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Fuelling the female athlete: carbohydrate and protein recommendations

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Abstract

The role of nutrition in modulating training adaptation and exercise performance is well established. From a macronutrient perspective, the exercise intensity inherent to the training and competitive scenarios typically undertaken by elite athletes is largely dependent on carbohydrate (CHO) metabolism. In addition, dietary protein provides the essential building blocks to facilitate post-exercise tissue remodelling. As such, the optimal approach to periodizing daily CHO and protein intakes in order to promote training adaptation and performance remains an active area of research. Nonetheless, the research base underpinning contemporary sport nutrition guidelines has largely been conducted on male populations, some of which may not always be applicable to the female athlete. In the present paper, we therefore provide a critical review of CHO and protein requirements for female athletes whilst also highlighting areas for future research. On the basis of current evidence, we consider it premature to substantiate that female athletes require sex specific guidelines in relation to CHO or protein requirements provided energy needs are met. Rather, there is a definitive need for further research using sport-specific competition and training related exercise protocols that rigorously control for prior exercise, CHO/energy intake, contraceptive use and phase of menstrual cycle. Moreover, our overarching recommendation is to adopt an individualised approach that takes into account athlete specific training and competition goals whilst also considering personal symptoms associated with the menstrual cycle.

Keywords: glycogen, muscle protein synthesis, performance, recovery

1. Introduction

Athlete performance and training adaptations are intimately linked to the adequate, periodized intake of energy and macronutrients. Carbohydrate (CHO) is the pre-eminent macronutrient that fuels high intensity exercise and permits athletes to train and compete at their peak capacity. To support the remodelling of muscle and body proteins that underpins the physiological adaptations to training, dietary protein is the principle macronutrient as it provides amino acids to support training-induced tissue remodelling. Sports nutrition guidelines have subsequently been developed for the optimal consumption of these macronutrients for athletes spanning the strength-endurance continuum (Thomas et al., 2016). However, a major limitation to sports science is the regrettable under-representation of female research participants (Costello et al., 2014).

Therefore, the aim of the present review is to outline the current understanding of periodized CHO and protein intake in athletes with a primary focus on females; where gaps in our knowledge exist, research in males will attempt to be translated to female nutrient requirements based on potential sex hormone-related differences in CHO and protein metabolism. Finally, CHO and protein requirements will primarily be discussed in relation to maintaining energy balance, although consideration to periods of planned, suboptimal energy intake (e.g. for the goal of weight loss) will be included as needed.

2. Energy requirements

Understanding energy requirements of female athletes is of importance not only for health and performance but also for its influence on macronutrient metabolism and requirements. Energy intake relative to expenditure influences not only body mass but also body composition, which may be relevant to sport performance. Intentional manipulation of energy balance may be used to augment lean mass or reduce fat mass, which may in turn influence strength, speed, or power-to-weight ratio. While careful adjustment of energy intake has the potential to enhance performance, extended periods of diminished energy intake may pose risks to health and performance associated with low energy availability (see Heikura et al in this issue). In addition, suboptimal energy intake may also compromise the ability to meet optimal CHO targets and can increase dietary protein requirements, as detailed below. Thus, matching energy intake to energy requirements should be a cornerstone for performance nutrition and optimal macronutrient intake.

Due to their typically shorter stature and lower body mass, female athletes would predictably have lower energy requirements than male athletes. Reduced levels of lean mass, both in an absolute (total kg) and relative ($kg \cdot m^{-2}$) sense also predicts reduced energy requirements for female athletes versus their male counterparts. Estimating energy requirements of athletes is conceptually relatively simple yet can be challenging in practice due to the various methodologies available including food frequency questionnaires, interviews, and food logs (for review, see Heikura and Areta). A systematic review of self-reported energy intake versus energy expenditure determined using doubly labeled water indicated that athletes underreport intake by an average of ~667 $kcal \cdot day^{-1}$ or ~19% of daily energy requirements (Capling et al., 2017). While under-reporting may be due to common issues with food logs and dairies, it is also possible that drive-for-thinness and a pressure to control diet and body mass may influence female athletes' tendency to under-report. Thus, the sports nutrition practitioner must be aware of the energy requirements of their athlete and be able to identify signs of energy deficiency (e.g. menstrual irregularities, endocrine and hematological changes, reduced bone mass, compromised performance and/or training adaptations (Mountjoy et al., 2018)) in order to provide a strong foundation on which to apply the recommended macronutrient intakes discussed below. An overview of the energy requirements of representative female athletes is provided in Table 1.

Changes in energy expenditure throughout the menstrual cycle has the potential to impact body mass and composition as well as macronutrient intake via changes in appetite and total energy requirements. It has previously been suggested that the resting metabolic rate (RMR) increases by ~100-300 kcal·day^{-1} in late luteal phase versus early follicular phase (Bisdee 1989, Curtis 1996), although recent studies with methodological improvements have not corroborated these results (Benton 2020). Barr et al (1995) reported that females spontaneously increase their energy intake by ~300 kcal·day^{-1} during the follicular phase, suggesting that women naturally experience appetite changes in accordance with this change in RMR. Conversely, (Kammoun et al., 2017) has reported that women tend to have a higher body mass at the end of the luteal phase versus the mid-follicular phase, suggesting either changes in energy intake or expenditure are occurring, or other hormonal factors, for example those affecting fluid retention, may be at play. However, it is important to consider that energy requirements can also vary with energy availability (see Heikura et al. This Issue). While the magnitude of RMR suppression can vary according to a number of factors, a reduction of up to 10% has been reported in amenorrheic vs. eumenorrheic female endurance athletes (Melin et al. 2015). Thus, athletes and practitioners must be critically aware of the total energy requirements (both basal and exercise-induced expenditure) of female athletes in order to maximize their health, performance, and recovery.

