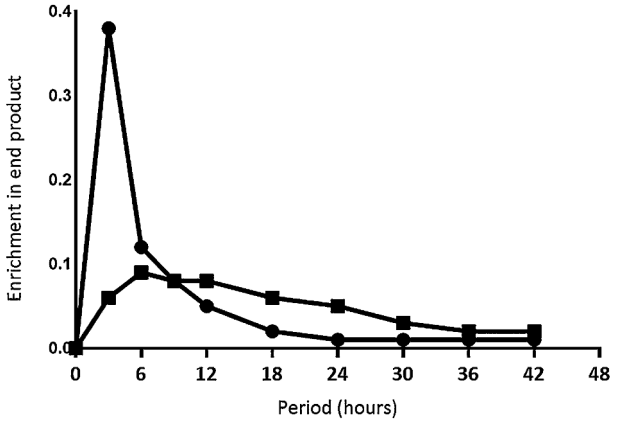

FIGURE 1 Mean ± SE pattern of urinary enrichment in ammonia (circles) and urea (squares) over 48 h following a single oral dose of 200 mg ^{15}N-glycine. Reproduced from Grove & Jackson (55), with permission.

The differences between flux and synthesis rates for the different amino acids are determined by: *1*) the metabolic proximity of the amino acid to the synthesis of urea and ammonia and, therefore, how much amino acid will be taken up for protein synthesis (44, 48), and *2*) how well the nitrogen metabolism from each amino acid represents nitrogen kinetics from the metabolic pool (48). The most frequently used tracer for measuring whole body protein turnover is ^{15}N-glycine (5, 35, 50–53), in fasted or fed states (46, 48, 54), and this tracer offers a practical, noninvasive method of dosing.

Dose

The end-product method typically involves administering a single dose of the stable isotope, either orally or intravenously (55). Given that the oral and intravenous delivery of ^{15}N-glycine give similar estimates of whole-body protein turnover (46, 48, 54), oral tracer administration offers a noninvasive, practical solution to dosing that is favorable for field research. Previous studies conducted during military field exercises have provided doses of ^{15}N-glycine relative to body mass (either 2 mg·kg^{-1} or 4 mg·kg^{-1}) (5, 6, 35). However, other studies have administered absolute doses of ∼200–300 mg ^{15}N-glycine (51, 52); administration of an absolute dose can be practically advantageous when studying large groups of people, or when operating in the field.

Timing

The time period that protein turnover can be measured over is dependent on the kinetics of the labeled nitrogen and, therefore, the selection of the end-product. These considerations will also determine the required urine sampling period. Most nitrogen-labeled ammonia is excreted 9–12 h after dosing, but the excretion of nitrogen-labeled urea is much slower and, therefore, requires a longer urine sampling period (≤24 h after dosing) (**Figure 1**). When using both nitrogen-labeled ammonia and urea as end-products, the sampling period can be restricted to 9–12 h (after dosing) if a blood sample is taken at the end of the urine collection protocol to determine the amount of nitrogen-labeled urea retained in the body pool (44). In this instance, nitrogen flux is based on the amount of excreted nitrogen-labeled ammonia in urine over 9–12 h, and the amount of nitrogen-labeled urea in a blood sample at the same time point. The loss of labeled nitrogen in urea can also be quantified from urine if a blood sample cannot be obtained, which is favorable for use in field research. The urine sampling period must, however, be of sufficient duration to ensure that the isotope is cleared from the body pool, with urine sampling over 9–24 h after dosing suitable to measure the amount of nitrogen-labeled urea (Figure 1) (55). Accordingly, most studies administering oral ^{15}N-glycine, and analyzing urea and ammonia as end-products, have adopted a 24-h urine collection to ensure clearance of both nitrogen-labeled urea and ammonia (32, 45, 48, 52).

Ruddick-Collins et al. (53) recently demonstrated that when measuring excreted ammonia only, the urine collection time can be reduced from 24 h to 9 or 12 h, and this approach provides estimates of whole-body protein turnover similar to the precursor method (56). Ruddick-Collins et al. (53) also investigated whether urine collection over 9 or 12 h provided a more valid measure of whole-body protein turnover in both men and women. Estimates of whole-body flux, protein synthesis, and protein balance were greater (11, 15, and 42%, respectively), and protein breakdown was lower (46%), over 9 compared with 12 h of urine sampling. The 9 h sampling time frame likely resulted in an overestimation of whole-body protein turnover (55), probably due to inadequate time for the turnover of the ammonia free-amino acid pool (53). Nine hours appears the minimum time for most ammonia to be cleared, although some is still excreted after 24 h (Figure 1). Although some studies have measured only 1 end-product (53), most studies calculate total ^{15}N-nitrogen enrichment over 24 h by averaging enrichment in the 2 end-products, urinary urea and ammonia (5, 35, 44, 48, 57). A 24 h urine collection period also allows estimates of nitrogen balance to be obtained (57). Nevertheless, whole-body protein turnover can be satisfactorily calculated using ^{15}N-glycine over a 12 h data collection period when only measuring urinary ammonia (53, 55).

