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REVIEW

# Protein: A nutrient in focus[1]

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**Abstract:** Protein is an essential component of a healthy diet and is a focus of research programs seeking to optimize health at all stages of life. The focus on protein as a nutrient often centers on its thermogenic and satiating effect, and when included as part of a healthy diet, its potential to preserve lean body mass. A growing body of literature, including stable isotope based studies and longer term dietary interventions, suggests that current dietary protein recommendations may not be sufficient to promote optimal muscle health in all populations. A protein intake moderately higher than current recommendations has been widely endorsed by many experts and working groups and may provide health benefits for aging populations. Further, consuming moderate amounts of high-quality protein at each meal may optimally stimulate 24-h muscle protein synthesis and may provide a dietary platform that favors the maintenance of muscle mass and function while promoting successful weight management in overweight and obese individuals. Dietary protein has the potential to serve as a key nutrient for many health outcomes and benefits might be increased when combined with adequate physical activity. Future studies should focus on confirming these health benefits from dietary protein with long-term randomized controlled studies.

*Key words:* protein, requirement, IAAO, aging, sarcopenia, intake distribution, satiety, weight management.

**Résumé :** Les protéines, des composantes essentielles d'un régime sain, sont au centre de programmes de recherche pour optimiser la santé à tous les stades de la vie. On met l'accent concernant les protéines en tant que nutriment surtout sur l'effet thermogène et rassasiant; intégrés dans un régime sain, les protéines pourraient préserver la masse corporelle maigre. De plus en plus d'études dont celles sur les isotopes stables et les interventions alimentaires à long terme suggèrent que les recommandations alimentaires actuelles en matière de protéines ne conviendraient pas à la promotion d'une santé musculaire optimale dans toutes les strates de la population. Un apport protéique modérément supérieur aux recommandations actuelles et soutenu par plusieurs experts et groupes de travail pourrait procurer des bienfaits sanitaires aux populations vieillissantes. De plus, la consommation de quantités modérées de protéines de haute qualité à chaque repas pourrait stimuler durant 24 h la synthèse des protéines musculaires et pourrait constituer une plateforme alimentaire favorisant le maintien de la masse et des fonctions musculaires tout en permettant une gestion du poids avec succès chez les personnes en surpoids et obèses. Les protéines alimentaires ont le potentiel de nutriments essentiels pour de nombreuses problématiques de santé et leurs bénéfices seraient accrus lorsque combinées à suffisamment d'activité physique. Les prochaines études devraient se concentrer sur la validation des bénéfices sanitaires des protéines alimentaires au moyen d'études à long terme contrôlées et aléatoires. [Traduit par la Rédaction]

*Mots-clés :* protéines, recommandations, OAAI, vieillissement, sarcopénie, distribution de l'apport, satiété, gestion du poids.

## Introduction

Protein is an essential component of a healthy diet and continues to be a focus of research programs that are seeking to optimize dietary protein consumption for different stages of life and health. The annual Symposium on Nutrition and Health hosted by the Dairy Farmers of Canada selects "hot" topics related to food, nutrition, and health issues as an opportunity for nutrition professionals to keep current with ongoing research. The 2013 symposium highlighted emerging topics in protein research and human health. The goal of this review is to summarize the key themes discussed during the symposium and provide science-based, translatable information that could be used by researchers and nutrition professionals alike to ensure that dietary protein is consumed in a way that optimizes health outcomes. Key themes included in this review are (*i*) novel methodologies for determining protein needs, particularly during growth and aging; (*ii*) the role of protein in healthy aging and the importance of protein intake distribution throughout the day; and (*iii*) the impact of protein on satiety and weight management.

## Protein requirements

### *Definition of protein needs*

Protein requirement is defined as the amount of protein that is necessary, as part of a nutritionally adequate diet, to achieve growth (neonates, children, pregnant women) and maintenance

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(adults and elderly). Protein intake recommendations are provided as the Estimated Average Requirement (EAR), and Recommended Dietary Allowance (RDA) (Dietary Reference Intakes (DRI) 2005). The EAR is the average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and sex group. The RDA is an estimate of the minimum daily average dietary intake level that meets the nutrient requirements of nearly all (97% to 98%) healthy individuals in a particular life stage (DRI 2005).