3. Carbohydrate Requirements

The primary nutritional consideration for athletic populations is often focused on the CHO requirements that are necessary to promote competitive performance as well as maintain the desired daily training intensities and volume. It is now also recognised (at least in males) that the strategic manipulation of CHO availability in a meal-by-meal and day-by-day manner (commonly referred to as CHO periodisation) can regulate training-induced oxidative adaptations of skeletal muscle, as mediated via activation of regulatory cell signalling pathways when exercise is completed in CHO restricted states (Impey et al., 2018). Contemporary guidelines for daily CHO intake therefore recognise the need for flexibility according to the metabolic demands of the exercise challenge as well as the individual athlete goals of promoting training quality versus stimulating adaptation (Burke et al., 2018; Thomas et al., 2016). Given the potential for sex-specific differences in CHO and fat metabolism during exercise (as reviewed by Issaco et al. This Issue), the practical question that arises therefore, is whether female athletes should follow sex-specific CHO guidelines in relation to CHO requirements before, during and after exercise (**Table 2**). The complexity of this issue is exacerbated by methodological differences between studies including exercise modality/intensity/duration, muscle group examined, participant training status, nutritional status, menstrual phase, and/or the use of hormonal contraception. Additionally, the effects of menstrual cycle phase on appetite regulation, gastrointestinal symptoms and food cravings (e.g. sweet foods) may also affect habitual energy and absolute CHO intake (Krishnan et al., 2016), which can impact "real world" fuelling.

3.1. CHO Loading

A reduced capacity of endurance trained females to store glycogen in the vastus lateralis muscle (as assessed in the follicular phase) when compared with males was initially reported by (Tarnopolsky et al., 1995). After a 3-day CHO loading protocol initiated by a glycogen depletion protocol and followed by increased CHO intake (55 to 75% of habitual energy intake), the authors observed a 150 mmol.kg^{-1} dw difference in resting glycogen storage between males (550 mmol.kg^{-1} dw) and females (400 mmol.kg^{-1} dw). It was suggested that such differences may be due to the combination of greater prior glycogen depletion and a higher absolute CHO intake in males (8 g/kg body mass equating to 610 g CHO) compared with females (6 g/kg body mass equating to 370 g CHO). Indeed, the same group later demonstrated that when females complete a 4 day CHO loading protocol whereby a higher relative (9 g/kg body mass) and absolute CHO intake was consumed (540 g CHO), no differences in glycogen concentration (>700 mmol.kg^{-1} dw) was apparent when compared with males who consumed a comparable absolute dose (600 g CHO equating to 8 g·kg^{-1} body mass) (Tarnopolsky et al., 2001). Furthermore, (James et al., 2001) reported equivalent glycogen storage in male and female endurance-trained females on oral contraceptives (OC) (878 and 839 mmol.kg^{-1} dw as assessed pre- and post-menses, respectively) (796 mmol.kg^{-1} dw) after 3 days of 12 g CHO·kg^{-1} fat-free mass per day. Collectively these data suggest that the capacity to "load" muscle glycogen is not sex dependent provided a sufficient CHO intake is met (i.e. 8-12 g·kg^{-1}, as recommended (Thomas et al., 2016).

A pertinent practical question is whether the capacity to store glycogen is altered during specific phases of the menstrual cycle. A preliminary study in recreationally active non-OC users reported that resting glycogen concentration was marginally but statistically greater in the mid-luteal (ML) phase when compared with the mid-follicular (MF) phase (443 and 391 mmol.kg^{-1} dw, respectively) (Hackney, 1990). In response to a 3-day sub-optimal CHO feeding protocol (4 g·kg^{-1}·d^{-1}) consumed after prior glycogen depleting exercise, (Nicklas et al., 1989) also reported in moderately trained non-OC users that glycogen storage was greater in the ML phase compared with the MF phase (383 and 313 mmol.kg^{-1} dw, respectively). (McLay et al., 2007) similarly observed that the lowest muscle glycogen concentration occurred during the MF phase under normal (5.2 g·kg^{-1}·d^{-1}) dietary CHO conditions (575 mmol·kg^{-1} dw) when compared with the MF phase under CHO loaded (8.4 g·kg^{-1}·d^{-1}) conditions (728 mmol.kg^{-1} dw) or the ML phase in either normal (761 mmol.kg^{-1} dw) or CHO loaded conditions (756 mmol.kg^{-1} dw). Considering that exercise performance may be trivially impaired in the early follicular phase (McNulty et al., 2020) coupled with females reporting negative physical symptoms at the onset or during menses (Findlay et al., 2020), these data suggest female athletes should pay attention to CHO availability during the follicular phase of the menstrual cycle, especially in competitive or training scenarios where absolute glycogen availability may be limiting to performance. Given that glycogen is stored within distinct sub-cellular pools in both type I and type II muscle fibres, future studies on female participants should also assess the effects of CHO loading on glycogen storage (and subsequent exercise-induced utilisation) within the subsarcolemmal, intramyofibrillar and intermyofibrillar pools.