During exercise, ammonia is lost in sweat (58) and produced in skeletal muscle (44). Exercise increases ammonia turnover, which can affect estimates of whole-body protein turnover (independent of actual changes to protein turnover) when only measuring ammonia as an end-product. Measuring, and averaging, nitrogen enrichment in urea and ammonia over 24 h attenuates the effect of exercise on estimates of whole-body protein turnover as a result of altered ammonia turnover. However, if only ammonia is being measured as an end-product over a 12 h period, the effect of the altered ammonia turnover on estimates of whole-body protein turnover is likely greater and restricting or controlling exercise should be considered.

Measurement of whole-body protein turnover in response to exercise and feeding

Whole-body protein turnover has been measured in free-living individuals during physiological stress, including Arctic exploration (59), spaceflight (60), altitude (61), energy and dietary manipulation (39, 52, 62), inactivity (49), acute exercise (37, 38, 58, 63–66), and exercise training (51, 67). During postabsorptive aerobic exercise (including prolonged, submaximal treadmill running or walking), whole-body protein synthesis is reduced (39, 55, 68) or unchanged (38, 64, 66), and protein breakdown is unchanged (38) or increased (65,69), resulting in a net negative whole-body protein balance. In contrast, during the post-exercise period, a net positive whole-body protein balance is typically observed due to a greater increase in whole-body protein synthesis compared with protein breakdown (65, 70). Dietary protein intake improved whole-body protein balance [measured using ^{15}N-glycine (2 mg·kg^{-1}) over 24 h] during a period of increased aerobic training volume in a dose-dependent manner, but only high protein intake (1.83 g·kg^{-1}·d^{-1}) maintained a net positive whole-body protein balance compared with low (0.94 g·kg^{-1}·d^{-1}) and moderate (1.20 g·kg^{-1}·d^{-1}) intakes (71). Both acute bouts of resistance exercise (72) and resistance training (73) improve skeletal muscle net protein balance, but very few studies have evaluated whole-body protein balance. West et al. (63), reported no effects of an acute resistance exercise bout or 25 g whey protein supplementation on whole-body protein turnover [measured using ^{15}N-glycine (2 mg·kg^{-1}) over 24 h] during an overnight fast. However, 24 h protein balance was more positive following resistance exercise and whey protein supplementation compared with rest (63). In another study, 12 weeks of resistance training with relatively constant protein intake (1.2 g·kg^{-1}·d^{-1}) reduced both protein synthesis and protein breakdown (51). Using the end-product method, an absolute dose (300 mg) of ^{15}N-glycine and 24 h urine collection, post-absorptive net protein balance was positive both pre- and post-training, but more positive post-training (51). These data indicate the importance of exercise and protein for promoting both skeletal muscle and whole-body positive protein balance, but direct translation to military-type exercise may be limited due to the unique demands of military activities (7).

Load carriage is an essential military task requiring multiple muscle groups, but the effect of load carriage on whole-body protein metabolism is not well studied. Load carriage generates different physiological and mechanical strains than typical endurance and resistance-type activities (7), and therefore, the effects of military-specific activity on protein turnover cannot be easily extrapolated from other exercise studies. To our knowledge, only 1 published study has investigated the effect of load carriage on whole-body protein turnover (measured using a constant infusion of L-[1–^{13}C]-leucine over 7 h) in a sample of men and women; whole-body protein turnover during recovery from exercise was not different between a single 90 min bout of load carriage exercise carrying 30% of body mass and an absolute $\dot{V}O_2$ matched bout of cycling (12). However, mixed muscle and myofibrillar, protein synthesis in the vastus lateralis were higher (31% and 56%, respectively) during load carriage exercise compared with cycling, with mixed muscle protein synthesis also remaining higher during the 3 h recovery period following load carriage exercise (12). Differences between muscle and whole-body protein turnover rates reflect the contribution of non-muscle tissues, including the gut or splanchnic region, and may indicate metabolic stress, altered gut microbiota activity, or energy imbalance (74, 75). Despite skeletal muscle accounting for ∼70% of body mass, the contribution of skeletal muscle protein turnover to whole-body protein turnover is relatively small (<30%) (76). During a 14 d period of bed rest to simulate spaceflight, the loss of lean body mass due to decreased muscle protein synthesis was captured at both the skeletal muscle and whole-body level (49), but whole-body measurements are unsuitable for inferring changes solely in muscle protein turnover in all scenarios. Given the findings of Pasiakos et al. (12), during military activities measurement of both whole-body protein turnover and body composition may be warranted to evaluate whole-body protein metabolism and changes in lean body mass (skeletal muscle protein). The impact of prolonged load carriage activities, which are more representative of military exercises and deployment, on whole-body protein turnover, and whether rates of whole-body protein turnover differ between women, who experience a greater physiological burden during load carriage (77), and men, remains unknown. With all roles now open to women in several militaries, understanding the protein requirements of military men and women is an important area of future research.