Application of the protein intake recommendations must be integrated with our knowledge of the capacity of dietary protein to meet protein synthetic requirements (and other amino acid requiring processes) of humans (Young and Borgonha 2000). Dietary protein contains most, if not all, of the 20 amino acids used to synthesize proteins in the human body (Reeds 2000). Amino acids are classified as indispensable (essential), conditionally indispensable, or dispensable (nonessential), based on the ability of the body to synthesize the amino acid from other carbon sources. Dietary protein sources are quite varied in their amino acid content; for example, lysine is limiting in cereal proteins, and methionine is limiting in legume proteins (Elango et al. 2009*a*). Thus protein quality, which is defined as the capacity of dietary protein sources to satisfy the metabolic needs for protein and essential amino acids, is also important when considering protein requirements (Food and Agricultural Organization (FAO) 2013).

## Traditional method to determine protein requirements

The nitrogen balance technique has long been regarded as the gold standard to determine nitrogen (protein) requirements (DRI 2005; Scrimshaw 1996; Waterlow 1999). However, this technique tends to underestimate protein needs because it overestimates nitrogen intake and underestimates nitrogen excretion (DRI 2005). Furthermore, 5 to 7 days of adaptation to test intakes are required for the equilibration of the large body urea pool (Rand et al. 1976), making the method cumbersome and time consuming, and therefore not suitable to study vulnerable populations. Limitations in data analysis and interpretation arise primarily because of the fact that the efficiency of protein utilization decreases near zero balance (Young et al. 1973) and as nitrogen intake increases, the nitrogen response curve is nonlinear. Most balance studies had test intakes at or near zero balance, and thus the intercept usually determined by linear interpolation leads to an underestimation of the true balance (Rand et al. 2003).

## Re-analysis of current recommendations

The current recommendations for protein requirements in adult humans are set at an EAR and RDA of 0.66 and 0.8 g/(kg·day)$^{-1}$, respectively, by both the DRI (2005) and FAO (2007) (Table 1). These recommendations are based on a detailed meta-analysis of nitrogen balance studies by Rand et al. (2003). The selected studies for the final analysis had test intakes around the expected requirements, and protein requirements were estimated by fitting a linear regression analysis model to the data with zero nitrogen balance as the criterion of nutritional adequacy. However, as explained above, the physiological response relationship between nitrogen intake and balance is not linear because of a decreased efficiency of protein utilization as zero balance approaches (Young et al. 1973). Humayun et al. (2007) performed a reanalysis of 28 nitrogen studies, including the 19 seminal studies used by Rand et al (2003) to set the RDA and EAR, and included studies that tested higher test protein intakes. Using a 2-phase linear regression analysis model resulted in the estimation of a breakpoint (EAR) of 0.91 g protein/(kg·day)$^{-1}$ and an upper 95% confidence interval (population-safe, equivalent to RDA) of 0.99 g/(kg·day)$^{-1}$. These values are significantly higher than the current EAR and RDA of 0.66 and 0.8 g/(kg·day)$^{-1}$ (DRI 2005).

The above, which described re-analysis of balance data, highlighted the need to develop and validate alternative methods to determine protein requirements. Indeed, the most recent FAO/World Health Organization (WHO)/United Nations University (UNU) (FAO 2007) report clearly identified that "…it (nitrogen balance) is an insensitive tool to use in defining protein requirements in adults and children, since it is derived by subtractingone large number (excretion) from another (intake)." The report further recommended that "Research should be undertaken to meet the urgent need for better techniques for assessing body protein homeostasis and balance that are sensitive enough to detect small changes that might be of significance for health."