3.2. Daily CHO Availability

The most meaningful practical challenge is matching the CHO cost of a sport to that of actual CHO availability according to training demands/goals. Surprisingly, little is known regarding the glycogen requirements of "real world" competitive events and/or training sessions that are typically completed by both amateur and elite athletes. To address this shortcoming, we recently subjected a cohort of male and female (OC users) recreationally active runners to three outdoor training sessions: 1) a 10-mile road run (10-mile) at lactate threshold, 2) 8 x 800 m track intervals (8 x 800 m) at $\dot{V}O_{2max}$ velocity and 3) 3 x 10 minute track intervals (3 x 10 min) at lactate turn point (Impey et al., 2020). Each training session was commenced after a standardised training session and 2 days of controlled diet (6 g $CHO.kg^{-1}$ body mass per day) with females studied during the MF phase. In accordance with previous studies utilising moderate daily CHO intakes (Tarnopolsky et al., 1995), we observed that resting glycogen concentration prior to all training sessions was reduced in the gastrocnemius muscle of females versus males (~400 and ~500 $mmol.kg^{-1}$ dw, respectively) (Impey et al., 2020). Nonetheless, such differences in absolute glycogen concentration were of no functional relevance considering that all female participants were able to maintain the desired intensities and workload associated with each training session. As such, we deemed it unlikely that such differences in glycogen storage (and subsequent utilisation patterns) would necessitate sex-specific practical recommendations, at least when considering the training protocols and training status of the participants under investigation. It is acknowledged, however, that future studies are required to further evaluate the glycogen requirements associated with other sport-specific training and competition scenarios.

3.3. CHO Feeding During Exercise

It is well accepted that CHO feeding during exercise is ergogenic to performance (Stellingwerff & Cox, 2014), likely underpinned by liver glycogen sparing (Gonzalez et al., 2015), maintenance of plasma glucose and elevated CHO oxidation rates (Coyle et al., 1986) and direct effects upon the central nervous system (CNS) (Carter et al., 2004). Contemporary guidelines for athletic populations (Thomas et al., 2016) currently recommend CHO mouth rinsing when exercise duration is <60 minutes, CHO intake at a rate of 30-60 $g \cdot h^{-1}$ (from single sources such as glucose or maltodextrin) during 1-2.5 h of endurance exercise and finally, up to 90 $g \cdot h^{-1}$ of multi-transportable CHO (glucose:fructose blends) when exercise duration is >2.5 h.

Although much of the foundation for CHO feeding guidelines are primarily based from research studies conducted on male participants, there is no conclusive evidence to suggest that practical strategies should be different for female athletes. For example, the metabolic responses to CHO feeding during exercise (90 g/h of a 10.9% glucose solution) are similar in trained males and females (MF) during a 2 h cycling protocol completed at 67% VO_{2max} with no effects of sex on the relative contribution of fat, exogenous CHO, liver derived glucose or muscle glycogen oxidation to total energy expenditure during the final 60 minutes of exercise (Wallis et al., 2006). Moreover, peak exogenous CHO oxidation rates were not significantly different between males and females (0.7 and 0.65 $g \cdot min^{-1}$, respectively). The ergogenic effect of CHO ingestion during exercise (67 g·h^{-1} during 2 h cycling at 70% VO_{2peak} followed by a 4 kJ/kg time trial) is also apparent in both the MF and ML phase of the menstrual cycle in endurance trained non-OC users with no differences in rate of glucose disappearance, plasma glucose oxidation or total CHO oxidation between phases (Campbell et al., 2001). In contrast to single source CHO solutions, it is not yet clear whether females retain the capacity to achieve superior exogenous CHO oxidation rates when consuming dual source blends. Although this has not been comprehensively examined within the same study, it is noteworthy that peak exogenous CHO oxidation rates have been reported at 1.03 g/min in females (O'Hara et al., 2019) and 1.42 g·min^{-1} in males (O'Hara et al., 2017) in response to consuming 1.8 g/min of CHO (2:1 glucose/fructose ratio) during 2 h cycling (55% W_{max}). It is, of course, difficult to directly compare between studies and it is noteworthy that the phase of menstrual cycle and prevalence of contraceptive use was not specified in the former study.

Using a cohort of highly trained male and female cross country skiers, (Pettersson et al., 2019) recently assessed the effects of an 18% maltodextrin and fructose solution (1:0.8 ratio with additional alginate and pectin) administered at a rate of 2.2 g/min during a 2 h sub-maximal (70% VO_{2max}) roller skiing protocol. While CHO ingestion suppressed endogenous CHO utilisation by a similar magnitude (~18%), peak rates of exogenous CHO oxidation tended (P=0.064) to be less in females (1.2 g/min) than males (1.5 g/min). However, the authors acknowledge several limitations in their design, namely they did not control for phase of menstrual cycle and three out of six females were contraceptive users. Nonetheless, these data suggest that highly trained female athletes are able to tolerate high doses of CHO feeding during exercise (albeit in cold ambient conditions) without experiencing gastrointestinal symptoms that limit performance.

3.4. Post-Exercise Muscle Glycogen Resynthesis

For athletes who compete in multi-day sporting events (e.g. cycling tours), undertake a congested competition schedule (e.g. soccer competitions), and/or undertake a high volume of training with multiple sessions in a 24-h period (e.g. distance runners, rowers, swimmers), the replenishment of endogenous glycogen stores after such events or between specific training sessions is of upmost importance to promote performance in the subsequent bout of exercise. It is well established that CHO ingestion rates of 1.2 $g{\cdot}kg^{-1}{\cdot}h^{-1}$ are considered optimal during the short-term (0-4 hours) recovery from exercise in males (Burke et al., 2017) and there is no convincing evidence to support sex-specific differences during similar recovery durations. For example, (Tarnopolsky et al., (1997) reported similar rates of muscle glycogen resynthesis (35-40 mmol.kg dw.h^{-1}) in moderately trained males and females (MF with 38% OC users) when ingesting 1 $g{\cdot}kg^{-1}$ of CHO immediately and 1 h post completion of a 90 minute cycling protocol. More recently, Flynn et al., (2020) also reported similar rates of muscle glycogen resynthesis in recreationally active males and females (OC users but without menstrual phase standardization) when ingesting 1.6 $g{\cdot}kg^{-1}$ CHO immediately and 2 h post-completion of a 90 minute cycling protocol. To the authors' knowledge, however, no researchers have yet tested the effects of menstrual cycle phase on rates of muscle glycogen resynthesis during the early post-exercise recovery period.

