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REVIEW

Effect of Exercise Intensity, Duration, and Volume on Protein Oxidation During Endurance Exercise in Humans: A Systematic Review With Meta-Analysis

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ABSTRACT

Proteins are degraded and amino acids are metabolized in different quantities during endurance exercise. However, a clear consensus on protein oxidation during exercise has yet to be established. The main objective was to calculate estimates of protein oxidation during endurance exercise using available data. Additionally, we aimed to investigate the effects of exercise intensity, duration, and volume on protein oxidation. We systematically searched for research studies published in English in the online databases PubMed and Google Scholar in March 2023. The inclusion criteria were: (1) measurement of protein metabolism with nitrogen excretion, leucine oxidation, or indicator amino acid utilization method; (2) inclusion of an endurance exercise condition and a control condition without exercise; (3) inclusion of a description of the endurance exercise protocol (duration, intensity); and (4) inclusion of healthy participants over the age of 18. Endurance exercises were defined as exercise periods of at least 60 min' duration of running, cycling, or cross-country skiing. We included 30 articles ($n = 286$ participants). Protein oxidation increased by 1.02 ± 0.06 mg•kg^{-1}•min^{-1} (95% CI [0.91, 1.14]) during endurance exercise, from the level of 0.81 ± 0.38 mg•kg^{-1}•min^{-1} measured without exercise. Protein contributed $3.28\% \pm 0.15\%$ (95% CI [2.97, 3.58]) of the total energy expenditure during exercise. Protein oxidation normalized by exercise duration significantly increased with exercise intensity. This review is the first to aggregate data on protein oxidation during endurance exercise, measured using different methods. Endurance exercise consistently increased protein oxidation, with protein metabolism more than doubling during exercise compared to rest. Protein oxidation increased with exercise intensity, but neither exercise duration nor volume augmented the rate of protein oxidation.

1 | Introduction

The view on protein as an energy source has changed dramatically over time. Justus von Liebig proposed in 1842 that protein served as the "only true nutrient providing both the machinery of the body and the fuel for its work" [1]. However, subsequent studies soon discredited Liebig's theory [2]. Since then, exercise physiologists, using indirect calorimetry, nitrogen excretion, and isotopic markers, have identified the distribution of substrate utilization during exercise [3, 4]. At rest, energy demand typically amounts to around 1.2 kcal•min^{-1}, with oxygen uptake at 0.25 L•min^{-1}, resulting in the oxidation of approximately 0.20 g carbohydrates (CHO) and 0.05 g fat in a 70 kg individual [5–7]. During exercise, energy demand escalates, and oxygen uptake rapidly reaches 4 L•min^{-1} in well-trained males [5, 8], elevating CHO oxidation rates to 2–3 g•min^{-1}. Protein metabolism is often omitted from calculations of substrate oxidation during exercise, despite evidence indicating its involvement during exercise [9, 10]. Proteins contain energy and constitute approximately 10%–20% of the

daily energy intake [11, 12]. The question remains: does protein oxidation also increase during exercise? There is indirect support for protein oxidation, since protein requirement is increased after an exhaustive endurance exercise and meeting this requirement improves the recovery of performance [5]. This could indicate that protein oxidation was increased and that protein was possibly used as an energy source. However, protein oxidation is not without consequences, as the body's ability to store amino acids is limited, and most proteins serve critical functional roles. Consequently, understanding protein metabolism during endurance exercise is crucial in sports physiology. It provides insights into how the body utilizes various energy sources during prolonged physical activity and how it affects muscle recovery, adaptation, and overall athletic performance. A comprehensive understanding of protein metabolism can inform effective dietary strategies, optimizing protein intake for enhanced endurance and recovery.

Exercise physiologists have established that the relative contributions of CHO and fats to energy production are contingent upon exercise intensity [3, 4, 13, 14]. As exercise intensity rises, CHO's contribution to energy supply increases while fat use diminishes. Consequently, CHO becomes the predominant energy source during moderate and high-intensity exercise, primarily derived from glycogen stores. However, glycogen stores are limited [15]. In addition, exercise performed with depleted glycogen stores heightens protein breakdown [9, 16]. These findings suggest that increased protein oxidation compensates for the energy deficit resulting from reduced CHO metabolism. Consequently, prolonged exercise, which depletes CHO stores, particularly glycogen, may exacerbate protein oxidation. Exercise intensity could further modulate protein metabolism by altering the relative contributions of energy substrates. Some data indicate that protein oxidation increases with exercise intensity, as shown by the positive correlation between leucine oxidation as a percentage of the leucine flux and the work rate during 30-min exercise bouts [17]. Finally, research indicates that increased energy expenditure during exercise could increase protein oxidation [18, 19]. In this review, exercise volume was defined as the total volume of oxygen consumed during exercise, thereby equating it with energy expenditure during exercise. To date, questions on the effects of exercise intensity, duration, and volume on protein oxidation remain unanswered.

Studying protein metabolism poses significant challenges [20]. Due to the many challenges involved, only a handful of studies have explored protein metabolism during endurance exercise, with disparities in the results [9, 21]. For example, measurements obtained from nitrogen excretion suffer from poor time resolution: urine is usually collected over 24 h, making it difficult to discern differences over shorter periods. Additionally, estimating protein intake from food presents considerable challenges [22]. Stable isotope techniques offer high resolution. While stable isotopes facilitate measurements over shorter periods, the large intracellular amino acid pools present a challenge, and the presence of 20 amino acids in proteins complicates this method. Variations in metabolic pathways between amino acids and their differential distribution across tissues further hinder the generalization of results measured from a single amino acid to whole-body protein metabolism.

Finally, protein metabolism during endurance exercise has seldom been the primary focus of previous studies, making it difficult to obtain a comprehensive overview of the results. As far as we are aware, no meta-analysis has been conducted to estimate protein oxidation during endurance exercise. Nevertheless, data on protein metabolism during exercise can be found in various published studies, although these data have not yet been used to assess protein oxidation. The principal objective of this review was to compile published findings and use available data to quantify protein oxidation during endurance exercise in healthy adults. Lemon et al. [23] conducted a review 40 years ago on protein metabolism and exercise, discussing the effects of exercise duration and intensity on amino acid oxidation during endurance exercise. However, they lacked sufficient data to draw definitive conclusions and did not discuss the effect of exercise volume. As a result, a secondary objective of this review was to determine the impact of exercise intensity, duration, and volume on protein metabolism.

2 | Methods to Study Protein Metabolism During Exercise

Several methods are used to quantify protein metabolism. We briefly describe the methods used to study protein metabolism during exercise and discuss their limitations and advantages.

2.1 | Measuring Nitrogen Excretion

This method for quantifying the contribution of proteins to total energy expenditure during endurance exercise is based on one chemical property: amino acids have an amino group in their chemical structure that is excreted and measured. All amino acids contain at least one nitrogen atom, and nitrogen excretion from amino acid metabolism is quantitatively the largest part of nitrogen excretion [20]. Thus, monitoring nitrogen excretion gives a good overview of the metabolism of amino acids.

Nitrogen excretion can be measured with the Kjeldahl method [24]. Most nitrogen is excreted in the urine, but nitrogen is also secreted in sweat, feces, hair, and skin loss [25]. Nitrogen excretion has been quantified in these different compartments. The total nitrogen excretion can be estimated with some correction factors [26–28]: ≈78% of the nitrogen is excreted in the urine, ≈13% in feces, ≈7% in sweat, and ≈2% in miscellaneous losses (Figure 1). We used these correction factors in this review when nitrogen excretion was not measured in all of these compartments. This results in:

$$\text{Total nitrogen excretion (g)} = \frac{\text{Urinary nitrogen excretion (g)}}{0.78}$$

$$\text{Total nitrogen excretion (g)} = \frac{\text{Urinary nitrogen excretion (g)} + \text{Sweat nitrogen excretion (g)}}{0.85}$$

In some studies, only nitrogen intake and balance are reported, but not nitrogen excretion. However, nitrogen excretion can be

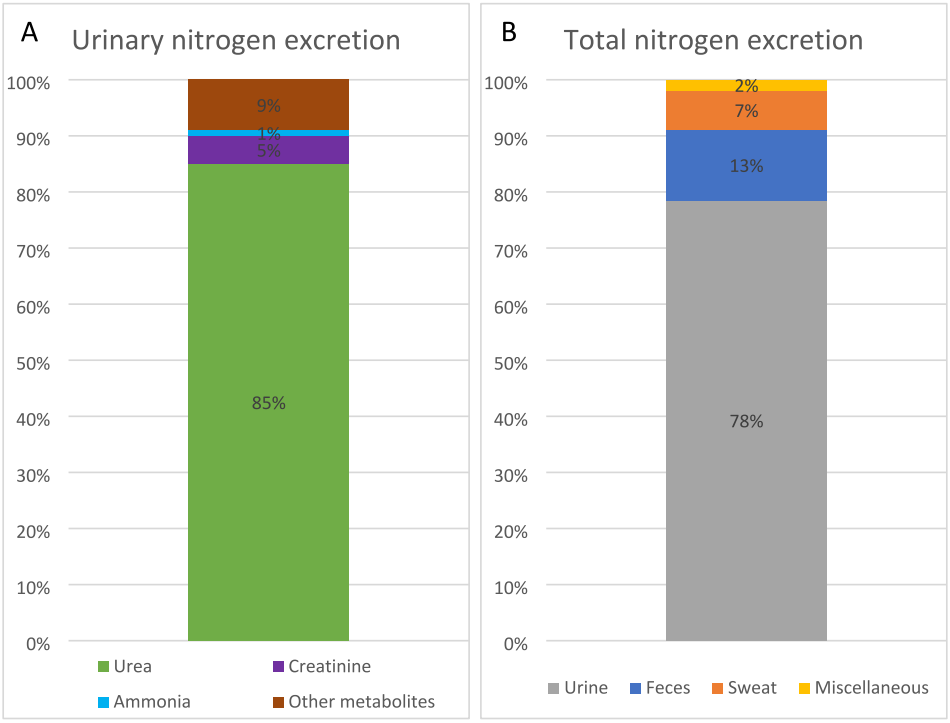

FIGURE 1 | (A) Distribution of nitrogen between the different metabolites in urine. (B) Contribution of the different compartments to total nitrogen excretion.

derived from nitrogen balance and nitrogen intake measurements when both are reported:

$$\text{Nitrogen excretion (g)} = \text{Nitrogen intake (g)} - \text{Nitrogen balance (g)}$$

The calculated nitrogen excretion, derived from nitrogen balance and nitrogen intake, was then used in the same manner as measured nitrogen in other studies. When necessary, corrections were applied to obtain nitrogen excretion in all compartments using the same correction factors as measured nitrogen in other studies.

Not all the studies measured the total nitrogen excretion in urine. Often only the urinary urea excretion is reported. However, it has been established that urinary urea excretion accounts for ≈85% of urinary nitrogen excretion [29], with variations between 80% and 90% [5, 8, 29] depending on nutritional intake and activity levels. This variation is not well described, so in this review we use the average proportion of 85% as a correction factor:

$$\text{Urinary nitrogen excretion (g)} = \frac{\text{Urinary urea excretion (g)}}{0.85}$$

2.2 | Measuring Leucine Oxidation by Stable Isotopes

Other methods do not use nitrogen exchanges to quantify the contribution of proteins to energy supply during exercise: they are based on the quantification of an amino acid of interest. By relating the quantified results to the relative proportion of this amino acid in the body, it is possible to estimate the overall metabolism of amino acids.

Such methods have some limitations. Indeed, the 20 amino acids usually found in proteins are metabolized differentially, and through transamination and oxidative deamination, can produce several metabolic intermediates. This, together with the fact that physical activity can have different effects on individual amino acids, makes it difficult to generalize the results from one amino acid to the whole protein metabolism. Nevertheless, this generalization works well for leucine.

Leucine and other amino acids are metabolized in skeletal muscles: it is estimated that about 50% of all branched-chain amino acids are metabolized in skeletal muscle [30]. The enzyme branched-chain amino acid aminotransferase is involved in this process. There are two isoforms of branched-chain amino acid aminotransferase: cytosolic and mitochondrial isoforms. This enzyme has an important role: deleting branched-chain amino acid aminotransferase reduces endurance capacity [31]. This happens because leucine oxidation increases during exercise to provide energy to the body. The human body cannot store amino acids, except in limited quantities in the free amino acid pool. To obtain sufficient quantities of amino acids, it must therefore degrade proteins. From the average composition of muscle fibers, it has been calculated that 1 g of protein contains 590 μmol leucine [32, 33]. This conversion factor comes from the average value of the protein leucine content of ≈7.8% in human and other mammalian muscles [33]. The mechanism for leucine oxidation involves the branched-chain alpha-keto acid dehydrogenase complex, in an irreversible reaction [34]. Therefore, no recycling of leucine is possible. Measuring the extra quantity of leucine oxidized during exercise compared to rest makes it possible to calculate the amount of protein degraded to obtain this quantity of leucine:

TABLE 1 | Strengths and weaknesses of the methods used to evaluate protein metabolism during exercise.