**Table 1.** Comparison of protein recommendations vs. requirements determined using the indicator amino acid oxidation method.*

| | DRI (2005) | IAAO† | |
|---|---|---|---|
| | (g/(kg·d)$^{-1}$ | (g/(kg·d)$^{-1}$ | % Energy |
| **Adults (*n* = 8)** | | | |
| EAR† | 0.66 | 0.93 | ~10% |
| RDA† | 0.80 | 1.2 | ~13% |
| **Children (6–10 y) (*n* = 7)** | | | |
| EAR | 0.76 | 1.3 | ~9% |
| RDA | 0.95 | 1.55 | ~10% |
| **Pregnant women (~16 wk gestation) (*n* = 17)** | | | |
| EAR | 0.88 | 1.22 | ~13% |
| RDA | 1.1 | 1.66 | ~18% |
| **Pregnant women (~36 wk gestation) (*n* = 19)** | | | |
| EAR | 0.88 | 1.52 | ~17% |
| RDA | 1.1 | 1.77 | ~20% |
| **Elderly women (>65 y) (*n* = 12)** | | | |
| EAR | 0.66 | 0.96 | ~13% |
| RDA | 0.80 | 1.29 | ~15% |
| **Elderly women (>80 y) (*n* = 6)** | | | |
| EAR | 0.66 | 0.85–0.97 | ~10–11.5% |
| RDA | 0.80 | 1.15–1.29 | ~13–15% |

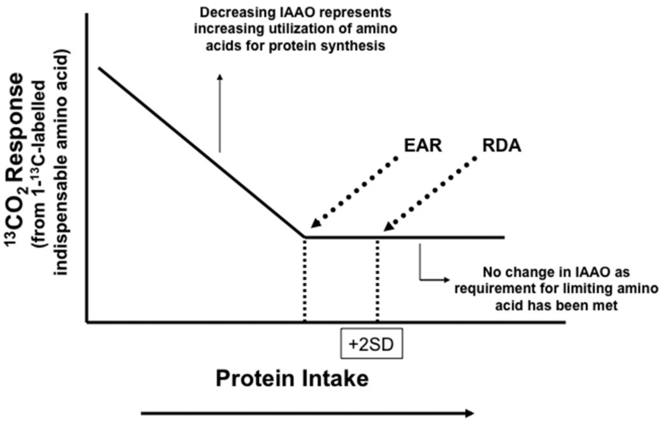
**Note:** DRI, dietary reference intakes; EAR, estimated average requirement; IAAO, indicator amino acid oxidation; RDA, recommended dietary allowance.

*Data from Humayun et al. (2007), Elango et al. (2011), Tang et al. (2014), and Stephens et al. (2015).

## Recent developments to define protein requirements:

Indicator amino acid oxidation (IAAO) is a minimally invasive technique that uses stable isotopes to determine amino acid requirements (Pencharz and Ball 2003), making it an attractive alternative for better assessing protein and essential amino acid needs in different populations (Elango et al. 2008). The method is based on the physiological principle that excess amino acids cannot be stored and therefore must be partitioned between incorporation into protein or oxidation. Therefore, when 1 indispensable amino acid is deficient for protein synthesis, then all other amino acids, including the indicator amino acid (another indispensable amino acid, usually L-1-$^{13}$C-phenylalanine), are in excess and therefore will be oxidized (Elango et al. 2008) (Fig. 1). With increasing intake of the limiting amino acid, oxidation of the indicator amino acid will decrease, reflecting increasing incorporation into protein (Fig. 1). Once the requirement is met for the limiting amino acid/protein, there will be no further change in the oxidation of the indicator amino acid with increasing intake of the test amino acid. The inflection point where the oxidation of the indicator amino acid stops decreasing and reaches a plateau is referred to as the "breakpoint" (Fig. 1). The breakpoint, identified with the use of 2-phase linear regression analysis, indicates the EAR of the limiting (test) amino acid/protein (Zello et al. 1995, Elango et al. 2012). Because IAAO requires only oral isotope administration (Kriengsinyos et al. 2002), collection of breath and urine samples (Bross et al. 1998) and a single study day adaptation (Elango et al. 2009*b*) it is