3.5. CHO Periodisation

In male participants, a growing body of literature demonstrates that deliberately commencing and/or recovering from training sessions with reduced CHO availability (the so-called *train low* paradigm) potentiates the activation of cell signalling pathways with regulatory roles in training adaptation (Impey et al., 2018). Accordingly, several weeks of train low protocols (e.g. twice per day training, fasted training, sleep-low:train-low) increases oxidative enzyme activity and protein content (Morton et al., 2009; Yeo et al., 2008), whole body (Yeo et al., 2008) and intramuscular lipid oxidation (Hulston et al., 2010) and may also improve exercise capacity (Hansen et al., 2005) and performance (Marquet et al., 2016). These data have been translated practically according to the "fuel for the work required" model whereby CHO availability is adjusted day-by-day and meal-by-meal according to the metabolic demands and personalised goals of the upcoming session (Impey et al., 2018). In relation to females, fasted training did not induce superior mitochondrial adaptations in skeletal muscle of obese females when compared with fed training (Gillen et al., 2013), though it is noteworthy that no comparable studies have yet been conducted in healthy females. As such, it is currently unclear if CHO restriction is beneficial, neutral, or potentially maladaptive for female athletic populations, the latter of which is especially relevant when considering the potential effects of reduced CHO availability on overall energy availability and the modulation of symptoms associated with RED-S (see Heikura et al. This Issue). Despite 54% of female athletes recently reporting they engage in "fasted" training (Rothschild et al., 2020), it is clear that the efficacy of train-low strategies in female athletes should be a targeted area for further research.

4. Protein requirements

Dietary protein provides the requisite building blocks to help repair and rebuild body and, especially, muscle protein after exercise, positioning it as a vital nutrient for active populations. It is generally accepted that protein requirements exceed the recommended dietary allowance of ~0.8 $g \cdot kg^{-1} \cdot d^{-1}$, which reduces the risk of protein malnutrition. Current recommendations suggest a broad range for athletes (i.e. 1.2-2.0 $g \cdot kg^{-1} \cdot d^{-1}$) (Thomas et al., 2016) that may not adequately reflect the different needs amongst athletes of varying disciplines and physiological requirements (**Figure 1**). Compounding the challenge of providing specific recommendations for female athletes is the relative dearth of research performed in this population as well as the potential impact of menstrual status or contraceptive use (for review, see (Mercer et al., 2020).

4.1. Daily protein requirements

Muscle growth with resistance training must be supported by sufficient protein and, arguably, energy intake (Slater et al., 2019). It has recently been demonstrated using stable isotope methodology that whole body protein synthesis and net protein balance (a surrogate marker for acute lean tissue 'growth') after resistance exercise is maximized at an estimated average requirement (EAR) of ~1.5 $g \cdot kg^{-1} \cdot d^{-1}$ in trained females (Malowany et al., 2019). This tracer-derived EAR is similar to the ~1.6 $g \cdot kg^{-1} \cdot d^{-1}$ that has been reported to optimize training-induced gains in fat-free mass in a mixed-sex meta-analysis (Morton et al., 2018). Accounting for a standard 12% variance (i.e. 1.24 x EAR), these daily protein estimates would translate into a recommended dietary intake (RDI) of 1.9-2.0 $g \cdot kg^{-1} \cdot d^{-1}$, which is at the upper range of current consensus recommendations for this macronutrient (Thomas et al., 2016). However, it is important to note that strength trained athletes typically consume >1.9 $g \cdot kg^{-1} \cdot d^{-1}$ (Malowany et al., 2018), which may increase the metabolic requirement for protein irrespective of the true requirement for muscle growth (Tinline-Goodfellow et al., 2020). Thus, it may be more relevant to consider the amount and pattern of meal protein intake (see below) to arrive at an optimal daily protein target.

Protein requirements in endurance athletes have been known to be elevated above the current RDA of 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ since Phillips et al., (1993) demonstrated that female recreationally trained athletes were not able to maintain nitrogen balance on this intake. Subsequent short term nitrogen balance studies in trained female cyclists and triathletes support this finding by showing that the EAR for nitrogen equilibrium is 1.3-1.6 $g \cdot kg^{-1} \cdot d^{-1}$ in the follicular phase (Houltham & Rowlands, 2014; Rowlands & Wadsworth, 2011), which is within the range previously suggested for endurance athletes based on nitrogen balance studies in males (i.e. 1.2-1.4 g protein/kg/d; (Tarnopolsky, 2004). However, recent estimates for male endurance athletes during training suggest an EAR of ~1.6 g protein/kg/d and an RDI of ~1.8 g protein/kg/d is required to maximize whole body protein synthesis (Kato et al., 2016), which is arguably more physiologically relevant for athletes than the 'black box' approach of nitrogen balance studies (i.e. nitrogen in minus nitrogen out). This elevated requirement, which is ~1.7-fold greater than the EAR for non-exercising males using identical methodology (Humayun et al., 2007), is primarily related to the need to replenish the exercise-induced oxidative loss of the branched chain amino acids (Kato et al., 2018). This is notable as estrogen has been shown to attenuate amino acid (and specifically leucine) oxidation during exercise at the expense of greater lipolysis and fatty acid oxidation (Hamadeh et al., 2005; Phillips et al., 1993). In contrast, a low estrogen:progesterone (E:P) ratio that is characteristic of the luteal phase can increase protein catabolism, amino acid oxidation, and, in exercising females, nitrogen excretion (Lamont et al., 1987; Lariviere et al., 1994). Thus, the greater estogen:progesterone (P:E) ratio of the follicular phase may have a 'protein-sparing' effect whereas the lower P:E ratio of the luteal phase may align female athlete protein requirements more closely to their male counterparts. For example, the EAR to maximize whole body protein synthesis (~1.4 vs. 1.2 $g \cdot kg^{-1} \cdot d^{-1}$)(Packer et al., 2017; Wooding et al., 2017) and net protein balance (~1.4 vs. ~1.8 $g \cdot kg^{-1} \cdot d^{-1}$)(Mazzulla et al., 2018) in female athletes during the mid-luteal phase performing a variable intensity, stop-and-go 'team sport' type exercise is broadly similar to active males, respectively. A final consideration for female athletes, perhaps more so during the luteal phase, is that low CHO availability training (reviewed above) may modestly increase daily protein requirements by ~12% to replenish a greater exercise-induced amino acid oxidative loss (Gillen et al., 2019).