Method	Strength	Weakness
Nitrogen excretion (urine, sweat, feces, miscellaneous)	• Good accuracy • Easy to implement • Possible to measure over a long period (of the order of magnitude of 1 day)	• Several approximations to estimate the total quantity of excreted nitrogen depending on the compartments measured • Difficult to implement depending on the compartments measured • The urea pool has a half-life of 8–10 h [39, 40]. Measurements over short periods are then inaccurate • Need for a ≈ 3 days equilibration period when changing the habitual protein intake
Leucine oxidation	• Possible to measure over a short period (of approximately 1 h)	• Approximation of a steady state necessary for the calculations • Difficult to implement

$$\text{Protein oxidation rate (g)} = \frac{\text{Leucine oxidation exercise (μmol)} - \text{Leucine oxidation rest (μmol)}}{590}$$

This measurement of the extra quantity of leucine oxidized during exercise is often carried out with a stable isotope of leucine (often L-[1-^{13}C] leucine). The inclusion of isotopes in specific molecules (tracers) provides identical molecules to the desired one (the tracee), with the same properties and metabolization pathways, except that the tracer can precisely be identified and quantified [35]. So, by infusing known quantities of labeled leucine and assuming a one-compartment model based on Steele's equations [36], the proportion of leucine oxidized can be estimated from all excretions/expirations of isotope derivatives. Leucine oxidation can be calculated from the measured flux from exhaled CO_2. While a steady state exists at rest, exercise alters numerous physiological parameters, including CO_2 excretion. Furthermore, bicarbonate in the blood can serve as a buffer for some of the CO_2 produced. In fact, Phillips et al. [37] reported that exercise significantly increased the background $^{13}CO_2$ enrichment in breath compared to rest, and that this background $^{13}CO_2$ enrichment varied during exercise. Exercise also caused a significant increase in bicarbonate retention following notably the buffering of lactic acid from anaerobic glycolysis. Hence, it becomes crucial to correct for background $^{13}CO_2$ enrichment and bicarbonate retention.

The indicator amino acid utilization method is another methodology used to measure the oxidation of a specific amino acid (see reference [38] for a detailed explanation of this method). We did not find any studies using the indicator amino acid utilization method that met the inclusion criteria; therefore, no further details on this methodology are provided (Table 1).

3 | Materials and Methods

This systematic review was performed following the checklist for the Preferred Reporting Items for Systematic reviews and Meta-Analyses 2020 (PRISMA) [41].

3.1 | Inclusion and Exclusion Criteria

Inclusion criteria for articles included in this systematic review were: (1) measurement of protein metabolism with one or more of these methods: nitrogen excretion, leucine oxidation, indicator amino acid utilization method (the methods are described previously); (2) inclusion of an endurance exercise condition and a control condition without exercise; (3) inclusion of a description of the endurance exercise protocol (duration, intensity); and (4) inclusion of healthy participants over the age of 18. Endurance exercises were defined as exercise periods of at least 60 min' duration of running, cycling, or cross-country skiing. Studies were excluded if they did not meet the inclusion criteria.

Only studies with a control condition were included to quantify the effect of exercise. This was done to distinguish amino acids' essential basal metabolism contributions and the additional contributions exerted by exercise. The control condition could have been total resting conditions (e.g., urine collection during one entire day without physical exercise and another whole day with exercise), or the measurement of a parameter of interest at rest before exercise (typically the oxidation rate of leucine during a steady state at rest). The extra contribution during exercise is calculated as the difference between the quantity that is oxidized and measured during exercise and the quantity that should have been oxidized at rest during the same period.

When nitrogen excretion was calculated from nitrogen intake and balance, only studies over a period without food intake or with the intake of identical diets between interventions were included, with the aim of standardization in relation to the digestibility of protein in food [42].

3.2 | Literature Search

We systematically searched in the online databases PubMed (Medline) and Google Scholar for research studies written in English in March 2023. We used the search terms: (protein OR

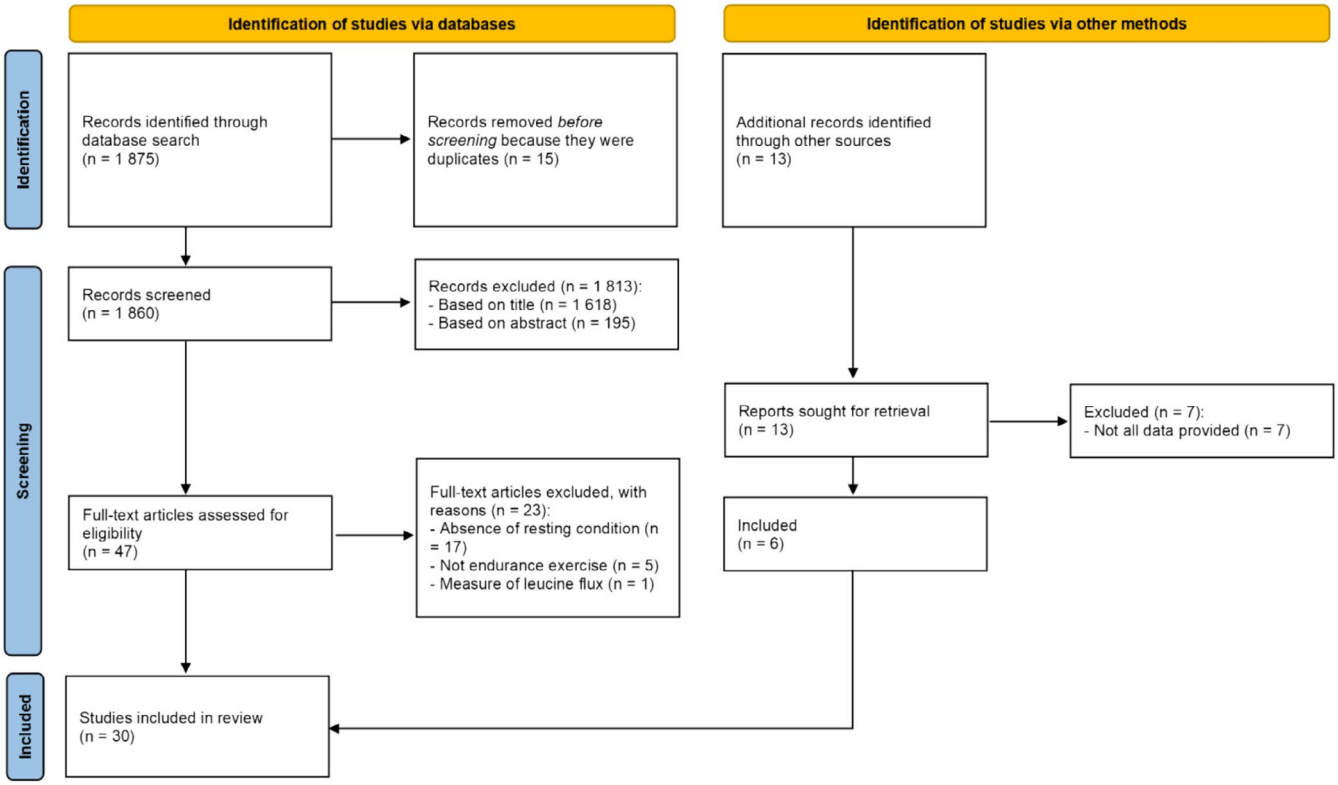

FIGURE 2 | PRISMA 2020 flow diagram for new systematic reviews that included searches of databases and other sources.

amino acids) AND (oxidation OR metabolism OR contribution to energy) AND (endurance exercise OR running OR cycling OR cross-country skiing). MC screened articles by titles and abstracts to determine initial eligibility. Blinding of authors was used to reduce bias during this process. Finally, MC and JJ reviewed the full texts of all articles to determine their eligibility for inclusion based on the inclusion criteria. MC and JJ performed independent data extraction, and differences were discussed until a consensus was reached.

As protein oxidation is often not the primary outcome of studies, we identified several articles through cross-reference checks, in addition to separate searches on authors with research papers already included in the database. We then used their data in the present review. A PRISMA flow diagram of the search strategy and study selection is shown in Figure 2.

3.3 | Risk-of-Bias and Publication Bias Assessment

Since the contribution of protein as an energy substrate during endurance exercise was seldom the primary focus in the included studies, most tools designed for assessing the risk of bias in intervention studies were not directly applicable to this review. A modified risk-of-bias tool [43] was used by MC to conduct the risk-of-bias assessment in this review, with criteria outlined in the Appendix S1. The quality of articles was evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system [44], resulting in an overall moderate grade of evidence. Given that the aim of this systematic review was to map and quantify protein's contribution as an energy substrate during endurance exercise, rather than to provide recommendations, no decisions based on assessed bias were used to exclude articles.

Moreover, since protein oxidation was rarely the primary focus in the included studies and since we calculated some of the protein's contributions, we saw no reason why studies reporting lower protein contributions would be less likely to be published than those reporting higher contributions. Therefore, it is unlikely that the included studies were affected by publication bias specifically related to 'protein oxidation outcomes.'

3.4 | Data Extraction

Data were extracted from the included research studies by MC and verified by JJ. Extracted information included the number and characteristics of participants, exercise duration, modality, and intensity (as a percentage of VO_{2max}), and exercise-related energy expenditure. From these data, we estimated the exercise volume as the total volume of oxygen consumed during exercise (multiplication of the exercise duration by the average oxygen consumption). In addition, from the included research studies, we also extracted the necessary data about protein metabolism (at rest and during exercise) and the method to determine protein oxidation.

In cases where studies included multiple conditions with the same participants, data were aggregated, and the average was calculated and used in the present review. Alternatively, when studies comprised several conditions with independent

participant groups and provided data for each group, the data for each condition were used independently.

A conversion factor of 6.25 was used in this review to convert nitrogen into protein (1 g of nitrogen measured indicates the presence of 6.25 g of protein in the measured sample) [45].

An energy equivalent of oxygen of 4.82 kcal per liter of oxygen was chosen in this review. It was based on the reported values for the exercise intensity range included in the present review [46].

When the raw data were not presented, the study authors were contacted to obtain them. If not received, these data were extracted from reported figures using WebPlotDigitizer (PlotDigitizer, Version 3.1.5, 2023).

If not all variables were reported in the research paper, the reported data were used to derive the missing values via the following formulas or combinations of formulas if possible:

$$\text{Total nitrogen excretion (g)} = \frac{\text{Urinary nitrogen excretion (g)}}{0.78}$$

$$\text{Urinary nitrogen excretion (g)} = \frac{\text{Urinary urea excretion (g)}}{0.85}$$

$$\text{Total nitrogen excretion (g)} = \frac{\text{Urinary nitrogen excretion (g)} + \text{Sweat nitrogen excretion (g)}}{0.85}$$

$$\text{Nitrogen excretion (g)} = \text{Nitrogen intake (g)} - \text{Nitrogen balance (g)}$$

$$\text{Protein oxidation rate (g)} = \text{Total nitrogen excretion (g)} \times 6.25$$

$$\text{Protein oxidation rate (g)} = \frac{\text{Leucine oxidation exercise (}\mu\text{mol)} - \text{Leucine oxidation rest (}\mu\text{mol)}}{590}$$

$$\text{Energy expenditure from protein oxidation (kcal)} = \text{Protein oxidation rate (g)} \times 4.09$$

$$\text{Total oxygen consumption (L)} = \text{Oxygen consumption } \left(\text{L} \bullet \text{min}^{-1}\right) \times \text{Exercise duration (min)}$$

$$\text{Total energy expenditure (kcal)} = \text{Total oxygen consumption (L)} \times 4.82$$

$$\text{Exercise volume (L)} = \text{Total oxygen consumption (L)}$$

$$\text{Contribution protein to energy supply (\%)} = \frac{\text{Energy expenditure from protein oxidation (kcal)}}{\text{Total energy expenditure (kcal)}}$$

3.5 | Statistical Analysis

All data are presented as mean ± SD. Protein oxidation was presented in a normalized format to mitigate the differences between the protocols of the included studies. Protein oxidation was presented relative to participants' body weight and exercise duration by dividing the average amount of protein oxidized by the average body weight of participants and by the average exercise duration. Standard deviations were extracted or calculated for each study to obtain the 95% confidence intervals (CIs) in the forest plots.

Data presented in the forest plots were calculated using the 'escalc' command of the 'metafor' package in R (Version 4.3.2, Vienna, Austria, 2023) [47]. The meta-analysis was completed with a random-effects model using the Restricted Maximum Likelihood (REML) method [48]. We used I^2 and τ^2 statistics to evaluate heterogeneity. The prediction interval for the mean effect was calculated from the point estimate of the mean effect, its standard error (SE) and the estimated τ^2 [49]. The standard deviation of the prediction interval was $\text{SD}_{PI} = \sqrt{\tau^2 + \text{SE}^2}$. The lower and upper limits of the 95% prediction intervals were $\text{mean} \pm t_{\frac{1-0.05}{2},df} \times \text{SD}_{PI}$, with $t_{\frac{1-0.05}{2},df}$ the t-value for a two-sided 95% interval at df degrees of freedom.