**Fig. 1.** Concept of the indicator amino acid oxidation (IAAO) method to determine protein requirements. With increasing intake of test protein, oxidation of the indicator amino acid (1-$^{13}$C-labeled indispensable amino acid) will decrease, reflecting increasing utilization of amino acids for protein synthesis. Once the requirement is met for the limiting amino acid and nitrogen (protein), there will be no further change in the oxidation of the indicator amino acid. The inflection point where the oxidation of the indicator amino acid stops decreasing and reaches a plateau indicates the Estimated Average Requirement (EAR) of protein; with the Recommended Dietary Allowance (RDA) being 2 standard deviations above. Source: Elango et al. (2012). IAAO, indicator amino acid oxidation.




well suited for studying protein requirements across the life cycle and in at-risk populations.

## IAAO studies to determine protein requirements

Since the first application of IAAO studies in humans to determine protein requirements in young men by Humayun et al. (2007), several populations (school-age children, pregnant women, elderly women >65 years, and female octogenarians) have been studied using this novel technique (Elango et al. 2011; Tang et al. 2014; Rafii et al. 2015; Stephens et al. 2015). In each of the studied populations the mean protein requirements determined by IAAO method exceed the requirements determined by traditional nitrogen balance studies by ~30%–40% (Table 1). It should be acknowledged that the application of the IAAO method to determine protein requirements has been criticized by some (Hoffer 2012; Millward and Jackson 2012). The reader is encouraged to read the letters and the responses to these letters, as they form an important part of the debate in reassessing the current protein intake recommendations (Hoffer 2012, author reply; Millward and Jackson 2012, author reply). One key discussion is whether the higher requirement estimates derived using the short-term IAAO method are relevant/significant to long-term health benefits (Fukagawa 2014). In a practical application, the discrepancy between the mean protein requirements determined by nitrogen balance and the IAAO method may have real consequences for vulnerable populations, such as older adults at risk for loss of lean mass. Epidemiological evidence indicates that protein intakes greater than the current RDA are beneficial for older adults (Houston et al. 2008; Bauer et al. 2013). In the longitudinal Health ABC study (Houston et al. 2008), it was shown that older subjects in the highest quintile of protein intake (~19% of energy) lost less lean body mass compared with the subjects in the lowest quintile (~11% of energy). In the recent PROT-AGE study group analysis (Bauer et al. 2013), 1.0–1.2 g/(kg·day)$^{-1}$ of protein intake was suggested as the least average daily intake to maintain and regain lean body mass and function. Together these data highlight the importance of the need for the continued implementation of methods that more adequately assess the protein needs of various populations.

## Protein and healthy aging

### *Effect of aging on building muscle in response to protein*

Skeletal muscle mass and functional capacity is regulated by the dynamic interaction of several factors, including diet and nutrition, physical activity, health status, and age. An undesirable yet common consequence of aging is the slow, progressive loss of muscle mass and physical function – sarcopenia. While the onset and progression of sarcopenia can be influenced by many factors, a compromised ability to mount a robust or youthful anabolic response following dietary protein ingestion (i.e., anabolic resistance) (Paddon-Jones and Rasmussen 2009) has become a key target for researchers seeking to identify deficits or mechanisms that may respond to behaviour/lifestyle modification. To this end, a central theme discussed during the symposium centered on establishing a dietary framework that includes consumption of a moderate amount (i.e., approximately 25–35 g) of high-quality protein at breakfast, lunch, and dinner.

Adults over 65 years of age account for 40% of all hospital admissions and are clearly at increased risk of being physically incapacitated or placed on bed rest for an extended period of time (Kozak et al. 2005; Wolfe et al. 2008; Fisher et al. 2011). Irrespective of age, physical inactivity alone has been repeatedly shown to facilitate or accelerate the deterioration of key health outcomes, including insulin signalling, muscle mass, motor control, and functional capacity (Clark et al. 2007; Paddon-Jones et al. 2005; Urso et al. 2006*a*, 2006*b*). Older adults appear to be particularly vulnerable to physical inactivity, even when anabolic stimuli, such as protein consumption approach or slightly exceed the RDA for protein (Sheffield-Moore et al. 2005).