4.2. Acute per meal protein requirements

Resistance-trained athletes are generally attuned to the need to consume protein after exercise as it has been known for decades that amino acid ingestion attenuates the normal exercise-induced increase in fasted muscle protein breakdown and stimulates muscle protein synthesis (Biolo et al., 1997), the latter of which is the primary regulated variable in healthy adults. This results in the requisite net positive muscle protein balance that supports muscle growth with training, especially within the myofibrillar (i.e. contractile) protein fraction given its synthesis is sustained for up to 24 h after resistance exercise with protein ingestion (West et al., 2012). Current evidence reveals that during energy balance a bolus ingestion of ~0.3 g protein·kg^{-1} of high quality protein (e.g. whey) maximizes myofibrillar protein synthesis (MyoPS) after resistance exercise with greater intakes merely being diverted to amino acid oxidation (Moore, 2019). Athletes who are purposely restricting energy availability as a strategy to alter body composition may require ~0.4-0.5 g protein·kg^{-1} to enhance MyoPS (Moore, 2019). While no study has specifically assessed the post-exercise dose-response in females, available evidence suggest that females obtain a similar benefit from post-exercise protein ingestion as males as MyoPS rates are indistinguishable between sexes over a range of protein intakes during energy balance (i.e. ~0.32-0.37 g protein/kg) (West et al., 2012) and energy deficit (i.e. 30 kcal/kg FFM/d; 0-0.8 g protein/kg FFM) (Areta et al., 2014). Moreover, there is no difference in MyoPS with the ingestion of ~0.37 g protein·kg^{-1} 24 h after resistance-type single leg kicking exercise in women in the luteal or follicular phase, suggesting acute protein requirements to support muscle protein repair and remodelling are generally consistent across the menstrual phase.

Post-exercise protein ingestion is also important for endurance athletes as dietary amino acids can replenish exercise-induced oxidative losses and represent important precursors for the remodelling and synthesis of new muscle proteins, including both myofibrillar and mitochondrial proteins (Churchward-Venne et al., 2020). Whereas the rate of mitochondrial protein synthesis does not appear to be regulated by dietary protein, MyoPS is stimulated in a dose-dependent manner up to a plateau of ~0.5 g protein/kg after 90 min of cycling (60%VO2peak) in male athletes (Churchward-Venne et al., 2020). Interestingly, endurance exercise is known to mobilize amino acids from the breakdown of muscle (primarily myofibrillar) protein and attenuate muscle protein synthesis, which based on leg phenylalanine kinetics could translate into an acute loss of ~0.1-0.2 g muscle protein/kg/h (Howarth et al., 2010). Given that the rate of leucine oxidation (as a marker of protein oxidation) during endurance exercise (i.e. running at ~70%VO_{2peak}) is of a similar magnitude as this muscle protein mobilization (i.e. ~0.1 g body protein·kg^{-1}·h^{-1})(Mazzulla et al., 2017), the relative difference in the maximal effective protein dose for MyoPS between resistance and endurance exercise may represent in part the need to replenish these amino acid oxidative losses (Moore, 2020). Thus, inasmuch as these oxidative losses influence the per meal protein requirement to maximize muscle, and primarily MyoPS, it is possible that female endurance athletes may require a slightly lower acute protein intake in the follicular phase when the E:P ratio is highest and amino oxidation is lowest. For exercise that incorporates both aerobic and resistive components, such as team sports characterised by high-intensity intermittent exercise, female athletes may wish to err on the side of caution with a post-exercise protein target of ~0.4 g protein·kg^{-1}.

4.3. Protein frequency and pattern

While there does not appear to be a defined 'window of opportunity' for post-exercise protein ingestion, female athletes should aim to consume a source of high quality protein immediately after exercise to replenish any exercise-induced amino acid oxidative losses and, more importantly, to initiate muscle protein remodelling and repair, a key aspect of the recovery process that can only be maximally supported by exogenous amino acids. Often to meet the high energy requirements of training, female athletes commonly consume 4-5 meals per day (Burke et al., 2003). Incidentally, MyoPS rates in male athletes are greatest over 12 h of recovery when consuming four moderate protein meals (~0.25 g protein·kg^{-1}) every 3 h as compared to the same quantity of protein in two large or 8 small meals (Areta et al., 2013). In addition, pre-sleep protein ingestion has also been shown to enhance overnight rates of MyoPS (Snijders et al., 2019), highlighting this as an opportunistic meal time. Therefore, in contrast to the typical skewed daily distribution (Gillen et al., 2017), female athletes should focus on consuming moderate (~0.3 g protein·kg^{-1}) protein-containing meals (perhaps with the exception of slightly larger intake immediately after endurance exercise) every 3-4 h to maximize muscle protein repair and remodelling and to minimize amino acid oxidation during the prolonged (>24 h) recovery period. Incidentally, using this 'muscle-centric' approach to optimized meal protein intake and pattern would provide a daily intake of 1.5-1.7 g protein·kg^{-1}·d^{-1} with 5 feeding occasions, which is similar to the daily EAR for resistance and endurance athletes discussed above.