The influence of exercise intensity, duration, and volume on the quantity of protein oxidized and the protein's contribution to energy supply during endurance exercise were investigated through meta-regressions [50]. Meta-regressions were performed with a random-effects model using the 'regplot' command in R. The REML method was used. The average regression line as well as the 95% CI are represented in each figure. All figures were produced in R (Version 4.3.2, Vienna, Austria, 2023).

4 | Results

This review focused on two parameters to study protein metabolism during exercise: the quantity of protein oxidized and the protein's contribution to energy supply during endurance exercise. As the data in the present review came from several studies with different participant characteristics and exercise durations, we present the quantity of protein oxidized during exercise normalized by the participants' body weight and exercise duration (quantity of protein oxidized in $\text{mg} \bullet \text{kg}^{-1} \bullet \text{min}^{-1}$). These parameters can be studied as a function of exercise intensity, duration, and volume.

The literature search yielded 1875 articles, of which 47 potentially met the inclusion criteria based on title and abstract screening. After full-text screening, 30 studies were confirmed to meet the inclusion criteria and were included ($n = 286$ participants). The average exercise duration of the included studies was 112 ± 72 min (range [60, 360]) and the average intensity was 54% ± 10% of $\text{VO}_{2\text{max}}$ (range [29, 73]). The studies included and their characteristics are presented in Table 2.

We did not find any studies using the indicator amino acid utilization method that met the inclusion criteria.

4.1 | Additional Quantity of Protein Oxidized During Exercise

One objective of this systematic review was to comprehensively assess and quantify the protein oxidized during endurance

TABLE 2 | Research papers included.

Research paper	n	Body weight (kg)	Method used to measure protein metabolism	Modality	Duration (min)	Intensity (%VO_{2max})
Rennie et al. 1981a [51]	4	74.3	Nitrogen excretion in urine	Running	225	57%
Rennie et al. 1981b [52]	4	74.3	Nitrogen excretion in urine and leucine oxidation	Running	120	57%
Tarnopolsky et al. 1990 [53]	6	66.9	Urea excretion in urine	Running	93	62%
Tarnopolsky et al. 1995 [54]	7	74.8	Urea excretion in urine	Cycling	76	73%
Tarnopolsky et al. 1997 [55]	8	72.9	Urea excretion in urine	Cycling	90	64%
Refsum and Strömme, 1974 [56]	22	75.0	Urea excretion in urine	Cross-country skiing	310	65%
Broad et al. 2008 [57]	10	75.7	Nitrogen excretion in urine	Cycling	90	70%
Lemon et al. 1984 [58]	5	55.5	Nitrogen excretion in sweat	Cycling	60	49%
Brouns et al. 1989 [59]	13	73.3	Nitrogen excretion in urine and sweat	Cycling	276	61%
Calles-Escandon et al. 1984 [60]	8	65.0	Nitrogen excretion in urine and sweat	Cycling	90	47%
Smith et al. 2009 [61]	5	74.0	Nitrogen excretion in all compartments	Cycling/ Running	128	58%
Pikosky et al. 2008 [62]	7	73.3	Nitrogen excretion in all compartments	Cycling/ Running	83	58%
Todd et al. 1984 [18] Diet A	6	75.1	Nitrogen excretion in all compartments	Cycling/ Running	60	50%
Todd et al. 1984 [18] Diet B	6	69.8	Nitrogen excretion in all compartments	Cycling/ Running	60	51%
Butterfield and Calloway, 1984 [19]	6	67.8	Nitrogen excretion in all compartments	Cycling/ Running	60	53%
Gontzea et al. 1968 [63]	30	68.2	Nitrogen excretion in all compartments	Cycling	113	61%
Phillips et al. 1993 [37]	6	64.1	Nitrogen excretion in all compartments and leucine oxidation	Cycling	90	64%
Lamont et al. 2001a [64]	16	68.9	Leucine oxidation	Cycling	60	42%
Lamont et al. 2001b [65]	7	77.4	Leucine oxidation	Cycling	60	40%
Lamont et al. 2003 [66]	4	76.0	Leucine oxidation	Cycling	60	50%
Lamont et al. 1999 [67] Trained	7	67.7	Leucine oxidation	Cycling	60	44%
Lamont et al. 1999 [67] Untrained	7	68.8	Leucine oxidation	Cycling	60	48%
McKenzie et al. 2000 [68]	6	78.8	Leucine oxidation	Cycling	90	62%
El-khoury et al. 1997 [69]	8	77.5	Leucine oxidation	Cycling	90	46%
Bowtell et al. 1998 [70] High protein diet	8	74.5	Leucine oxidation	Running	120	60%
Bowtell et al. 1998 [70] Low protein diet	8	71.9	Leucine oxidation	Running	120	60%

(Continues)

TABLE 2 | (Continued)

Research paper	n	Body weight (kg)	Method used to measure protein metabolism	Modality	Duration (min)	Intensity (%VO_{2max})
Bowtell et al. 2000 [71] High protein diet	8	74.8	Leucine oxidation	Running	120	60%
Bowtell et al. 2000 [71] Low protein diet	8	72.8	Leucine oxidation	Running	120	60%
Wolfe et al. 1982 [72]	4	69.8	Leucine oxidation	Cycling	105	29%
Knapik et al. 1991 [73]	7	79.1	Leucine oxidation	Cycling	125	48%
Howarth et al. 2010 [16]	6	80.0	Leucine oxidation	Two-leg knee extensor	120	33%
Koopman et al. 2004 [74]	8	72.4	Leucine oxidation	Cycling/ Running	360	50%
Mazzulla et al. 2017 [75]	7	72.4	Leucine oxidation	Running	60	69%
Forslund et al. 1999 [76] High protein diet	6	80.0	Leucine oxidation	Cycling	90	47%
Forslund et al. 1999 [76] Normal protein diet	8	78.0	Leucine oxidation	Cycling	90	46%

Abbreviation: *n*, number of participants.

exercise. To achieve this, summary statistics for each study, including mean values and 95% CIs, are depicted on the right side of Figure 3.

In the studies included in the present review, protein oxidation increased by 1.02 ± 0.06 mg•kg^{-1}•min^{-1} (95% CI [0.91, 1.14]) during endurance exercise (Figure 3), from the level of 0.81 ± 0.38 mg•kg^{-1}•min^{-1} measured without exercise. Variation across studies presents considerable heterogeneity with an I^2 of 99.1% and a τ^2 of 0.10. To present the range of effects in a way that acknowledges this heterogeneity, we used the prediction interval. To account for this heterogeneity, we used the prediction interval to present the range of outcomes. The prediction interval of the increase in protein oxidation is [0.37, 1.67] mg•kg^{-1}•min^{-1}.

4.2 | Protein's Contribution to Total Energy During Exercise

Protein contributed $3.28\% \pm 0.15\%$ (95% CI [2.97, 3.58]) of the total energy expenditure during endurance exercise (Figure 4). The prediction interval is [1.53, 5.03] %. The I^2 is 98.1%, and τ^2 is 0.72. In the included studies, the contribution of protein to the total energy expenditure was $22.17\% \pm 0.60\%$ without exercise.

4.3 | Effect of Exercise Intensity

An increase in exercise intensity was associated with a greater quantity of protein oxidized during exercise when normalized by body weight and exercise duration ($r^2 = 0.254$; $p = 0.001$) (Figure 5A). Exercise intensity had no effect on the protein's contribution to energy supply ($r^2 = 0.018$; $p = 0.205$) (Figure 5B).

4.4 | Effect of Exercise Duration

Exercise duration had no effect on the quantity of protein oxidized when normalized by body weight and exercise duration ($r^2 = 0$; $p = 0.505$) (Figure 6A). Exercise duration was not associated with the contribution of the protein to energy supply ($r^2 = 0.020$; $p = 0.189$) (Figure 6B).

4.5 | Effect of Exercise Volume

Exercise volume had no effect on the quantity of protein oxidized when normalized by body weight and exercise duration ($r^2 = 0.062$; $p = 0.099$) (Figure 7A). Exercise volume was not associated with the protein's contribution to energy supply ($r^2 = 0.046$; $p = 0.102$) (Figure 7B). The results obtained are summarized in Table 3.

5 | Discussion

5.1 | Meta-Analysis of the Protein Oxidation During Endurance Exercise

The main finding was that endurance exercise increased protein oxidation, with an average of 1.02 ± 0.06 mg•kg^{-1}•min^{-1} (95% CI [0.91, 1.14]) of additional protein being oxidized during

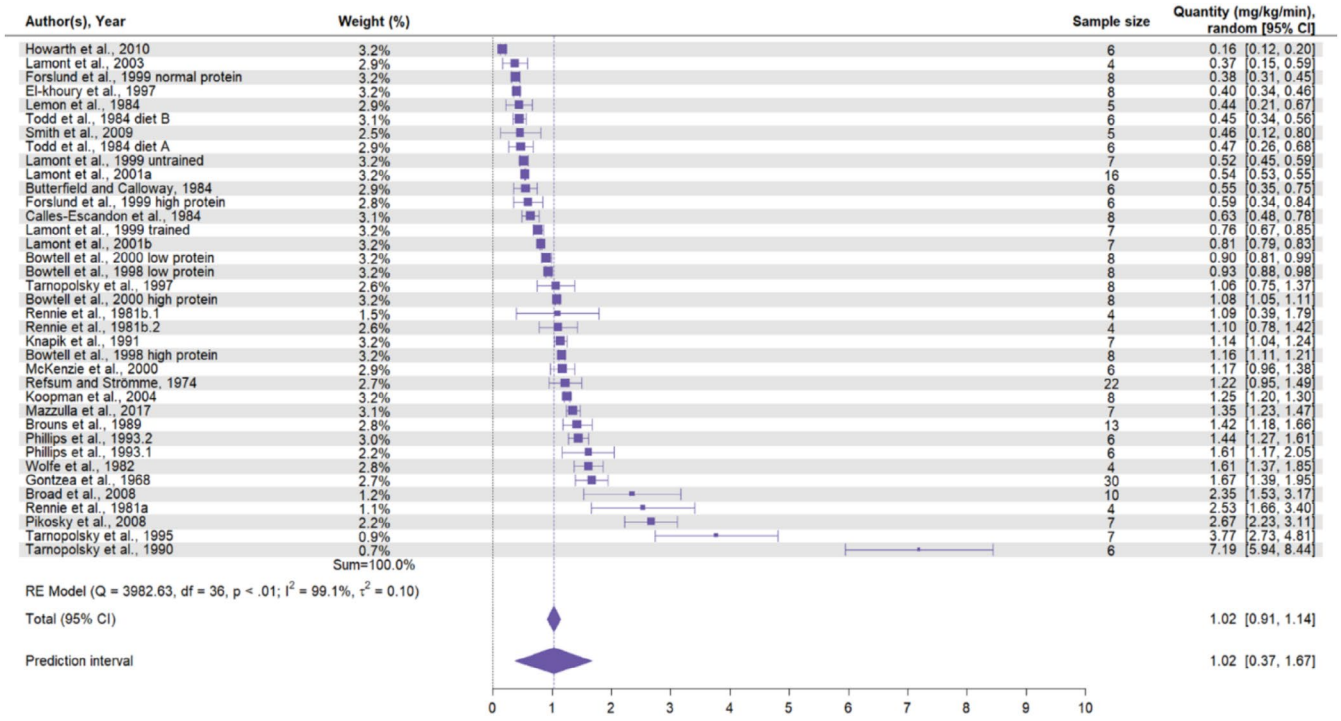

FIGURE 3 | Forest plot of the random-effects meta-analysis of the additional quantity of protein oxidized during exercise, normalized by body weight and exercise duration ($mg \bullet kg^{-1} \bullet min^{-1}$).