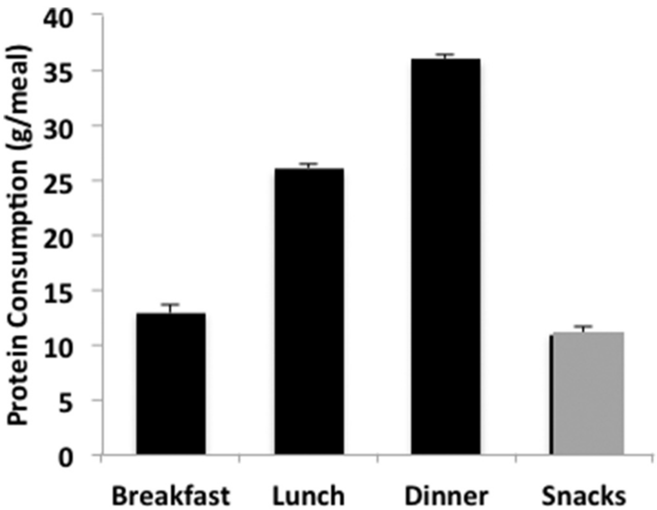
## Consequences of age-related muscle loss

The consequences of accelerated muscle loss during periods of physical inactivity are complicated by the fact that a large proportion of older adults (aged ≥65 years) can already be characterized as sarcopenic (Janssen 2006). Although the clinical definition of sarcopenia continues to be refined, most researchers and clinicians agree that it is characterized by progressive, insidious loss of muscle mass, beginning in middle-age. Importantly, sarcopenia typically includes a loss of physical function/capacity that is not explained by the loss of muscle mass alone. Thus, reductions in muscle mass and function that may be initially imperceptible can be physically and metabolically debilitating over a period of years (English and Paddon-Jones 2010). Furthermore, with advancing age, even brief periods of inactivity become increasingly likely to accelerate the loss of muscle mass and functional capacity, a condition from which some may not fully recover (Hirsch et al. 1990; Covinsky et al. 2003; English and Paddon-Jones 2010). Suboptimal nutrition and/or protein consumption clearly contributes to the onset and progression of sarcopenia. Many current dietary interventions, while intuitively appropriate and well intentioned, often fail when confronted with the practical realities of a physically inactive or aging population. While nutrition is only one of several factors that may modulate the onset and progression of sarcopenia, a number of recent studies and review papers have explored the concept of daily protein distribution (Paddon-Jones and Rasmussen 2009; Mamerow et al. 2014).

## Protein dose and distribution

While the RDA for protein focuses on daily aggregate protein intake and has considerable policy and population health relevance, it is questionable if it represents the best metric for individual dietary protein prescription at specific meals. In the United States, the average protein intake for men over 20 years is approximately 98 g/day and for women ~68 g/day (US Department of Agriculture – Agricultural Research Service 2012). Protein consump-

**Fig. 2.** Protein consumption (g/meal) for adults (aged ≥19 years) in the United States. Data are from the National Health and Nutrition Examination Survey 2001–2008 (US Department of Agriculture – Agricultural Research Service 2012) and are presented as means plus SE.




tion in adults over the age of 71 years decreases to 66 g/day, although this represents a median intake of 1.0 g/kg body weight, which exceeds the RDA (Fulgoni 2008). Despite this outward appearance of adequacy, a disproportionate amount of daily protein is consumed during the evening meal (>60%) with protein (US Department of Agriculture – Agricultural Research Service 2012) (Fig. 2).