4.4. Protein type

Consuming a protein source that is rapidly digested and enriched in the essential amino acid leucine is generally regarded as the most effective means to 'turn on' and support maximal rates of muscle protein remodelling immediately after exercise (Stokes et al., 2018). However, the remodelling of skeletal muscle and replenishment of body protein stores can occur for up to 24 h after exercise (West et al., 2012), which may reduce the importance of this immediate post-exercise window if there is sufficient time between training bouts for recovery and refuelling (e.g. CHO replenishment; see above). Certainly within this prolonged recovery window athletes should prioritize the consumption of nutrient dense whole foods, which are a relatively understudied aspect of sports nutrition in comparison to protein supplements. In some cases, the food matrix of a protein source may be more anabolic than the sum of its parts (for review, see: (Burd et al., 2019)).

5. Summary

On the basis of current evidence, we consider it premature to substantiate that female athletes require sex-specific guidelines in relation to CHO or protein requirements provided energy needs are met. Rather, there is a definitive need for further research using sport-specific competition and training related exercise protocols that rigorously control for prior exercise, CHO/energy intake, contraceptive use and phase of menstrual cycle. Until such data exists, it remains prudent for female athletes to therefore adhere to previously published best practice guidelines that are generalised to athletic populations. However, our overarching recommendation is to adopt an individualised approach that takes into account athlete specific training and competition goals whilst also considering personal symptoms associated with the menstrual cycle.

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Declaration of interest statement

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Table 1: Energy expenditure of representative female athletes.

Sport Type	Discipline	Level	N	Body mass (kg)	Body fat (%)	Energy Expenditure, (kcal/day)	Method	Menstrual phase	Reference
Endurance	Artistic swimming	Elite (national team)	9 (4 senior, 5 junior)	52.5±2.7	N/R	2738±672	$^{2}H^{18}O$	N/R	(Ebine et al., 2000)
	Cross-country skiing	Elite (national team)	4	54.4±5.1	~17.5	4373±525	$^{2}H^{18}O$	N/R	(Sjödin et al., 1994)
	Rowing, lightweight	Elite	7	60.9±2.3	22.8±5.1	3957±1219	$^{2}H^{18}O$	N/R	(Hill & Davies, 2002)
	Running, endurance	Elite	9	53±4	12±3	2826±315	$^{2}H^{18}O$	N/R	(Schulz et al., 1992)
	Running, endurance	Sub-elite (university)	9	55.3±6.2	13.0±3.2	2990±415	$^{2}H^{18}O$	N/R	(Edwards et al., 1993)
Mixed	Basketball	Sub-elite (junior national)	7	64.0±5.4	~20.3	2497±242	$^{2}H^{18}O$	N/R	(Silva et al., 2013)
	Dance, ballet	Sub-elite (university)	12	N/R	N/R	~3176	$^{2}H^{18}O$	N/R	(Hill & Davies, 1999)
	Soccer	Elite	8	65.1±5.9	23.2±6.2	2863±439	ACC	N/R	(Mara et al., 2015)
Strength	Resistance training	Trained	10	59.4±5.7	15.4±2.9	~2796	IC	Follicular	(Binzen et al., 2001)

Mixed athletes = discipline requires both strength and endurance and typically features stop-and-go exercise. ACC = accelerometer; IC = indirect calorimetry; N/R = not reported. Values are mean ± standard deviation. N = number of female athletes.

Table 2. Daily and periodized carbohydrate recommendations.

Scenario	**CHO Recommendations**	**Sex-Specific Considerations**	**Directions for Further Research**
Daily CHO Requirements: *Daily CHO intake should be adjusted in accordance with the associated daily training loads and total daily energy expenditure as well as any individualised training goals (e.g. CHO periodisation strategies or body composition goals).*			
Light: Low-intensity or skill-based activities	4 g/kg	• Consider the phase of menstrual cycle (e.g. during menses) and potential effects of individualised physical and mental symptoms on ability to achieve these daily CHO targets. • Consider that glycogen storage may be reduced in the follicular phase. • Consider the phase of menstrual cycle in relation to appetite regulation, food cravings and potential effects on habitual absolute CHO intake.	*Where relevant and possible, future studies should control for prior exercise, energy / CHO intake, menstrual cycle phase and contraceptive use.* • Effects of menstrual cycle phase on training adherence and habitual nutritional intakes, food / taste preferences and gut health / function. • Effects of menstrual cycle on glycogen storage in sub-cellular pools. • Assessment of the glycogen cost (in sub-cellular pools) of typical training sessions completed by amateur and elite athletes. • Evaluation of the efficacy of train-low and CHO periodisation strategies that manipulate CHO intake "day-by-day and meal-by-meal".
Moderate: Moderate duration (≈ 1 h) and intensity	4-6 g/kg		
High: Longer duration (≈ 1-3 h) and periods of high intensity activity	6-8 g/kg		
Very High: Extreme duration (>4-5 h) with periods of high intensity activity	8-12 g/kg		
Acute Fuelling Strategies: *CHO intake should be adjusted in accordance with the associated energetic demands of the upcoming training session or competitive event as well as any individualised training goals (e.g. CHO periodisation strategies or body composition goals).*			