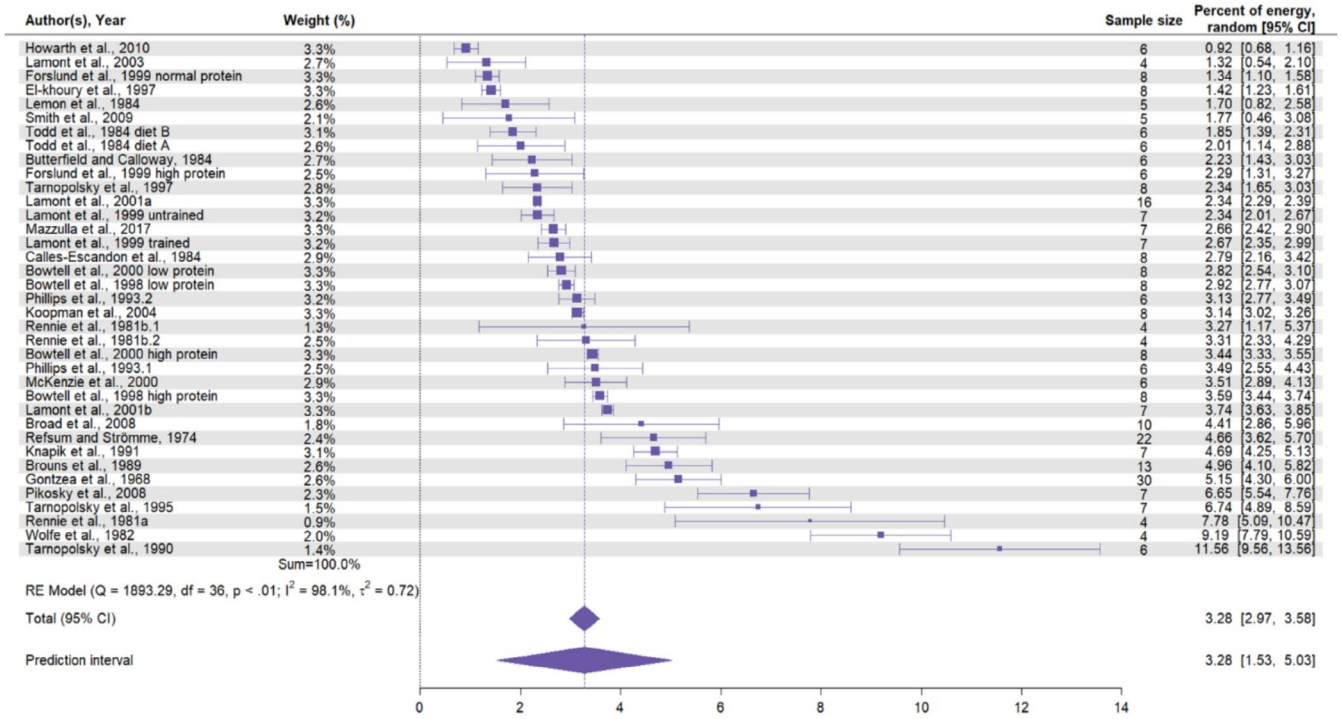

FIGURE 4 | Forest plot of the random-effects meta-analysis of protein's contribution to energy supply during exercise.

exercise (Figure 3). This showed that protein metabolism more than doubled during exercise, since protein oxidation on control days without exercise was $0.81 \pm 0.38\ mg \bullet kg^{-1} \bullet min^{-1}$, corresponding to a daily protein oxidation of 82 g for a 70 kg person. Every study included in the present review demonstrated an elevation in protein oxidation during endurance exercise. However, the energy contribution from protein metabolism during endurance exercise was low and covered only $3.28\% \pm 0.15\%$ of the energy expenditure during endurance exercise (Figure 4), with a range of 0.92%–11.56% across all included studies. This is in the same order of magnitude as previous studies calculating the contribution of protein as an energy source during endurance exercise [9, 21, 77]. Oxidation of CHO and fat increases much more than protein oxidation

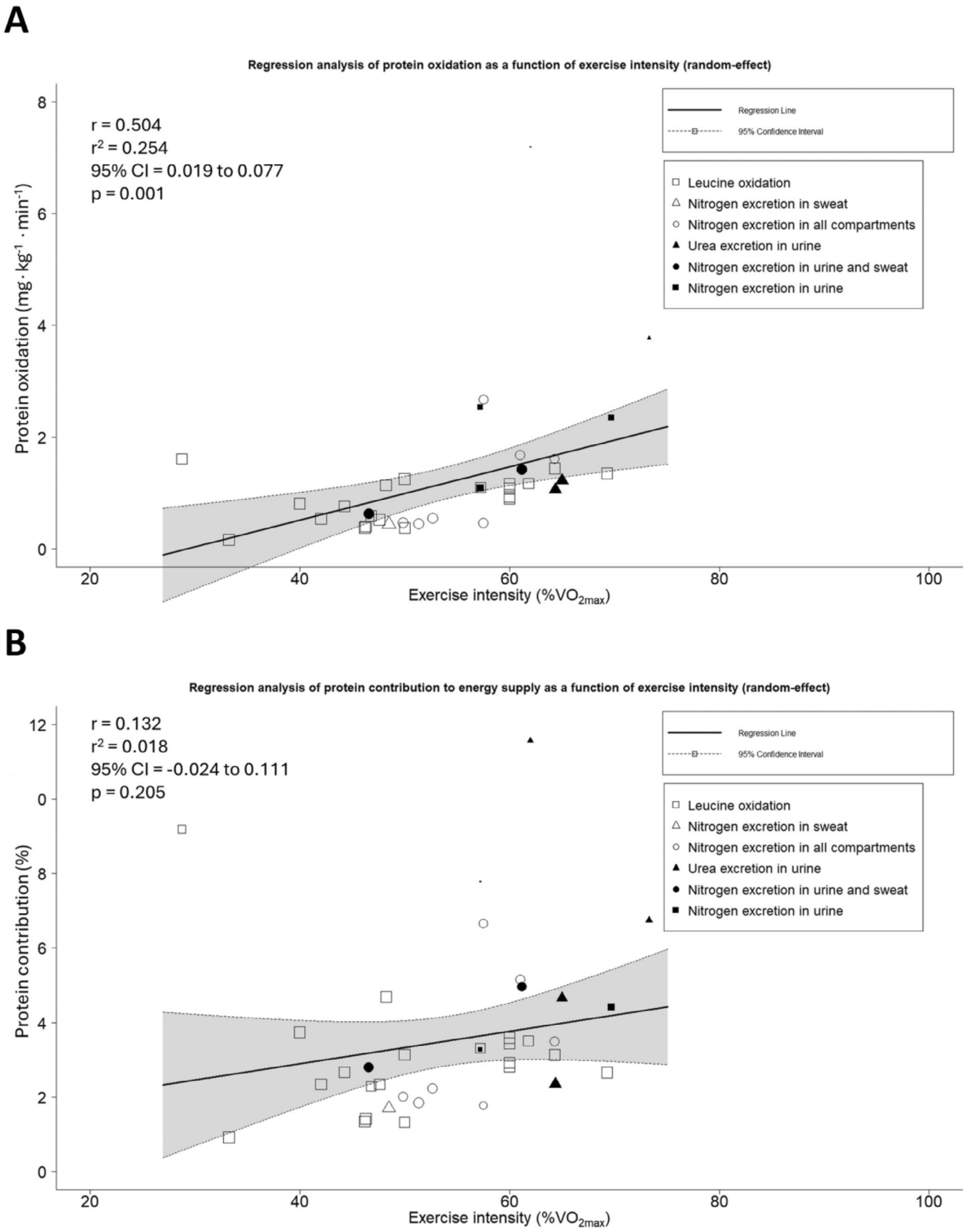

FIGURE 5 | Random-effects meta-regressions of the effect of exercise intensity on (A) protein oxidation normalized by body weight and exercise duration ($mg \bullet kg^{-1} \bullet min^{-1}$); and (B) protein's contribution to energy supply (%).

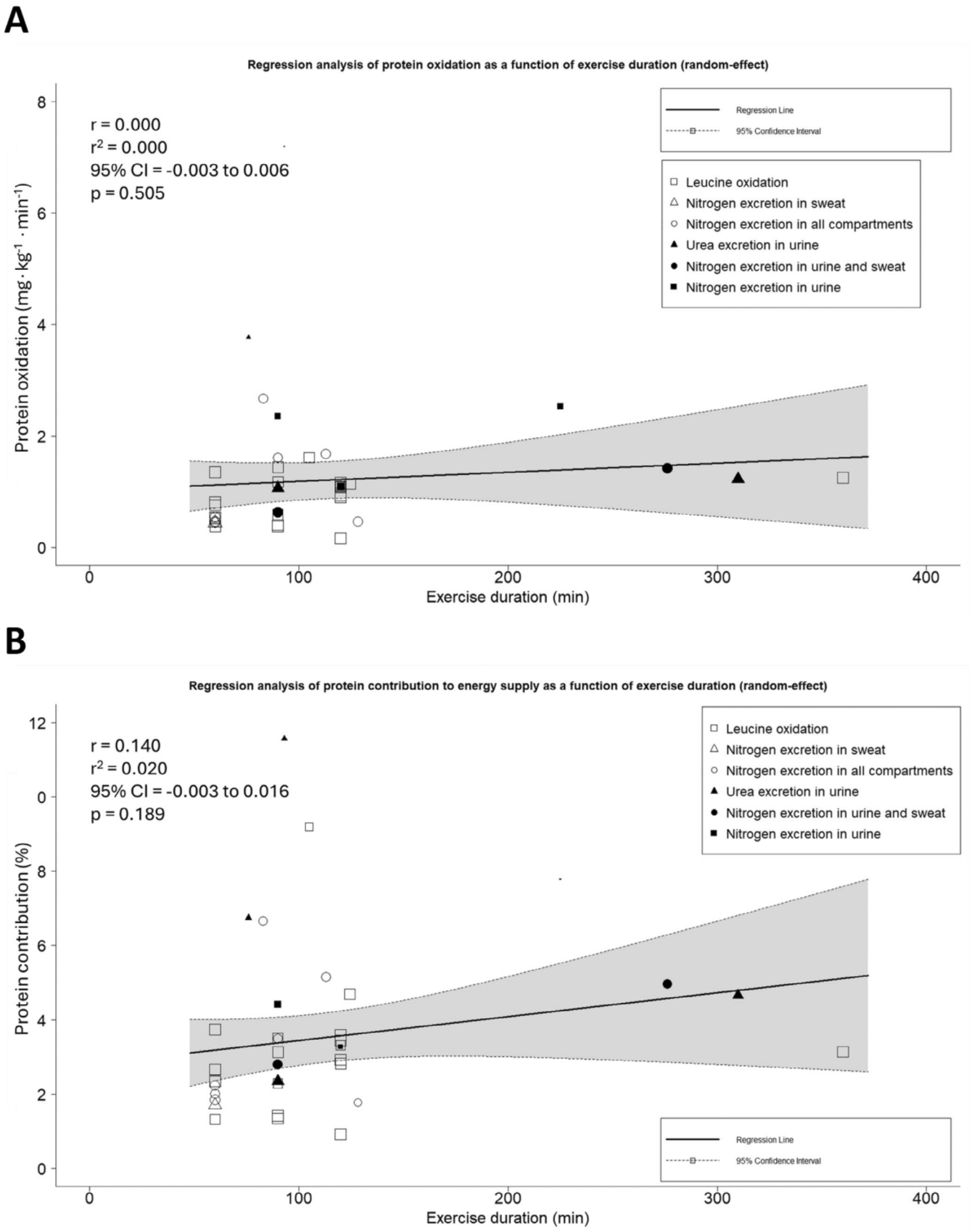

FIGURE 6 | Random-effects meta-regressions of the effect of exercise duration on (A) protein oxidation normalized by body weight and exercise duration (mg•kg^{-1}•min^{-1}); and (B) protein's contribution to energy supply (%).