The unequal distribution of protein intake over a 24-h period may reduce the effectiveness of the total daily protein intake for promoting muscle health. The human body has a limited ability to store excess protein/amino for later anabolic use. Therefore, there may be no protein synthetic advantage gained by consuming overly large servings of protein in a single meal (Paddon-Jones and Rasmussen 2009; Symons et al. 2009*a*, 2009*b*). Mamerow et al. (2014) recently reported that consuming a moderate amount of protein at each meal stimulated 24-h muscle protein synthesis more effectively than skewing the same total amount of protein intake towards the evening meal. While some data supporting a pulsed (i.e., skewed) delivery of protein appear to superficially conflict with the even-distribution theory, many of the conclusions are in fact supportive and likely only hidden by related issues, such as the quantity of protein consumed at each meal (Arnal et al. 1999; Bouillanne et al. 2013). For example, in a 6-week trial in hospitalized, older adults, Bouillanne et al. (2013) noted that patients who consumed a "pulsed" protein diet (0800 hours: 4.5 g; 1200 hours: 47.8 g; 1600 hours: 2.3 g; 1900 hours: 10.9 g protein) experienced a modest, but significant improvement in lean mass compared with the "spread" protein diet (0800 hours: 12.2 g; 1200 hours: 21 g; 1600 hours: 13.5 g; 1900 hours: 21.2 g protein). In this patient population, we would argue that the quantity of protein consumed at each meal in the "spread/distributed" protein group (i.e., 12–21 g/meal) was likely insufficient to optimally stimulate muscle protein synthesis across all meals.

For many individuals, starting with a meal plan that includes a moderate amount of high-quality protein 3 times a day may offer an effective strategy for optimizing muscle protein synthesis and potentially protecting muscle mass and function, but clearly longer duration, outcome-focused trials in a variety of populations is needed before definitive recommendations can be made. Several studies agree that consuming 25–35 g of protein (Symons et al. 2009*b*; Phillips et al. 2012; Volpi et al. 2013) in a single meal maximally stimulates muscle protein synthesis. There are also data suggesting that these moderate- to higher protein meals increase muscle protein synthesis to a similar degree in both young and older adults (Volpi et al. 1998; Symons et al. 2009*b*). Although in highly controlled and carefully screened healthy research subjects aging does not necessarily impair the ability to mount an anabolic response to a protein-rich meal, there is recent evidence that suggests older adults exhibit a less robust anabolic response to meals with a lower protein content or a mix of protein and carbohydrates (Glynn et al. 2013).

A limitation of many protein metabolism studies is the difficulty in concurrently measuring muscle protein synthesis and breakdown over multiple meals. It has been suggested that increasingly larger protein meals may provide a greater net anabolic effect by progressively inhibiting breakdown while still providing a maximal protein synthetic stimulus. The theory has merit and may indeed be beneficial in situations where the ingestion of additional protein does not exceed daily energy requirements, compromise the intake of other necessary nutrients, or unduly burden the individual (cost, satiety, etc.). While 25–35 g of protein per meal is likely sufficient for the majority of the adult population, some groups may clearly benefit from more, or in some cases less, protein based on their energy requirements, activity levels, body size, and health status.

Recent epidemiological studies have suggested that consuming large quantities of protein, animal protein in particular, could be linked to an increase in the risk for adverse health outcomes such as cancer or diabetes (Levine et al. 2014; van Nielen et al. 2014). While valuable, these studies cannot establish causation and should not be immediately interpreted to mean that protein has negative health outcomes. In contrast, many support the theme of moderation, where consuming moderate portions of high-quality protein is desirable. The inclusion of high-quality animal proteins or combinations of plant-based proteins at each meal can efficiently stimulate muscle anabolism while being mindful of total macronutrient and energy consumption. The message of protein at each meal in moderation is a paradigm shift from the typical nonspecific, default recommendation of a large, global increase in protein intake for populations actively seeking to increase or maintain muscle mass and function. This balanced and moderate protein message should be placed in the context of total energy intake to ensure appropriate energy balance. Limiting energy intake from protein would by default increase energy intake from carbohydrates and fat, which is important when considering the complex issue of obesity.

## Protein and weight management

The global obesity epidemic is an issue that commands the resources of many public health organizations and solicits the input of health professionals and scientists to develop new approaches to prevent and/or treat obesity. It also invites researchers to revisit some previously demonstrated effects of protein on energy balance to determine if greater than expected benefits might be obtained from diet manipulations. As discussed in this section, contemporary research has reexamined the effects of variations in protein intake on body weight management with the perspective to provide tools to obese individuals preoccupied by weight loss and maintenance.