General Fuelling: 18-24 h before a key training session or competitive event <90 min in duration	6-8 g/kg	• Consider the phase of menstrual cycle (e.g. during menses) and associated effects of individualised physical and mental symptoms on ability to achieve these daily CHO targets. • Consider that glycogen storage may be reduced in the follicular phase • Consider the phase of menstrual cycle in relation to appetite regulation, food cravings and potential effects on habitual absolute CHO intake.	*Where relevant and possible, future studies should control for prior exercise, energy / CHO intake, menstrual cycle phase and contraceptive use.* • Effects of menstrual cycle phase on training adherence and habitual nutritional intakes, food / taste preferences and gut health / function. • Effects of menstrual cycle on glycogen storage in sub-cellular pools. • Assessment of the glycogen cost (sub-cellular pools) of typical training sessions and competitive events completed by amateur and elite athletes. • Evaluation of the efficacy of train-low and CHO periodisation strategies that manipulate “pre-exercise CHO availability”.
General: Fuelling 18-24 h before a key training session or competitive event >90 min in duration.	8-12 g/kg		
CHO Loading: 1-3 days extreme fuelling before a key competitive event >90 min in duration.	10-12 g/kg		
Pre-Exercise Meal: 1-4 h before training or competition.	1-4 g/kg		
CHO During Exercise: *CHO intake should be adjusted in accordance with the associated energetic demands of the training session or competitive event as well as any individualised training goals (e.g. CHO periodisation strategies or body composition goals).*			
Short duration exercise: <45 minutes	Not needed or CHO mouth rinse	• Consider the phase of menstrual cycle (e.g. during menses) and associated effects of individualised physical and mental symptoms on ability to achieve “in-exercise” CHO targets. • Consider that glycogen storage may be reduced in	*Where relevant and possible, future studies should control for prior exercise, energy / CHO intake, menstrual cycle phase and contraceptive use.* • Effects of menstrual cycle phase on training adherence and habitual CHO intake preferences during exercise
Sustained high-intensity exercise: 45-75 minute	CHO mouth rinse and/or 30 g/h		
Moderate intensity exercise and high-intensity intermittent exercise: 1-2.5 h	30-60 g/h		
Endurance exercise: > 2.5 h	90 g/h (dual source CHO blends)		

		the follicular phase and that CHO intake during exercise may be more crucial to maintain sufficient CHO availability.	(e.g. dose, format, source, taste) and associated gastrointestinal symptoms and gut function. • Assessment of maximal rates of exogenous CHO oxidation (using dual source blends). • Effects of menstrual cycle phase on exogenous rates of CHO oxidation (using dual source blends). • Evaluation of the efficacy of train-low and CHO periodisation strategies that manipulate CHO intake "during" exercise.
CHO Intake Post-Exercise: *CHO intake should be adjusted in accordance with the associated energetic demands and time-scale of when the next training session or competitive event occurs as well as any individualised training goals (e.g. CHO periodisation strategies or body composition goals).*			
Maximal recovery: 0-4 h post-exercise	1.2 g/kg/h	• Consider the phase of menstrual cycle (e.g. during menses) and associated effects of individualised physical and mental symptoms on ability to achieve "in-exercise" CHO targets. • Consider that glycogen storage may be reduced in the follicular phase and that CHO intake during this early post-exercise period may be even more crucial to optimise glycogen storage in accordance with the time-scale of the next training	*Where relevant and possible, future studies should control for prior exercise, energy / CHO intake, menstrual cycle phase and contraceptive use.* • Effects of menstrual cycle phase on habitual CHO intake preferences post exercise (e.g. dose, format, source, taste) and associated gastrointestinal symptoms and gut function. • Effects of menstrual cycle on maximal rates of glycogen re-synthesis in sub-cellular pools.

		session or competitive event.	• Evaluation of the efficacy of CHO periodisation strategies that manipulate “post-exercise CHO availability”.

Table 3: Meal protein intakes for female athletes

	Post-exercise[1]	**Daily[2]**	**Considerations**
Endurance training[3]	• 0.5 g/kg per meal • Rapidly digested, leucine-enriched	• 0.3 g/kg per meal • High quality, nutrient dense (e.g. whole foods) • Enriched in branched chain amino acids • Consume 4-5 equally spaced meals	• Post-exercise requirements may be slightly lower in the follicular phase • Include ~10% buffer with lower quality proteins (e.g. plant-based) • Requirements may be increased ~10-15% with low CHO availability training • Consume adequate energy • If tolerable, target last meal ~1-2 h before sleep
Resistance training[4]	• 0.3 g/kg per meal • Rapidly digested, leucine-enriched	• 0.3 g/kg per meal • High quality, nutrient dense (e.g. whole foods) • Consume 4-5 equally spaced meals	• Include ~10% buffer with lower quality proteins (e.g. plant-based) • Post-exercise requirements may be 0.4-0.5 g/kg in energy deficit (e.g. weight loss) • If tolerable, target last meal ~1-2 h before sleep
Mixed training[5]	• 0.4 g/kg per meal • Rapidly digested, leucine-enriched	• 0.3 g/kg per meal • High quality, nutrient dense (e.g. whole foods) • Consume 4-5 equally spaced meals	• Post-exercise requirements may be slightly lower in the follicular phase • Include ~10% buffer with lower quality proteins (e.g. plant-based) • Requirements may be increased ~10-15% with low CHO availability training • If tolerable, target last meal ~1-2 h before sleep

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[1] Post-exercise refers to the first meal after exercise, preferably within 1h after training cessation to maximize muscle protein synthesis.