To the authors knowledge, only 3 studies have used the end-product method to measure whole-body protein turnover during military field exercises, 2 of which utilized ^{15}N-glycine (Table 1). Margolis et al. (5) investigated the effect of 7 d Arctic training (4 d of military task training consisting of weapons familiarization, mountainous terrain navigation, and general winter survival training and 3 d of loaded ski marching) on protein turnover in a group of male Norwegian soldiers. Whole-body protein turnover was measured via ingestion of ^{15}N-glycine (2 mg·kg^{-1}) and urine collected over a 10 h period. Despite dietary protein intake being ∼200% of the recommended daily allowance (∼1.7 g·kg^{-1}·d^{-1}), whole-body protein breakdown (+27%) was elevated to a greater extent than whole-body protein synthesis (+18%), resulting in a net negative whole-body protein balance. The same group of researchers (35) conducted a similar study assessing the effects of a 4 d loaded (45 kg) 51 km ski march in 73 Norwegian soldiers on whole-body protein turnover (4 mg·kg^{-1} of ^{15}N-glycine, with 24 h urine collection). The ski march also led to reductions in whole-body protein balance, but whole-body protein synthesis and protein breakdown were instead both reduced following 4 d of training. The reduction in protein synthesis (−1.62 ± 2.41 g·kg^{-1}·d^{-1}) was greater than protein breakdown (−1.47 ± 2.49 g·kg^{-1}·d^{-1}), resulting in an overall

TABLE 2 Summary of key information, advantages and disadvantages of methods to measure whole-body protein turnover

	Precursor method	End-product method
Key information	• Predominately uses leucine or phenylalanine as tracers, given as an infusion (44). • Requires frequent blood and breath samples. • Takes several hours to reach enrichment steady-state, which can be reduced by administering a priming dose. • Calculations involve both plasma α-ketoisocaproate and breath $^{13}CO_2$ values (78).	• ^{15}N-glycine (or equivalent tracer) given as an oral or intravenous dose. • Tracer can be administered as a single dose (5). • Urine measured for 24 h and ammonia and urea measured. Sampling period can be reduced to 9 or 12 h and a blood sample taken at 9 h if measuring both ammonia and urea (recommended to minimize bias) (44). Measuring ammonia flux only via a 12-h urine collection is acceptable (53). • Quantifying nitrogen intake and excretion carefully is vital for model accuracy and interpretation.
Advantages	• Current gold-standard. • No time-consuming urine samples. • Ideal for small research projects. • Muscle protein turnover can also be measured with the addition of muscle biopsies.	• Noninvasive as tracer can be administered orally and end-products measured in urine (5, 35). • Participants can be free-living, and the technique can measure protein status during high-stress states, e.g. military activity, bed rest/immobilization. • Protein synthesis and breakdown values are comparable to those obtained by precursor methods. • No requirement for expensive, and tightly regulated, isotope compounding and infusion. • Glycine is cheap and easy to manufacture (44). • Glycine readily transaminates with most amino acids (68). • Test retest variability of <5% (55).
Disadvantages	• Expensive and complex equipment needed. • Requires clinical setting, limiting participant activity. • Requires continuous breath sampling. • Invasive as uses blood samples and intravenous infusions. • Measurements are acute.	• Assumes a simplified amino acid pool, which may not represent the complex amino acid dynamics, and steady-state conditions (68). • Time-consuming, and logistically challenging, as all urine must be collected over prolonged periods (12 to 24 h). • Inability to measure tissue-specific changes in protein metabolism. • Measurements are acute.

net negative protein balance. Finally, Berryman et al. (6) assessed whole-body protein turnover using ingestion of ^{15}N-alanine (4 mg·kg^{-1}), with 10–12 h urine collection in 63 male US marines pre and post 18 days of survival, evasion, resistance, and escape training. Whole-body protein balance was reduced following training as a result of increased whole-body protein breakdown (+19.3%) and reduced protein synthesis (−8.2%). Results from these studies suggest that extreme exercise and severe energy deficit results in a negative whole-body protein balance, with amino acids likely either oxidized or mobilized as substrate for gluconeogenesis (6, 35). We propose that the end-product method of measuring whole-body protein turnover can be used to advance our understanding of the metabolic demands of military training and operational stress; evaluating the protein status of free-living, military personnel to inform improved feeding practices.