### High-protein diet and weight loss

Physiological research has clearly established that dietary protein represents the macronutrient with the greatest thermogenic potential (Flatt 1978) and most pronounced satiating effects; thereby implying that calorie for calorie, a high-protein diet should be expected to facilitate appetite control and reduce subsequent energy intake. When applied to a weight-loss program, these principles support the idea that a high-protein diet should accentuate the weight-reducing effect of a diet-based obesity treatment. The classical study of Skov et al. (1999) tested the effect of 2 diets differing in the contribution of protein and carbohydrate to en-

**Table 2.** Body weight/fat loss induced by diet over 6 mo in relation to protein intake.*

| Variables | High-protein diet | Normal-protein diet |
|---|---|---|
| Protein intake (% E) | 25 | 12 |
| Carbohydrate intake (% E) | 45 | 58 |
| Fat intake (% E) | 30 | 30 |
| Energy intake (MJ/d) | 5.0 | 6.2 |
| Δ Body weight (kg)† | −8.9 | −5.1 |
| Δ Fat mass (kg)† | −7.6 | −4.3 |
| Energy equivalent of Δ fat mass (kJ/d) | 1643 | 930 |

**Note:** E, energy.
*Data from Skov et al. (1999).
†Change from baseline.

ergy intake on the response of energy balance and body weight/fat during a 6-month intervention. In accordance with their hypothesis, the high-protein, reduced-carbohydrate diet induced a significant decrease in daily energy intake concurrent with greater weight and fat loss (Table 2). Daily energy deficit almost doubled under a high-protein, reduced-carbohydrate condition, as reflected by the estimation of the daily energy equivalent of fat loss. This research group subsequently extended its proof of concept in a second study confirming that a 6-month, controlled, high-protein diet significantly increased body weight loss in overweight and obese individuals (Due et al. 2004). They also added to their protocol an additional 6-month nutrition counselling period after which a difference in abdominal fat persisted between the medium and the high-protein diet, whereas the between-group difference in body weight was not further significant. Thus, as with other weight-loss modalities, a regular and sustained support of a health professional, i.e., a dietitian in this case, seems to be necessary to get an optimal outcome from a high-protein diet.

The findings reported by Skov et al. (1999) are indirectly supported by data pertaining to the effects of dairy food, and particularly dairy protein, on appetite control, energy intake, and body composition. In the study by Zemel et al. (2004), body weight and fat loss over a supervised 6-month diet was significantly greater when a standardized calcium supplementation was provided via a dairy food compared with a nondairy supplement. This suggests that other factors were partly responsible for the accentuation of weight loss. In this regard, the fact that the percentage of energy as protein was comparable in each condition does not exclude a role for the well-documented satiating properties of the main dairy proteins, i.e., casein and whey protein (Hall et al. 2003; Anderson and Moore 2004). In addition, other nutrients such as vitamin D and fatty acids might have also contributed to the impact of dairy supplementation in this study. Other recent studies show that a dairy protein supplement favourably influences appetite sensations and time of request of a meal (Douglas et al. 2013) as well as subsequent energy compensation (Akhavan et al. 2010).

### Protein and body weight maintenance in the reduced-obese state

Our research experience shows that a substantial weight loss approaching a threshold of spontaneous resistance to further lose fat promotes a greater than predicted decrease in energy expenditure, be it at rest (Doucet et al. 2001) or in an active state (Doucet et al. 2003), as well as an increase in hunger and desire to eat (Doucet et al. 2000). These observations demonstrate the regulatory effects of variations of body fat on energy balance and highlight that the loss of fat-related regulatory impact must be compensated by other factors to prevent weight regain in a reduced-obese state.