[2] Daily meals refer to all meals throughout the day with the exception of the post-exercise meal

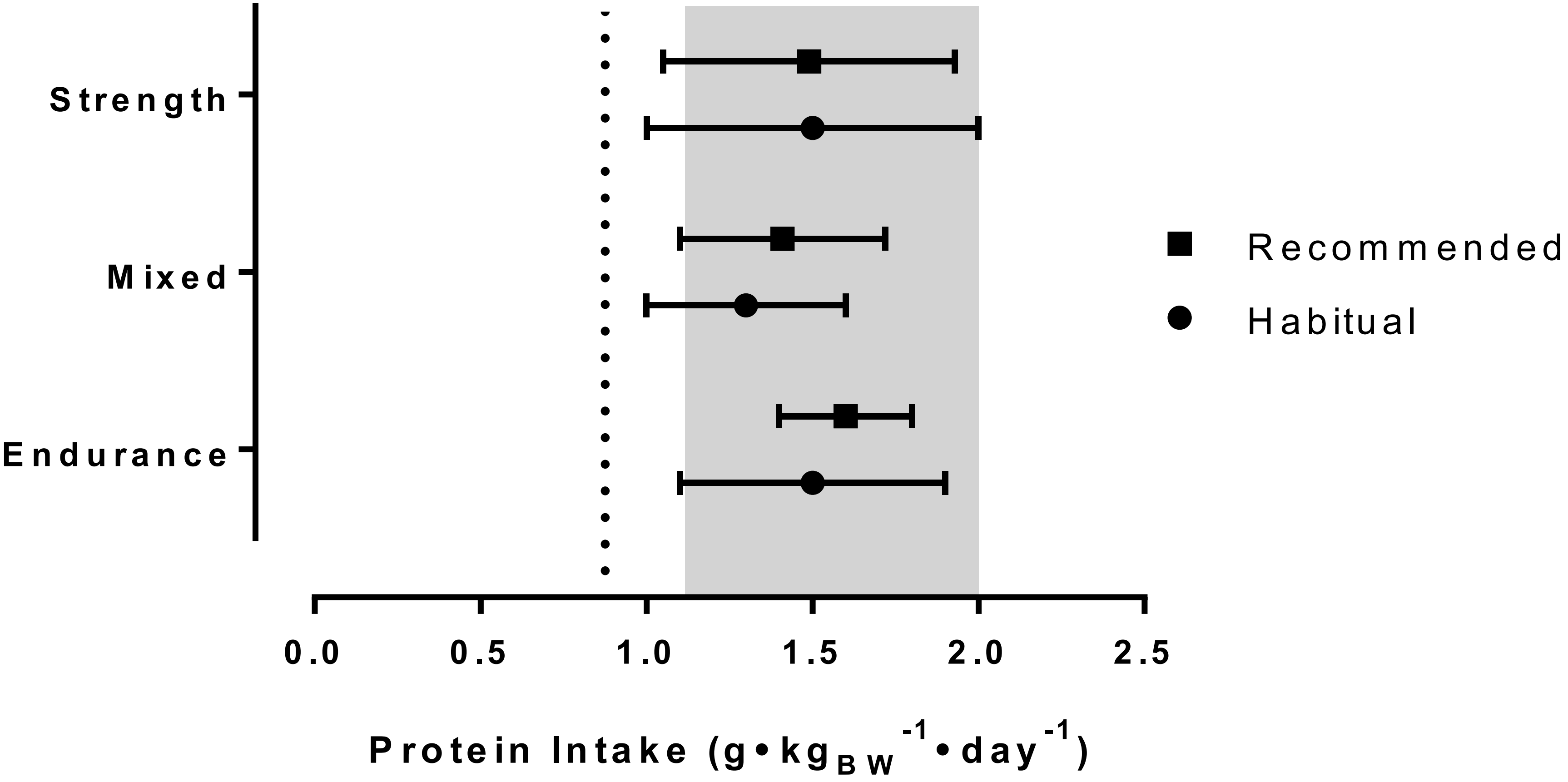
[3] Endurance training refers to aerobic-based exercise of moderate-high intensity (e.g. $\geq 70\% VO_{2peak}$)

[4] Resistance training refers to high effort, externally loaded muscle contractions (e.g. weight lifting)

[5] Mixed training refers to mixed aerobic/anaerobic exercise typically with weight-bearing stop-and-go decelerations and accelerations, such as that common to many team sports (e.g. soccer, rugby, ice hockey)

Figure 1. Daily habitual (mean±SD) and recommended (estimated average requirement±95% confidence interval) protein intakes in endurance, mixed, and strength athletes. Mixed athletes refers to mixed aerobic/anaerobic exercise typically with weight-bearing stop-and-go decelerations and accelerations, such as that common to many team sports (e.g. soccer, rugby, ice hockey). Habitual intakes for female athletes from (Gillen et al., 2017). Recommended protein intakes determined to maximize whole body protein synthesis and anabolism during recovery as determined by stable isotope methodology for female strength athletes (Malowany et al., 2019), female mixed athletes after a simulated soccer match (Wooding et al., 2017), and male endurance athletes (Kato et al., 2016) given the lack of research in females. Dashed line represent the recommended dietary allowance. Shaded area represents athlete nonspecific range according to current sports nutrition guidelines (Thomas et al., 2016).

Figure 1.


Strength
Mixed
Endurance
0.0
0.5
1.0
1.5
2.0
2.5
Protein Intake (g•kg$_{BW}^{-1}$•day^{-1})
■ Recommended
● Habitual