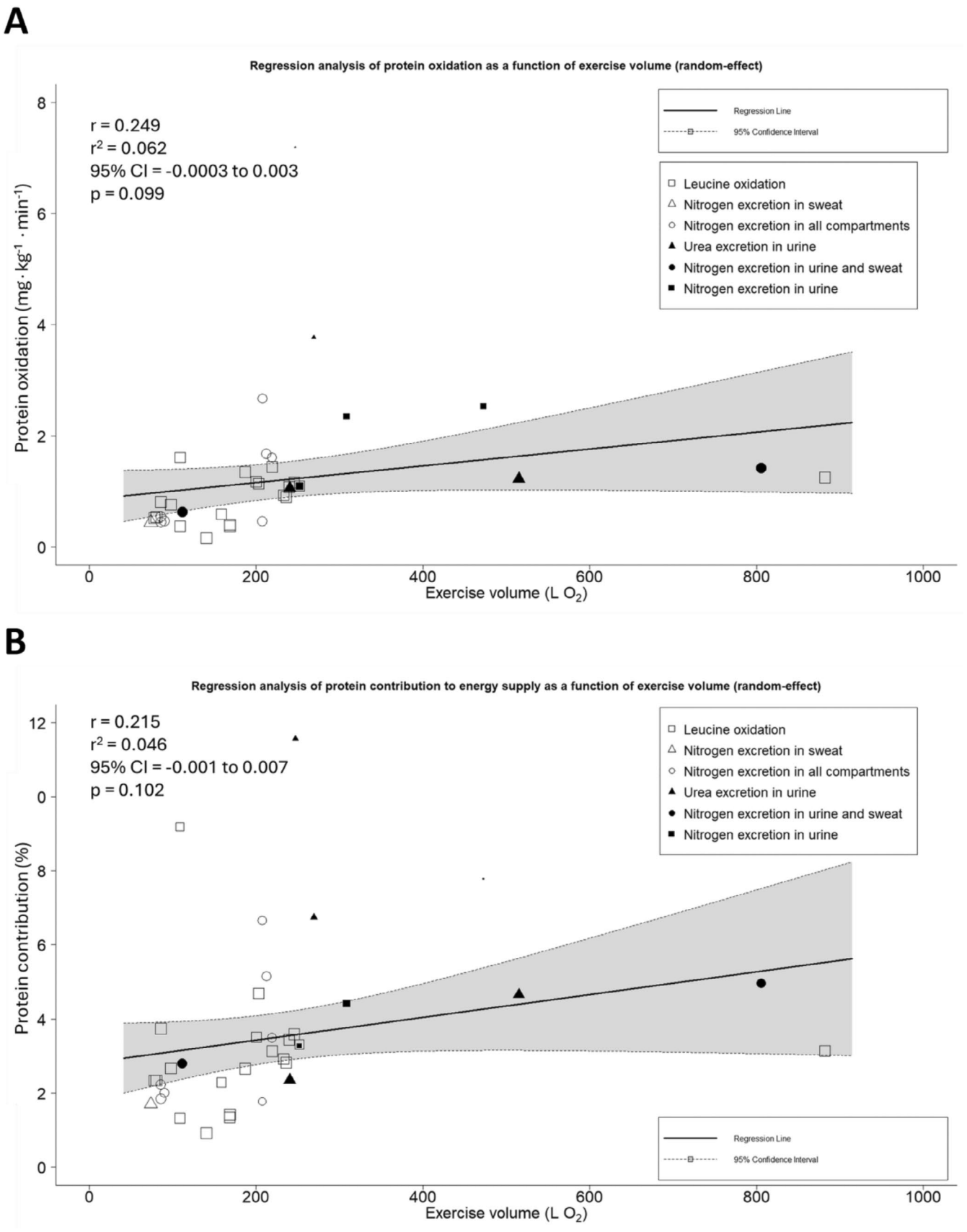

FIGURE 7 | Random-effects meta-regressions of the effect of exercise volume on (A) protein oxidation normalized by body weight and exercise duration (mg•kg^{-1}•min^{-1}); and (B) protein's contribution to energy supply (%).

TABLE 3 | Summary of results.

Parameter	Exercise intensity	Exercise duration	Exercise volume
Protein oxidation normalized by body weight and exercise duration ($mg•kg^{-1}•min^{-1}$)	Significant effect ($p = 0.001$)	No effect ($p = 0.505$)	No effect ($p = 0.099$)
Protein's contribution to energy supply (%)	No effect ($p = 0.205$)	No effect ($p = 0.189$)	No effect ($p = 0.102$)

during endurance exercise and provides more than 95% of the energy [3, 4].

5.2 | Effect of Exercise Intensity on Protein Oxidation

In this review, protein oxidation was positively correlated with exercise intensity. However, protein's contribution to energy supply during exercise remained constant regardless of the exercise intensity. Importantly, no human study to date has investigated the effect of exercise intensity on protein oxidation by performing the same exercise at different intensities. Nevertheless, rat studies using similar exercises at both easy and hard exercise intensities have shown that protein oxidation increased with higher exercise intensities, related to the increased metabolic rate [78, 79]. From the same data [79], it is also possible to calculate that the relative contribution of protein to energy supply remained constant, aligning with our findings. The studies included in the present meta-analysis used exercise intensities ranging from 29% to 73% of VO_{2max}. It is well documented in humans that as exercise intensity rises, a larger proportion of CHO is used relative to fat, reaching over 75% of the energy supply at the higher intensities of the included studies [3, 4]. Within the range of exercise intensities included in this review, both CHO and fat were primarily metabolized through the tricarboxylic acid (TCA) cycle [80]. Interestingly, our data suggest that protein metabolism also increased with rising exercise intensity, but the relative contribution did not significantly increase. While our data do not provide any mechanistic insights, they hopefully inspire further investigation into the effect of exercise intensity on protein oxidation directly.

It is well documented that the concentration of the sum of the TCA cycle intermediates increases during exercise [81]. Although pyruvate is the main contributor to anaplerosis, protein metabolism also contributes, mainly through the alanine aminotransferase reaction [82]. Interestingly, the increase in TCA cycle intermediates is augmented with rising exercise intensity [81]. Thus, a higher quantity of protein may be necessary to replenish TCA cycle intermediates at higher exercise intensity. Lamont et al. [64] also observed that protein oxidation increased with energy expenditure during exercise, as leucine oxidation was significantly positively correlated with oxygen consumption during exercise. This indicates that protein oxidation is related to aerobic energy pathways.

Interestingly, studies that reported improved performance following protein intake after exhaustive exercise used high-intensity exercises (> 70% of VO_{2max}) [5, 8, 83, 84]. The improvement in performance was greater the higher the intensity of the exhaustive exercise. This finding aligns with our meta-analysis, which showed that high-intensity exercise was associated with high protein requirements, as demonstrated by high protein oxidation during exercise above 70% of VO_{2max}. Furthermore, if we calculate protein oxidation during the exhaustive exercises in these studies [5, 8] using data from the present review (1.97 and 2.03 $mg•kg^{-1}•min^{-1}$ at 72% and 73% of VO_{2max} respectively) and add it to protein oxidation during control days without exercise from the present review (≈1.15 $g•kg^{-1}•d^{-1}$), these data accurately predict negative nitrogen balances when consuming a carbohydrate drink during recovery. Conversely, these data also accurately predict positive nitrogen balances when protein intake is increased during recovery [5, 8].

5.3 | Effect of Exercise Duration on Protein Oxidation

Protein oxidation was not correlated with exercise duration. This means that the quantity of protein oxidized per unit of time did not increase as the exercise period was extended. This is surprising because glycogen stores decrease with exercise duration [83, 85, 86] and some studies manipulating glycogen stores observed an increase in protein oxidation when glycogen stores were low. When starting exercise with different glycogen levels, protein oxidation during exercise was higher (+2130% [16]) when beginning with low glycogen levels compared to higher glycogen levels. Only one study found a lower protein oxidation (−47%) when starting with low glycogen levels [54]. However, participants cycled 45% longer before exhaustion with high glycogen levels, making comparisons of the effect of glycogen concentration not straightforward in this study. A study by Lemon and Mullin [9], which did not meet the inclusion criteria due to the absence of a control condition without exercise, also found higher protein oxidation (+89%) with low glycogen levels during a 1-h cycle ergometer exercise at 61% VO_{2max} following CHO loading and CHO depletion. A possible explanation for the discrepancy between our results on the effect of exercise duration and the results of studies manipulating glycogen stores may be the time point at which muscles reach low glycogen levels and the adaptations that result. In the studies described above, the muscle glycogen stores were manipulated before exercise and were low from the start of exercise, whereas in most of the studies included in the present review, the muscle glycogen stores were normal at the start of the intervention and decreased gradually during exercise until possibly becoming depleted. When starting

exercise with low glycogen stores, the participants had to rely on other energy substrates from the start of the exercise, leading to higher protein oxidation during exercise. Supporting this hypothesis are patients with McArdle's disease, who are unable to use muscle glycogen as an energy source during exercise. These patients have a five-to-tenfold larger leg release of both ammonia and glutamine during exercise compared with healthy individuals [87]. They also have a larger uptake of branched-chain amino acids in exercising leg muscles and show a more rapid activation of the muscle branched-chain 2-oxoacid dehydrogenase complex, a key enzyme in the degradation of the branched-chain amino acids [87]. In the included studies, glycogen degradation occurred gradually. Glycogen degradation depends on the exercise intensity, but low glycogen stores were reached from 1 to 3 h of exercise [83, 85, 86]. This means that participants in the included studies could rely on glycogen as an energy source at least in the first hours of exercise before having to rely on other energy sources. The increase in protein as an energy substrate therefore only occurs after the first hours of exercise and in a progressive manner. This may potentially explain the discrepancy in the reported effect of exercise duration.

Protein oxidation studied with stable isotopes has a high temporal resolution and allows investigation of the rate of protein oxidation change during prolonged exercise. Leucine oxidation during exercise was found to be higher than at rest, but it remained unchanged throughout exercise periods of 120 min [52] and 225 min [51]. This was confirmed by El Khoury et al. [69], who found similar levels of leucine oxidation throughout a 90-min exercise period and during the repetition of the same exercise 6 h later. Our analyses agree with these results, examining the effect of exercise duration on protein oxidation for this method separately. This confirms that protein oxidation did not change with exercise duration. Analysis of the nitrogen excretion studies also did not indicate an effect of exercise duration on protein oxidation. Unfortunately, this cannot be confirmed by results from simple studies because nitrogen excretion has low temporal resolution, making the monitoring of changes in nitrogen excretion over time during exercise not feasible. Indeed, during exercise, both kidney blood flow and urine flow fall [88], leading to a reduction in urea clearance. As a result, the excretion of urea is delayed until after exercise [51].

5.4 | Effect of Exercise Volume on Protein Oxidation

In this review, exercise volume was defined as the total volume of oxygen consumed during exercise, equating it with energy expenditure during exercise. Protein oxidation was not correlated with exercise volume. This outcome is unsurprising as the duration of exercise was the main determinant of the exercise volume. The inclusion criteria contributed to this relationship between exercise duration and volume by restricting variations in exercise intensity, given the minimum exercise duration requirement of 60 min.

Previous studies indicate that energy balance impacts protein oxidation [18, 19]. Protein oxidation increased when participants were in a negative energy balance during exercise and was unchanged when the additional energy expended during exercise was compensated for by increased energy intake. Further studies are necessary to determine the effect of energy expenditure during exercise on protein oxidation.

5.5 | Consequences of Protein Oxidation During Endurance Exercise

The degree of protein oxidation during endurance exercise, as calculated in this review, may have significant consequences. For instance, using the linear regression obtained in Figure 5A, 120 min of endurance exercise at 60% VO_{2max} equates to ≈180 mg•kg^{-1} or ≈12.4 g of protein oxidized for a 70 kg individual. Considering that the body's protein stores are limited and primarily functional, increased protein oxidation during exercise could have negative consequences for prolonged endurance exercise or exercises conducted over several consecutive days without adequate nutritional intake.

These findings underscore the importance of adequate protein intake for endurance athletes. The World Health Organization's (WHO) [89] protein recommendations of 0.83 g•kg^{-1}•d^{-1} for sedentary adults are lower than our data on the control days without exercise, which indicated ≈1.16 g•kg^{-1}•d^{-1} of protein oxidized. As protein oxidation increased with endurance exercise, the WHO's protein recommendations appear to be too low for endurance athletes, as also mentioned by other authors [37]. Data from the present review indicated that ≈1.32 g•kg^{-1}•d^{-1} of protein were oxidized on the exercise days (in the included studies, the average exercise duration during the exercise days was 112 ± 72 min and the average intensity was 54% ± 10% of VO_{2max}). This estimate is consistent with recommendations for endurance athletes to maintain zero balance [5, 8]. This estimate is also consistent with protein intake recommendations for endurance athletes of 1.6–1.8 g•kg^{-1}•d^{-1} using nitrogen balance [27, 90] and 1.65 g•kg^{-1}•d^{-1} using the indicator amino acid method [91, 92].

5.6 | Effect of Carbohydrate Ingestion During Exercise on Protein Oxidation

Carbohydrate ingestion during exercise and immediately afterward reduced protein oxidation, as measured by both nitrogen excretion [52, 59] and leucine oxidation [52, 70, 71]. In these studies [52, 59, 70, 71], the contribution of protein to total energy was reduced due to the higher availability of carbohydrates, which have been shown to increase carbohydrate oxidation and thus contribute more to the energy supply [85, 93]. While carbohydrate intake during exercise typically maintains plasma glucose concentration [85, 93], maintaining plasma glucose concentration during exercise, with or without carbohydrate intake, was not associated with reduced protein oxidation in the included studies [53, 55, 57, 64, 65, 67, 70, 71, 75].

Another potential mechanism is the increased energy balance with carbohydrate intake. Energy balance, defined as the difference between energy expenditure and energy intake, was positive when carbohydrates were ingested [52, 59, 70, 71]. In some

of the included studies investigating the effect of energy balance on protein oxidation, it was demonstrated that protein oxidation increased when participants were in a negative energy balance due to exercise [18, 19]. However, protein oxidation was no longer elevated when the extra energy expenditure from exercise was compensated for by additional energy intake [18, 19].

Finally, another explanation for the reduction in protein oxidation with carbohydrate supplementation during exercise is that carbohydrate intake can suppress the exercise-induced activation of mitochondrial branched-chain alpha-keto acid dehydrogenase [94]. Branched-chain alpha-keto acid dehydrogenase is the rate-limiting enzyme in BCAA metabolism [34, 95, 96], and its activity increases during exercise, enabling greater oxidation of BCAAs [97–101]. Reduced activation of branched-chain alpha-keto acid dehydrogenase could result in lower protein oxidation. Further studies are necessary to elucidate the exact mechanisms behind the effect of carbohydrate ingestion on protein oxidation.