In the context of preventing weight regain in the reduced-obese state the thermogenic and satiety advantages of protein can also contribute to body weight maintenance. Lejeune et al. (2005) investigated this in obese individuals who first underwent a standardized energy deficit designed to trigger adaptations favoring weight regain. This was followed by a 12-month follow-up, during which a subgroup of subjects consumed a high-protein diet whereas other participants consumed a standard control diet. The main finding of this study was that the mean weight regain was lower by 2.9 kg in individuals subjected to the high-protein diet.

**Table 3.** Changes (baseline – postexercise) in body weight and composition in adult women after 16 wk of consuming reduced-energy diets with a high- or low-protein intake (1.6 vs. 0.8 g/(kg·d⁻¹)) with or without a supervised exercise program (5 d/wk walking and 2 d/wk resistance training).*

| Group (kg)† | High protein | High protein+ exercise | Low protein | Low protein+ exercise |
|---|---|---|---|---|
| Δ Body weight | 8.7 | 9.8 | 7.8 | 6.7 |
| Δ Fat mass | 5.9 | 8.8 | 5.0 | 5.5 |
| Δ Lean mass | 2.0 | 0.4 | 2.7 | 1.0 |

*Data from Layman et al. (2005).
†Change from baseline.

The ability of a high-protein diet to contribute to the prevention of weight regain in reduced-obese individuals was also observed in the Diogenes Study (Larsen et al. 2010). In this case, a diet combining high protein and low-glycemic index (GI) foods totally prevented weight regain over a 26-week follow-up. This is concordant with our research experience, which demonstrated that the high-protein–low-GI–low-fat diet promotes a lower daily energy intake compared with a normal protein–high-carbohydrate–low-fat diet (Dumesnil et al. 2001).

This is evidence that an increase in the protein content of the diet may at least partly compensate for the biological vulnerability of the weight-reduced obese individual towards the risk of weight regain. As recently reviewed, there is no clear indication that any particular protein source might produce a more pronounced effect than another (Gilbert et al. 2011).

### High-protein diet and physical activity

While increasing protein intake during weight loss offers some benefits, the combination of physical activity and protein enhances weight-loss efforts. This has been specifically investigated by Layman et al. (2005), who recruited obese women in a 16-week weight-loss program who were assigned to 4 conditions differing by the exercise prescription and the protein content of the diet. As shown in Table 3, the high-protein–exercise combination increased mean body-fat loss by about 3 kg compared with other conditions while preventing loss of lean body mass. This agrees with data recently reported by Josse et al. (2011), who showed that combining exercise training with a higher protein primarily derived from dairy foods enhances body weight loss accompanied by a greater fat/fat-free mass ratio.

Thus, the latter observation together with the studies of the Astrup group (Skov et al. 1999; Due et al. 2004) suggest that doubling the relative contribution of protein to energy intake, e.g., up to about 25% kcal, appears to be a safe dietary modification to increase the outcome of a diet-based weight-reducing program. According to Westerterp-Plantenga et al. (2009), a daily protein intake under 2.8 g/kg was shown to have no negative effects on renal function in athletes and may be considered as safe for healthy individuals. Furthermore, the adherence to an exercise program including resistance training seems to accentuate the benefits of a high-protein diet on body composition.

## Conclusions

Protein is a nutrient deserving of focus, because it is a macronutrient that has the most pronounced thermogenic and satiating effects, and the most promising nutrient to help preserve lean body mass. Current recommendations for protein intake through the life-cycle are based primarily on the nitrogen balance method in adults, the limitations of which are well known. Recent methods to determine protein requirements using stable isotope-based methods during different life-stages suggest that current recommendations are underestimated, and a reassessment of the recommendations may be timely. There is evidence that consuming a moderate amount (25–35 g) of high-quality protein during each meal stimulates muscle protein synthesis and promotes muscle health and plays a role in preservation of lean body mass with increasing age. Furthermore, an increase in the proportion of dietary protein (about 25% kcal) during a hypo-energetic diet regimen favours an accentuation of body weight/fat loss in obese individuals and helps in the prevention of weight regain. These benefits might be increased when high-protein intake is combined with exercise training. Future studies should be conducted to confirm these recommendations, which are based on short-term studies with longer randomized controlled studies.

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