Logistical comparison of precursor-product and end-product methods for measuring whole-body protein turnover, and practical recommendations

There are advantages and disadvantages to both precursor-product and end-product methods of measuring whole-body protein turnover (51) (**Table 2**). The end-product method offers a practical method for measuring acute whole-body protein turnover (over 9–24 h) in field studies of free-living individuals, typical of those in military populations, where the procedures must be minimally invasive. Ideally, a single oral dose of ^{15}N-glycine (both relative and absolute dosing strategies are feasible) is administered, and urine is collected for 24 h. Total nitrogen enrichment of urinary ammonia and urea should be measured to minimize bias of enrichment portioning between the 2 metabolic pools (35). Energy intake must also be monitored during 24 h urine collections, and participants should follow their normal activities. Whole-body protein flux (Q), protein synthesis, protein breakdown, and protein balance can be calculated as:

[1] $\mathbf{Q\ (gN{\cdot}kg^{-1}{\cdot}d^{-1})}$ = d/(corrected tr:T)/24 × body mass (BM)

[2] **Whole-body protein synthesis** $\mathbf{(g{\cdot}kg^{-1}{\cdot}d^{-1})}$ = Q-(E/24× BM) × 6.25

[3] **Whole-body protein breakdown** $\mathbf{(g{\cdot}kg^{-1}{\cdot}d^{-1})}$ = Q-(I/24× BM) × 6.25

[4] **Whole-body protein balance** $\mathbf{(g{\cdot}kg^{-1}{\cdot}d^{-1})}$ = whole-body protein synthesis–whole-body protein breakdown

Where: Q = whole-body protein flux, d = ^{15}N oral dose (g glycine × 0.1972), tr:T = ratio of tracer to tracee (corrected for background isotope enrichment), E = 24 h urinary nitrogen excretion, I = 24 h nitrogen intake, BM = body mass, 6.25 = conversion factor for nitrogen to protein (5, 35, 51).

If the data collection period is limited, or a 24 h collection period is logistically difficult, ammonia alone should be used as the end-product. In this case, total urine should be collected for 12 h following isotope tracer ingestion. Energy intake must be recorded and physical activity should be controlled to minimize the effect of exercise on ammonia production. In this instance, whole-body protein flux, protein synthesis, protein breakdown, and protein balance can be calculated as:

[1] **Q (g·h^{-1}) =** d × E_x/e_x so Q = d/En
[2] **Whole-body protein synthesis (g·h^{-1}) =** Q-E
[3] **Whole-body protein breakdown (g·h^{-1}) =** Q-I
[4] **Whole-body protein balance (g·h^{-1}) =** whole-body protein synthesis–whole-body protein breakdown

Where: *Q = whole-body protein flux,* d = *^{15}N oral dose, E_x = excretion of ammonia, e_x = amount of ^{15}N in ammonia, En = enrichment corrected for background abundance of ^{15}N, E = total urinary nitrogen excretion, I = total protein intake on test day* (45, 52).

Conclusion

Military training involves exposure to a high degree of metabolic stress and, therefore, adequate methods for measuring protein turnover are required. Protein turnover can be measured with precursor-product and end-product methods at the tissue and whole-body level. The logistical constraints of field research preclude the use of some of these techniques in free-living individuals, such as those in military training. Whole-body protein turnover methods cannot determine tissue-specific changes in protein turnover; however, military training includes a wide range of exercises that utilize upper body, lower body, and core muscle groups. Measures of whole-body protein turnover may, therefore, be more appropriate for evaluating protein metabolism during military activity, with the end-product method of measuring whole-body protein turnover considered both practical and appropriate for military field research. Future studies conducted in a military environment, and other field research, should consider using this noninvasive method to better understand protein kinetics in free-living environments.

Acknowledgments

We are grateful to Oliver Witard for his insightful review of the manuscript prior to submission. The authors' contributions were as follows—KLH: performed the literature searches and drafted the manuscript; KLH, TJO'L, JPG, and SLW: were involved in the design, review, and editing of the manuscript, and have read and approved the final manuscript.

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