5.7 | Methodological Considerations

The present review included studies measuring protein oxidation during exercise with different methods. Some authors have previously argued that different methods can yield varying results in terms of the effect of exercise [102, 103]. However, comparing the estimates of protein oxidation derived from each method in our data, we did not observe any significant differences between these estimates. This was confirmed by studies using several methods. Phillips et al. [37] found that protein oxidation was $1.61 \pm 0.55\,mg \bullet kg^{-1} \bullet min^{-1}$ with nitrogen excretion in all compartments and $1.44 \pm 0.21\,mg \bullet kg^{-1} \bullet min^{-1}$ with leucine oxidation, and these were not significantly different. Similarly, protein oxidation was not significantly different when calculated from data in Rennie et al. [52]. We calculated $1.09 \pm 0.71\,mg \bullet kg^{-1} \bullet min^{-1}$ with nitrogen excretion in urine corrected for all compartments and $1.10 \pm 0.33\,mg \bullet kg^{-1} \bullet min^{-1}$ with leucine oxidation. Lastly, el-Khoury et al. [69] found that the irreversible protein nitrogen loss was not significantly different when it was derived from nitrogen excretion in all compartments and from leucine oxidation. Only the data on protein oxidation with leucine oxidation from this study are presented in this review because the nitrogen excretion data lacked a resting control condition.

5.8 | Strengths and Limitations

In the present review, the majority of included studies are more than 25 years old. However, inclusion criteria guaranteed the inclusion of studies with data that still represent high-quality evidence based on current scientific standards. Some of the oldest studies were the ones with the highest control over study conditions and longest study duration [18, 19, 63]. These can be used as references to verify our estimates. In particular, Gontzea et al. [63] measured nitrogen excretion in all compartments under highly controlled conditions. During the study period, all feces and urine were collected in 24-h batches for measurement. Sweat was collected and analyzed both during rest and exercise periods. This was achieved while each participant remained in the laboratory for the entire duration of the experiments, which lasted between 28 and 32 days and occasionally extended to 52 days. We found no differences in protein oxidation between this study and our estimates of protein oxidation from studies reporting nitrogen excretion in some compartments, which we converted to encompass all compartments.

This review has some limitations. First, despite our comprehensive search strategy involving a variety of search terms, multiple databases, and manual scanning of reference lists, there is a chance that we may have missed some relevant articles. A potential limitation of our literature search was that it was confined to only PubMed and Google Scholar databases. The process of conducting a systematic review typically involves searching multiple databases, including EMBASE, Cochrane Library, and others, to ensure comprehensiveness and reduce bias. By casting a wider net, we increase the chance of capturing all relevant evidence on a given topic. Therefore, by restricting our search to only two databases, we may have inadvertently omitted pertinent studies, potentially impacting the comprehensiveness and unbiased nature of our review. Future reviews should consider utilizing multiple databases to ensure a more exhaustive and unbiased collection of relevant literature. It is also plausible that we may have missed some relevant articles given that the quantification of protein oxidation during endurance exercise is often not a primary outcome of many studies.

Second, our inclusion criteria were specifically designed to focus on studies that included an endurance exercise and a control condition. Consequently, we excluded studies lacking such a control condition ($n = 17$ studies). As a result, our selection was narrowed down, encompassing only studies that allowed for correction of nitrogen losses by obligatory losses without exercise.

Third, since protein oxidation during endurance exercise was rarely a primary outcome in the included studies, experimental conditions were neither standardized nor optimized for this outcome. For example, the included studies had different protein and energy intakes, and different metabolic states of the subjects (fasting or fed). These differences could account for the considerable heterogeneity observed in the study results regarding both the quantity of protein oxidized and the protein's contribution to energy supply during endurance exercise. Feeding status and protein intake directly impact protein oxidation during endurance exercise. For example, protein oxidation during exercise was higher in the fed state compared to the fasted state, with an increase of 136% on a high-protein diet and 45% on a normal-protein diet [76]. In another included study, protein oxidation during exercise was 66% higher in the fed state compared to the fasted state [69]. When comparing results between studies with quite similar measurement methods, exercise protocols, and participant characteristics, protein oxidation was higher in the fed state compared to the fasted state [37, 68], even though the study conducted in the fed state involved lower dietary protein intake [37]. This review includes studies conducted in both the fed and fasted states. However, it is important to note that most practitioners do not exercise in a fasted state; they typically focus on nutrition before and during exercise. As a result, the findings of this review may underestimate protein oxidation in real-world conditions where dietary intake occurs before exercising. With regard to protein intake, the high-protein diet resulted in an 87% higher protein oxidation rate compared to the normal-protein diet [76]. Similarly, some studies found that when transitioning

from a high to low protein intake, or vice versa, the transition impacted protein metabolism and it took ≈3 days before a new equilibrium was reached [104, 105]. Most of the included studies maintained the protein intake constant before the intervention days [18, 19, 37, 73, 51, 54, 55, 60–71, 75, 76], but some modified the protein intake for periods of < 3 days before the intervention days [53, 57, 59, 74, 77, 106] or did not control for protein intake [16, 58, 72, 107]. However, Pacy et al. [108] found that participants well accustomed to a certain dietary protein intake did not exhibit a significant change in the daily protein degradation rate at rest, regardless of the absolute value of protein intake. Similarly, participants demonstrated an increase in protein oxidation in response to exercise, irrespective of both very low and very high protein intakes [109]. Furthermore, correcting with a control intervention without exercise, as performed in this review, also reduced the effect of this factor on our conclusions. This indicates that differences in the protein intake between studies likely did not significantly impact our conclusions. Finally, the intensity, nature, and duration of exercise differed between studies, which could contribute to the observed heterogeneity among studies.

6 | Perspective

Gaining a comprehensive understanding of protein metabolism during endurance exercise is essential for elucidating the complex interactions among various energy sources during prolonged physical activity and their subsequent effects on muscle recovery and adaptation. Such knowledge can contribute to the development of optimal nutritional strategies, ensuring adequate protein intake to enhance endurance performance and facilitate effective recovery.

This review represents the first attempt to gather and calculate estimates of protein oxidation during endurance exercise using available data. Although most of the results of this review are in agreement with results found previously, this review also opens the door for new studies having as their main objective the study of protein oxidation. The finding that neither exercise duration nor volume increased protein oxidation seems contradictory to studies that manipulated glycogen stores and observed an increase in protein oxidation with lower glycogen levels. Further research, comparing the same participants across diverse exercise durations and volumes, is required to establish definitive conclusions regarding these relationships. Likewise, further investigation into the effect of exercise intensity on protein oxidation is necessary to provide mechanistic insights.

7 | Conclusion

The primary finding was that endurance exercise increases protein oxidation, with roughly 1 $mg \cdot kg^{-1} \cdot min^{-1}$ of additional protein oxidized during exercise. This signifies that protein metabolism more than doubles during exercise, given that protein oxidation without exercise is around 0.8 $mg \cdot kg^{-1} \cdot min^{-1}$. Still, the energy contribution from protein metabolism during endurance exercise remains modest, constituting approximately 3.3% of the energy supply. This estimate is consistent across various measurement methods. Protein oxidation increased with exercise intensity, though its relative contribution to energy supply did not significantly increase. Interestingly, neither exercise duration nor volume augmented absolute protein oxidation. Considering that the body's protein stores are limited and primarily functional, increased protein oxidation during exercise could have negative consequences. Therefore, adequate protein intake is necessary during endurance exercise.

Disclosure

Registration and protocol: The review was not registered, and a protocol was prepared but not accessible online. No amendments to the information provided in the protocol were performed.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

References

1. J. F. von Liebig, *Animal Chemistry, or Organic Chemistry in Its Applications to Physiology and Pathology* (Taylor and Walton, 1842).

2. D. A. Fick and D. J. Wislicenus, "LXX. On the Origin of Muscular Power," *London and Edinburgh Philosophical Magazine and Journal of Science* 31 (1866): 485–503, https://doi.org/10.1080/14786446608644105.

3. J. A. Romijn, E. F. Coyle, L. S. Sidossis, et al., "Regulation of Endogenous Fat and Carbohydrate Metabolism in Relation to Exercise Intensity and Duration," *American Journal of Physiology* 265 (1993): E380–E391, https://doi.org/10.1152/ajpendo.1993.265.3.E380.

4. L. J. C. van Loon, P. L. Greenhaff, D. Constantin-Teodosiu, W. H. Saris, and A. J. Wagenmakers, "The Effects of Increasing Exercise Intensity on Muscle Fuel Utilisation in Humans," *Journal of Physiology* 536 (2001): 295–304, https://doi.org/10.1111/j.1469-7793.2001.00295.x.

5. P. I. Rustad, M. Sailer, K. T. Cumming, et al., "Intake of Protein Plus Carbohydrate During the First Two Hours After Exhaustive Cycling Improves Performance the Following Day," *PLoS One* 11 (2016): e0153229, https://doi.org/10.1371/journal.pone.0153229.

6. M. D. Mifflin, S. T. St Jeor, L. A. Hill, B. J. Scott, S. A. Daugherty, and Y. O. Koh, "A New Predictive Equation for Resting Energy Expenditure in Healthy Individuals," *American Journal of Clinical Nutrition* 51 (1990): 241–247, https://doi.org/10.1093/ajcn/51.2.241.

7. K. Melzer, "Carbohydrate and Fat Utilization During Rest and Physical Activity," *E-SPEN, the European e-Journal of Clinical Nutrition and Metabolism* 6 (2011): e45–e52, https://doi.org/10.1016/j.eclnm.2011.01.005.

8. O. Sollie, P. B. Jeppesen, D. S. Tangen, et al., "Protein Intake in the Early Recovery Period After Exhaustive Exercise Improves Performance the Following Day," *Journal of Applied Physiology* 125 (2018): 1731–1742, https://doi.org/10.1152/japplphysiol.01132.2017.

9. P. W. Lemon and J. P. Mullin, "Effect of Initial Muscle Glycogen Levels on Protein Catabolism During Exercise," *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology* 48 (1980): 624–629, https://doi.org/10.1152/jappl.1980.48.4.624.

10. P. W. Lemon and F. J. Nagle, "Effects of Exercise on Protein and Amino Acid Metabolism," *Medicine and Science in Sports and Exercise* 13 (1981): 141–149.

11. J. B. Gillen, J. Trommelen, F. C. Wardenaar, et al., "Dietary Protein Intake and Distribution Patterns of Well-Trained Dutch Athletes," *International Journal of Sport Nutrition and Exercise Metabolism* 27 (2017): 105–114, https://doi.org/10.1123/ijsnem.2016-0154.

12. B. Smeuninx, C. A. Greig, and L. Breen, "Amount, Source and Pattern of Dietary Protein Intake Across the Adult Lifespan: A Cross-Sectional Study," *Frontiers in Nutrition* 7 (2020): 25, https://doi.org/10.3389/fnut.2020.00025.

13. E. F. Coyle, "Substrate Utilization During Exercise in Active People," *American Journal of Clinical Nutrition* 61 (1995): 968S–979S, https://doi.org/10.1093/ajcn/61.4.968S.

14. A. E. Jeukendrup and G. A. Wallis, "Measurement of Substrate Oxidation During Exercise by Means of Gas Exchange Measurements," *International Journal of Sports Medicine* 26, no. 1 (2005): S28–S37, https://doi.org/10.1055/s-2004-830512.

15. D. H. Wasserman, "Four Grams of Glucose," *American Journal of Physiology. Endocrinology and Metabolism* 296 (2009): E11–E21, https://doi.org/10.1152/ajpendo.90563.2008.

16. K. R. Howarth, S. M. Phillips, M. J. MacDonald, D. Richards, N. A. Moreau, and M. J. Gibala, "Effect of Glycogen Availability on Human Skeletal Muscle Protein Turnover During Exercise and Recovery," *Journal of Applied Physiology* 109 (2010): 431–438, https://doi.org/10.1152/japplphysiol.00108.2009.

17. D. J. Millward, C. T. Davies, D. Halliday, S. L. Wolman, D. Matthews, and M. Rennie, "Effect of Exercise on Protein Metabolism in Humans as Explored With Stable Isotopes," *Federation Proceedings* 41 (1982): 2686–2691.

18. K. S. Todd, G. E. Butterfield, and D. H. Calloway, "Nitrogen Balance in Men With Adequate and Deficient Energy Intake at Three Levels of Work," *Journal of Nutrition* 114 (1984): 2107–2118, https://doi.org/10.1093/jn/114.11.2107.

19. G. E. Butterfield and D. H. Calloway, "Physical Activity Improves Protein Utilization in Young Men," *BJN* 51 (1984): 171, https://doi.org/10.1079/BJN19840021.

20. L. J. Hoffer, "Human Protein and Amino Acid Requirements," *Journal of Parenteral and Enteral Nutrition* 40 (2016): 460–474, https://doi.org/10.1177/0148607115624084.

21. D. S. Rowlands and W. G. Hopkins, "Effects of High-Fat and High-Carbohydrate Diets on Metabolism and Performance in Cycling," *Metabolism* 51 (2002): 678–690, https://doi.org/10.1053/meta.2002.32723.

22. E. M. Widdowson, "Assessment of the Energy Value of Human Foods," *Proceedings of the Nutrition Society* 14 (1955): 142–154, https://doi.org/10.1079/pns19550031.

23. P. W. Lemon, K. Yarasheski, and D. Dolny, "The Importance of Protein for Athletes," *Sports Medicine* 1 (1984): 474–484, https://doi.org/10.2165/00007256-198401060-00006.

24. J. Kjeldahl, "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern [New method for determining nitrogen in organic bodies]," *Fresenius' Zeitschrift fuer Analytische Chemie* 22 (1883): 366–382, https://doi.org/10.1007/BF01338151.

25. D. H. Calloway, A. C. Odell, and S. Margen, "Sweat and Miscellaneous Nitrogen Losses in Human Balance Studies," *Journal of Nutrition* 101 (1971): 775–786, https://doi.org/10.1093/jn/101.6.775.

26. C. F. Consolazio, R. A. Nelson, L. O. Matoush, R. S. Harding, and J. E. Canham, "Nitrogen Excretion in Sweat and Its Relation to Nitrogen Balance Requirements," *Journal of Nutrition* 79 (1963): 399–406, https://doi.org/10.1093/jn/79.4.399.

27. M. A. Tarnopolsky, J. D. MacDougall, and S. A. Atkinson, "Influence of Protein Intake and Training Status on Nitrogen Balance and Lean Body Mass," *Journal of Applied Physiology* 64 (1988): 187–193, https://doi.org/10.1152/jappl.1988.64.1.187.

28. W. M. Rand, P. L. Pellett, and V. R. Young, "Meta-Analysis of Nitrogen Balance Studies for Estimating Protein Requirements in Healthy Adults," *American Journal of Clinical Nutrition* 77 (2003): 109–127, https://doi.org/10.1093/ajcn/77.1.109.

29. S. A. Bingham, R. Williams, T. J. Cole, C. P. Price, and J. H. Cummings, "Reference Values for Analytes of 24-h Urine Collections Known to be Complete," *Annals of Clinical Biochemistry* 25, no. Pt 6 (1988): 610–619, https://doi.org/10.1177/000456328802500603.

30. Y. Duan, F. Li, Y. Li, et al., "The Role of Leucine and Its Metabolites in Protein and Energy Metabolism," *Amino Acids* 48 (2016): 41–51, https://doi.org/10.1007/s00726-015-2067-1.

31. P. She, Y. Zhou, Z. Zhang, K. Griffin, K. Gowda, and C. J. Lynch, "Disruption of BCAA Metabolism in Mice Impairs Exercise Metabolism and Endurance," *Journal of Applied Physiology* 108 (2010): 941–949, https://doi.org/10.1152/japplphysiol.01248.2009.

32. R. R. Wolfe, "Tracers in Metabolic Research: Radioisotope and Stable Isotope/Mass Spectrometry Methods," *Laboratory and Research Methods in Biology and Medicine* 9 (1984): 1–287.

33. D. E. Matthews, K. J. Motil, D. K. Rohrbaugh, J. F. Burke, V. R. Young, and D. M. Bier, "Measurement of Leucine Metabolism in Man From a Primed, Continuous Infusion of L-[1-^{3}C]Leucine," *American Journal of Physiology* 238 (1980): E473–E479, https://doi.org/10.1152/ajpendo.1980.238.5.E473.

34. Y. Shimomura, T. Murakami, N. Nakai, M. Nagasaki, and R. A. Harris, "Exercise Promotes BCAA Catabolism: Effects of BCAA Supplementation on Skeletal Muscle During Exercise," *Journal of Nutrition* 134 (2004): 1583S–1587S, https://doi.org/10.1093/jn/134.6.1583S.

35. A. Vella and R. A. Rizza, "Application of Isotopic Techniques Using Constant Specific Activity or Enrichment to the Study of Carbohydrate Metabolism," *Diabetes* 58 (2009): 2168–2174, https://doi.org/10.2337/db09-0318.

36. R. Steele, "Influences of Glucose Loading and of Injected Insulin on Hepatic Glucose Output," *Annals of the New York Academy of Sciences* 82 (1959): 420–430, https://doi.org/10.1111/j.1749-6632.1959.tb44923.x.

37. S. M. Phillips, S. A. Atkinson, M. A. Tarnopolsky, and J. D. MacDougall, "Gender Differences in Leucine Kinetics and Nitrogen Balance in Endurance Athletes," *Journal of Applied Physiology* 75 (1993): 2134–2141, https://doi.org/10.1152/jappl.1993.75.5.2134.

38. R. Elango, R. O. Ball, and P. B. Pencharz, "Indicator Amino Acid Oxidation: Concept and Application," *Journal of Nutrition* 138 (2008): 243–246, https://doi.org/10.1093/jn/138.2.243.

39. E. A. Jones, A. Craigie, A. S. Tavill, W. Simon, and V. M. Rosenoer, "Urea Kinetics and the Direct Measurement of the Synthetic Rate of Albumin Utilizing [14C]Carbonate," *Clinical Science* 35 (1968): 553–564.

40. D. E. Matthews and R. S. Downey, "Measurement of Urea Kinetics in Humans: A Validation of Stable Isotope Tracer Methods," *American Journal of Physiology* 246 (1984): E519–E527, https://doi.org/10.1152/ajpendo.1984.246.6.E519.

41. M. J. Page, J. E. McKenzie, P. M. Bossuyt, et al., "The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews," *BMJ* 372 (2021): n71, https://doi.org/10.1136/bmj.n71.

42. FAO, *FAO Expert Consultation Dietary Protein Quality Evaluation in Human Nutrition* (Food and Agriculture Organization of the United Nations, 2013).

43. D. Hoy, P. Brooks, A. Woolf, et al., "Assessing Risk of Bias in Prevalence Studies: Modification of an Existing Tool and Evidence of Interrater Agreement," *Journal of Clinical Epidemiology* 65 (2012): 934–939, https://doi.org/10.1016/j.jclinepi.2011.11.014.

44. D. Atkins, D. Best, P. A. Briss, et al., "Grading Quality of Evidence and Strength of Recommendations," *BMJ* 328 (2004): 1490, https://doi.org/10.1136/bmj.328.7454.1490.

45. F. Mariotti, D. Tomé, and P. P. Mirand, "Converting Nitrogen Into Protein—Beyond 6.25 and Jones' Factors," *Critical Reviews in Food Science and Nutrition* 48 (2008): 177–184, https://doi.org/10.1080/10408390701279749.

46. F. Péronnet and D. Massicotte, "Table of Nonprotein Respiratory Quotient: An Update," *Canadian Journal of Sport Sciences* 16 (1991): 23–29.

47. W. Viechtbauer, "Conducting Meta-Analyses in R With the Metafor Package," *Journal of Statistical Software* 36 (2010): 1–48, https://doi.org/10.18637/jss.v036.i03.

48. W. Viechtbauer, "Bias and Efficiency of Meta-Analytic Variance Estimators in the Random-Effects Model," *Journal of Educational and Behavioral Statistics* 30 (2005): 261–293, https://doi.org/10.3102/10769986030003261.

49. J. IntHout, J. P. A. Ioannidis, M. M. Rovers, and J. J. Goeman, "Plea for Routinely Presenting Prediction Intervals in Meta-Analysis," *BMJ Open* 6 (2016): e010247, https://doi.org/10.1136/bmjopen-2015-010247.

50. S. G. Thompson and J. P. T. Higgins, "How Should Meta-Regression Analyses be Undertaken and Interpreted?," *Statistics in Medicine* 21 (2002): 1559–1573, https://doi.org/10.1002/sim.1187.

51. M. J. Rennie, R. H. Edwards, S. Krywawych, et al., "Effect of Exercise on Protein Turnover in Man," *Clinical Science* 61 (1981): 627–639, https://doi.org/10.1042/cs0610627.

52. M. J. Rennie, D. Halliday, C. T. M. Davies, et al., "Exercise Induced Increase in Leucine Oxidation in Man and the Effect of Glucose," in *Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids* (Elsevier, 1981).

53. L. J. Tarnopolsky, J. D. MacDougall, S. A. Atkinson, M. A. Tarnopolsky, and J. R. Sutton, "Gender Differences in Substrate for Endurance Exercise," *Journal of Applied Physiology* 68 (1990): 302–308, https://doi.org/10.1152/jappl.1990.68.1.302.

54. M. A. Tarnopolsky, S. A. Atkinson, S. M. Phillips, and J. D. MacDougall, "Carbohydrate Loading and Metabolism During Exercise in Men and Women," *Journal of Applied Physiology* 78 (1995): 1360–1368, https://doi.org/10.1152/jappl.1995.78.4.1360.

55. M. A. Tarnopolsky, M. Bosman, J. R. Macdonald, D. Vandeputte, J. Martin, and B. D. Roy, "Postexercise Protein-Carbohydrate and Carbohydrate Supplements Increase Muscle Glycogen in Men and Women," *Journal of Applied Physiology* 83 (1997): 1877–1883, https://doi.org/10.1152/jappl.1997.83.6.1877.

56. H. E. Refsum and S. B. Strömme, "Urea and Creatinine Production and Excretion in Urine During and After Prolonged Heavy Exercise," *Scandinavian Journal of Clinical and Laboratory Investigation* 33 (1974): 247–254, https://doi.org/10.1080/00365517409082493.

57. E. M. Broad, R. J. Maughan, and S. D. R. Galloway, "Carbohydrate, Protein, and Fat Metabolism During Exercise After Oral Carnitine Supplementation in Humans," *International Journal of Sport Nutrition and Exercise Metabolism* 18 (2008): 567–584, https://doi.org/10.1123/ijsnem.18.6.567.

58. P. W. Lemon, D. M. Orenstein, and K. G. Henke, "Protein Metabolism During Moderate Exercise in Cystic Fibrosis Patients," *Journal of Sports Sciences* 2 (1984): 47–55, https://doi.org/10.1080/02640418408729695.

59. F. Brouns, W. H. Saris, J. Stroecken, et al., "Eating, Drinking, and Cycling. A Controlled Tour de France Simulation Study, Part II. Effect of Diet Manipulation," *International Journal of Sports Medicine* 10 (1989): 41–48, https://doi.org/10.1055/s-2007-1024953.

60. J. Calles-Escandon, J. J. Cunningham, P. Snyder, et al., "Influence of Exercise on Urea, Creatinine, and 3-Methylhistidine Excretion in Normal Human Subjects," *American Journal of Physiology* 246 (1984): E334–E338, https://doi.org/10.1152/ajpendo.1984.246.4.E334.

61. T. J. Smith, M. A. Pikosky, A. Grediagin, et al., "Aerobic Fitness Does Not Modulate Protein Metabolism in Response to Increased Exercise: A Controlled Trial," *Nutrition & Metabolism* 6 (2009): 28, https://doi.org/10.1186/1743-7075-6-28.

62. M. A. Pikosky, T. J. Smith, A. Grediagin, et al., "Increased Protein Maintains Nitrogen Balance During Exercise-Induced Energy Deficit," *Medicine and Science in Sports and Exercise* 40 (2008): 505–512, https://doi.org/10.1249/MSS.0b013e31815f6643.

63. I. Gontzea, P. Sutzesco, and S. Dumitrache, "Recherches Concernant l'influence de l'activité Musculaire Sur Le Métabolisme Azoté et Sur Le Besoin En Protéines de l'homme [Research Concerning the Influence of Muscular Activity on Nitrogen Metabolism and on the Human Protein Requirement]," *Ann Nutr Alim* 22 (1968): 183–238.

64. L. S. Lamont, A. McCullough, and S. Kalhan, "Relationship Between Leucine Oxidation and Oxygen Consumption During Steady-State Exercise," *Medicine and Science in Sports and Exercise* 33 (2001): 237–241.

65. L. S. Lamont, A. J. McCullough, and S. C. Kalhan, "Gender Differences in Leucine, but Not Lysine, Kinetics," *Journal of Applied Physiology* 91 (2001): 357–362, https://doi.org/10.1152/jappl.2001.91.1.357.

66. L. S. Lamont, A. J. McCullough, and S. C. Kalhan, "Gender Differences in the Regulation of Amino Acid Metabolism," *Journal of Applied Physiology* 95 (2003): 1259–1265, https://doi.org/10.1152/japplphysiol.01028.2002.

67. L. S. Lamont, A. J. McCullough, and S. C. Kalhan, "Comparison of Leucine Kinetics in Endurance-Trained and Sedentary Humans," *Journal of Applied Physiology* 86 (1999): 320–325, https://doi.org/10.1152/jappl.1999.86.1.320.

68. S. McKenzie, S. M. Phillips, S. L. Carter, S. Lowther, M. J. Gibala, and M. A. Tarnopolsky, "Endurance Exercise Training Attenuates Leucine Oxidation and BCOAD Activation During Exercise in Humans," *American Journal of Physiology. Endocrinology and Metabolism* 278 (2000): E580–E587, https://doi.org/10.1152/ajpendo.2000.278.4.E580.

69. A. E. el-Khoury, A. Forslund, R. Olsson, et al., "Moderate Exercise at Energy Balance Does Not Affect 24-h Leucine Oxidation or Nitrogen Retention in Healthy Men," *American Journal of Physiology* 273 (1997): E394–E407, https://doi.org/10.1152/ajpendo.1997.273.2.E394.

70. J. L. Bowtell, G. P. Leese, K. Smith, et al., "Modulation of Whole Body Protein Metabolism, During and After Exercise, by Variation of Dietary Protein," *Journal of Applied Physiology* 85 (1998): 1744–1752, https://doi.org/10.1152/jappl.1998.85.5.1744.

71. J. L. Bowtell, G. P. Leese, K. Smith, et al., "Effect of Oral Glucose on Leucine Turnover in Human Subjects at Rest and During Exercise at Two Levels of Dietary Protein," *Journal of Physiology* 525 (2000): 271–281, https://doi.org/10.1111/j.1469-7793.2000.00271.x.

72. R. R. Wolfe, R. D. Goodenough, M. H. Wolfe, G. T. Royle, and E. R. Nadel, "Isotopic Analysis of Leucine and Urea Metabolism in Exercising Humans," *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology* 52 (1982): 458–466, https://doi.org/10.1152/jappl.1982.52.2.458.

73. J. Knapik, C. Meredith, B. Jones, R. Fielding, V. Young, and W. Evans, "Leucine Metabolism During Fasting and Exercise," *Journal of Applied Physiology* 70 (1991): 43–47, https://doi.org/10.1152/jappl.1991.70.1.43.

74. R. Koopman, D. L. E. Pannemans, A. E. Jeukendrup, et al., "Combined Ingestion of Protein and Carbohydrate Improves Protein Balance During Ultra-Endurance Exercise," *American Journal of Physiology. Endocrinology and Metabolism* 287 (2004): E712–E720, https://doi.org/10.1152/ajpendo.00543.2003.

75. M. Mazzulla, J. T. Parel, J. W. Beals, et al., "Endurance Exercise Attenuates Postprandial Whole-Body Leucine Balance in Trained Men," *Medicine and Science in Sports and Exercise* 49 (2017): 2585–2592, https://doi.org/10.1249/MSS.0000000000001394.

76. A. H. Forslund, A. E. El-Khoury, R. M. Olsson, A. M. Sjödin, L. Hambraeus, and V. R. Young, "Effect of Protein Intake and Physical Activity on 24-h Pattern and Rate of Macronutrient Utilization," *American Journal of Physiology* 276 (1999): E964–E976, https://doi.org/10.1152/ajpendo.1999.276.5.E964.

77. F. Brouns, W. H. Saris, E. Beckers, et al., "Metabolic Changes Induced by Sustained Exhaustive Cycling and Diet Manipulation," *International Journal of Sports Medicine* 10, no. 1 (1989): S49–S62, https://doi.org/10.1055/s-2007-1024954.

78. T. P. White and G. A. Brooks, "[U-14C]Glucose, -Alanine, and -Leucine Oxidation in Rats at Rest and Two Intensities of Running," *American Journal of Physiology* 240 (1981): E155–E165, https://doi.org/10.1152/ajpendo.1981.240.2.E155.

79. P. W. Lemon, F. J. Nagle, J. P. Mullin, and N. J. Benevenga, "In Vivo Leucine Oxidation at Rest and During Two Intensities of Exercise," *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology* 53 (1982): 947–954, https://doi.org/10.1152/jappl.1982.53.4.947.

80. M. Hargreaves and L. L. Spriet, "Skeletal Muscle Energy Metabolism During Exercise," *Nature Metabolism* 2 (2020): 817–828, https://doi.org/10.1038/s42255-020-0251-4.

81. T. E. Graham and M. J. Gibala, "Anaplerosis of the Tricarboxylic Acid Cycle in Human Skeletal Muscle During Exercise. Magnitude, Sources, and Potential Physiological Significance," *Advances in Experimental Medicine and Biology* 441 (1998): 271–286, https://doi.org/10.1007/978-1-4899-1928-1_25.

82. A. J. Wagenmakers, "Protein and Amino Acid Metabolism in Human Muscle," *Advances in Experimental Medicine and Biology* 441 (1998): 307–319, https://doi.org/10.1007/978-1-4899-1928-1_28.

83. M. A. Dahl, J. L. Areta, P. B. Jeppesen, et al., "Coingestion of Protein and Carbohydrate in the Early Recovery Phase, Compared With Carbohydrate Only, Improves Endurance Performance Despite Similar Glycogen Degradation and AMPK Phosphorylation," *Journal of Applied Physiology* 129 (2020): 297–310, https://doi.org/10.1152/japplphysiol.00817.2019.

84. M. B. Williams, P. B. Raven, D. L. Fogt, and J. L. Ivy, "Effects of Recovery Beverages on Glycogen Restoration and Endurance Exercise Performance," *Journal of Strength and Conditioning Research* 17 (2003): 12–19, https://doi.org/10.1519/1533-4287(2003)017<0012:eorbog>2.0.co;2.

85. E. F. Coyle, A. R. Coggan, M. K. Hemmert, and J. L. Ivy, "Muscle Glycogen Utilization During Prolonged Strenuous Exercise When Fed Carbohydrate," *Journal of Applied Physiology* 61 (1986): 165–172, https://doi.org/10.1152/jappl.1986.61.1.165.

86. L. Hermansen, E. Hultman, and B. Saltin, "Muscle Glycogen During Prolonged Severe Exercise," *Acta Physiologica Scandinavica* 71 (1967): 129–139, https://doi.org/10.1111/j.1748-1716.1967.tb03719.x.

87. A. J. Wagenmakers, J. H. Coakley, and R. H. T. Edwards, "Metabolism of Branched-Chain Amino Acids and Ammonia During Exercise: Clues From McArdle's Disease," *International Journal of Sports Medicine* 11 (1990): S101–S113, https://doi.org/10.1055/s-2007-1024861.

88. J. Castenfors, "Renal Function During Prolonged Exercise," *Annals of the New York Academy of Sciences* 301 (1977): 151–159, https://doi.org/10.1111/j.1749-6632.1977.tb38194.x.

89. Joint WHO/FAO/UNU Expert Consultation, *Protein and Amino Acid Requirements in Human Nutrition* (WHO, 2007), 1–265.

90. C. N. Meredith, M. J. Zackin, W. R. Frontera, and W. J. Evans, "Dietary Protein Requirements and Body Protein Metabolism in Endurance-Trained Men," *Journal of Applied Physiology* 66 (1989): 2850–2856, https://doi.org/10.1152/jappl.1989.66.6.2850.

91. H. Kato, K. Suzuki, M. Bannai, and D. R. Moore, "Protein Requirements Are Elevated in Endurance Athletes After Exercise as Determined by the Indicator Amino Acid Oxidation Method," *PLoS One* 11 (2016): e0157406, https://doi.org/10.1371/journal.pone.0157406.

92. E. Williamson, H. Kato, K. A. Volterman, K. Suzuki, and D. R. Moore, "The Effect of Dietary Protein on Protein Metabolism and Performance in Endurance-Trained Males," *Medicine and Science in Sports and Exercise* 51 (2019): 352–360, https://doi.org/10.1249/MSS.0000000000001791.

93. M. Clauss, Ø. Skattebo, M. R. Dæhli, et al., "Carbohydrate Ingestion During Prolonged Cycling Improves Next-Day Time Trial Performance and Alters Amino Acid Concentrations," *Medicine & Science in Sports & Exercise* 55, no. 12 (2023): 2228–2240, https://doi.org/10.1249/MSS.0000000000003264.

94. A. J. Wagenmakers, E. J. Beckers, F. Brouns, et al., "Carbohydrate Supplementation, Glycogen Depletion, and Amino Acid Metabolism During Exercise," *American Journal of Physiology. Endocrinology and Metabolism* 260 (1991): E883–E890, https://doi.org/10.1152/ajpendo.1991.260.6.E883.

95. S. M. Hutson, "Branched Chain Alpha-Keto Acid Oxidative Decarboxylation in Skeletal Muscle Mitochondria. Effect of Isolation Procedure and Mitochondrial Delta pH," *Journal of Biological Chemistry* 261 (1986): 4420–4425.

96. F. L. Shinnick and A. E. Harper, "Branched-Chain Amino Acid Oxidation by Isolated Rat Tissue Preparations," *Biochimica et Biophysica Acta* 437 (1976): 477–486, https://doi.org/10.1016/0304-4165(76)90016-7.

97. G. J. Kasperek, "Regulation of Branched-Chain 2-Oxo Acid Dehydrogenase Activity During Exercise," *American Journal of Physiology. Endocrinology and Metabolism* 256 (1989): E186–E190, https://doi.org/10.1152/ajpendo.1989.256.1.E186.

98. G. J. Kasperek, G. L. Dohm, and R. D. Snider, "Activation of Branched-Chain Keto Acid Dehydrogenase by Exercise," *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 248 (1985): R166–R171, https://doi.org/10.1152/ajpregu.1985.248.2.R166.

99. G. J. Kasperek and R. D. Snider, "Effect of Exercise Intensity and Starvation on Activation of Branched-Chain Keto Acid Dehydrogenase by Exercise," *American Journal of Physiology* 252 (1987): E33–E37, https://doi.org/10.1152/ajpendo.1987.252.1.E33.

100. Y. Shimomura, H. Fujii, M. Suzuki, T. Murakami, N. Fujitsuka, and N. Nakai, "Branched-Chain Alpha-Keto Acid Dehydrogenase Complex in Rat Skeletal Muscle: Regulation of the Activity and Gene Expression by Nutrition and Physical Exercise," *Journal of Nutrition* 125 (1995): 1762S–1765S, https://doi.org/10.1093/jn/125.suppl_6.1762S.

101. G. van Hall, D. A. MacLean, B. Saltin, and A. J. Wagenmakers, "Mechanisms of Activation of Muscle Branched-Chain Alpha-Keto Acid Dehydrogenase During Exercise in Man," *Journal of Physiology* 494, no. Pt 3 (1996): 899–905, https://doi.org/10.1113/jphysiol.1996.sp021542.

102. A. J. Wagenmakers, "Tracers to Investigate Protein and Amino Acid Metabolism in Human Subjects," *Proceedings of the Nutrition Society* 58 (1999): 987–1000, https://doi.org/10.1017/S0029665199001305.

103. A. J. Wagenmakers, D. L. E. Pannemans, A. E. Jeukendrup, et al., "Effect of Prolonged Exercise on Protein Metabolism in Trained Men Ingesting Carbohydrates," *Clinical Nutrition* 16 (1997): 25, https://doi.org/10.1016/S0261-5614(97)80135-5.

104. D. J. Millward, "The Metabolic Basis of Amino Acid Requirements," in *Protein-Energy Interactions* (United Nations International Dietary Energy Consultative Group, 1991).

105. M. R. Quevedo, G. M. Price, D. Halliday, P. J. Pacy, and D. J. Millward, "Nitrogen Homoeostasis in Man: Diurnal Changes in Nitrogen Excretion, Leucine Oxidation and Whole Body Leucine Kinetics During a Reduction From a High to a Moderate Protein Intake," *Clinical Science* 86 (1994): 185–193, https://doi.org/10.1042/cs0860185.

106. F. Brouns, W. H. Saris, J. Stroecken, et al., "Eating, Drinking, and Cycling. A Controlled Tour de France Simulation Study, Part I,"

International Journal of Sports Medicine 10 (1989): 32–40, https://doi.org/10.1055/s-2007-1024952.

107. T. P. Stein, R. W. Hoyt, M. O. Toole, et al., "Protein and Energy Metabolism During Prolonged Exercise in Trained Athletes," *International Journal of Sports Medicine* 10 (1989): 311–316, https://doi.org/10.1055/s-2007-1024920.

108. P. J. Pacy, G. M. Price, D. Halliday, M. R. Quevedo, and D. J. Millward, "Nitrogen Homeostasis in Man: The Diurnal Responses of Protein Synthesis and Degradation and Amino Acid Oxidation to Diets With Increasing Protein Intakes," *Clinical Science* 86 (1994): 103–116, https://doi.org/10.1042/cs0860103.

109. R. A. Kocher, "Über Die Große Des Eiweißzerfalls Bei Fieber Und Bei Arbeitsleistung [About the Extent of Protein Breakdown during Fever and during Work]," *Deutsches Archiv für Klinische Medizin* CXV (1914): 82.

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