LJMU Research Online

Moore, DR, Sygo, J and Morton, JP

 Fuelling the female athlete: Carbohydrate and protein recommendations

http://researchonline.ljmu.ac.uk/id/eprint/18221/

Article

For more information please contact researchonline@ljmu.ac.uk

1 **Fuelling the female athlete: carbohydrate and protein recommendations**

2

3 Daniel R. Moore[1]*, Jennifer Sygo[2], James P. Morton[3]

4

5 [1]Faculty of Kinesiology and Physical Education, University of Toronto, Toronto, Canada

6 [2]Athletics Canada, Ottawa, Canada

7 [3] Research Institute for Sport and Exercise Sciences, Liverpool John Mores University,

8 Liverpool, United Kingdom

9

10

*Corresponding author:

Daniel R. Moore, PhD

100 Devonshire Place

Toronto, ON, M5S 2C9, CAN

Phone: +1 (416) 946-4088

E-mail: dr.moore@utoronto.ca

Word count: 4813;

Number of tables: 3

Number of figures: 1

Running title: Carbohydrate and protein for female athletes

11

12

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.

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

59   influence strength, speed, or power-to-weight ratio. While careful adjustment of energy intake

60   has the potential to enhance performance, extended periods of diminished energy intake may

61   pose risks to health and performance associated with low energy availability (see Heikura et al

62   in this issue). In addition, suboptimal energy intake may also compromise the ability to meet

63   optimal CHO targets and can increase dietary protein requirements, as detailed below. Thus,

64   matching energy intake to energy requirements should be a cornerstone for performance

65   nutrition and optimal macronutrient intake.

66   Due to their typically shorter stature and lower body mass, female athletes would

67   predictably have lower energy requirements than male athletes. Reduced levels of lean mass,

68   both in an absolute (total kg) and relative ($kg \cdot m^{-2}$) sense also predicts reduced energy

69   requirements for female athletes versus their male counterparts. Estimating energy

70   requirements of athletes is conceptually relatively simple yet can be challenging in practice

71   due to the various methodologies available including food frequency questionnaires,

72   interviews, and food logs (for review, see Heikura and Areta). A systematic review of self-

73   reported energy intake versus energy expenditure determined using doubly labeled water

74   indicated that athletes underreport intake by an average of ~667 $kcal \cdot day^{-1}$ or ~19% of daily

75   energy requirements (Capling et al., 2017). While under-reporting may be due to common

76   issues with food logs and dairies, it is also possible that drive-for-thinness and a pressure to

77   control diet and body mass may influence female athletes' tendency to under-report. Thus, the

78   sports nutrition practitioner must be aware of the energy requirements of their athlete and be

79   able to identify signs of energy deficiency (e.g. menstrual irregularities, endocrine and

80   hematological changes, reduced bone mass, compromised performance and/or training

81   adaptations (Mountjoy et al., 2018)) in order to provide a strong foundation on which to apply

82   the recommended macronutrient intakes discussed below. An overview of the energy

83   requirements of representative female athletes is provided in Table 1.

84   Changes in energy expenditure throughout the menstrual cycle has the potential to

85   impact body mass and composition as well as macronutrient intake via changes in appetite and

86   total energy requirements. It has previously been suggested that the resting metabolic rate

87   (RMR) increases by ~100-300 kcal·day^{-1} in late luteal phase versus early follicular phase

88   (Bisdee 1989, Curtis 1996), although recent studies with methodological improvements have

89   not corroborated these results (Benton 2020). Barr et al (1995) reported that females

90   spontaneously increase their energy intake by ~300 kcal·day^{-1} during the follicular phase,

91   suggesting that women naturally experience appetite changes in accordance with this change

92   in RMR. Conversely, (Kammoun et al., 2017) has reported that women tend to have a higher

93   body mass at the end of the luteal phase versus the mid-follicular phase, suggesting either

94   changes in energy intake or expenditure are occurring, or other hormonal factors, for example

95   those affecting fluid retention, may be at play. However, it is important to consider that energy

96   requirements can also vary with energy availability (see Heikura et al. This Issue). While the

97   magnitude of RMR suppression can vary according to a number of factors, a reduction of up

98   to 10% has been reported in amenorrheic vs. eumenorrheic female endurance athletes (Melin

99   et al. 2015). Thus, athletes and practitioners must be critically aware of the total energy

100   requirements (both basal and exercise-induced expenditure) of female athletes in order to

101   maximize their health, performance, and recovery.

102

103   **3. Carbohydrate Requirements**

104   The primary nutritional consideration for athletic populations is often focused on the CHO

105   requirements that are necessary to promote competitive performance as well as maintain the

106   desired daily training intensities and volume. It is now also recognised (at least in males) that

107   the strategic manipulation of CHO availability in a meal-by-meal and day-by-day manner

108   (commonly referred to as CHO periodisation) can regulate training-induced oxidative

109   adaptations of skeletal muscle, as mediated via activation of regulatory cell signalling pathways

110   when exercise is completed in CHO restricted states (Impey et al., 2018). Contemporary

111   guidelines for daily CHO intake therefore recognise the need for flexibility according to the

112   metabolic demands of the exercise challenge as well as the individual athlete goals of

113   promoting training quality versus stimulating adaptation (Burke et al., 2018; Thomas et al.,

114   2016). Given the potential for sex-specific differences in CHO and fat metabolism during

115   exercise (as reviewed by Issaco et al. This Issue), the practical question that arises therefore, is

116   whether female athletes should follow sex-specific CHO guidelines in relation to CHO

117   requirements before, during and after exercise (**Table 2**). The complexity of this issue is

118   exacerbated by methodological differences between studies including exercise

119   modality/intensity/duration, muscle group examined, participant training status, nutritional

120   status, menstrual phase, and/or the use of hormonal contraception. Additionally, the effects of

121   menstrual cycle phase on appetite regulation, gastrointestinal symptoms and food cravings (e.g.

122   sweet foods) may also affect habitual energy and absolute CHO intake (Krishnan et al., 2016),

123   which can impact "real world" fuelling.

124

125   *3.1. CHO Loading*

126   A reduced capacity of endurance trained females to store glycogen in the vastus lateralis muscle

127   (as assessed in the follicular phase) when compared with males was initially reported by

128   (Tarnopolsky et al., 1995). After a 3-day CHO loading protocol initiated by a glycogen

129   depletion protocol and followed by increased CHO intake (55 to 75% of habitual energy

130   intake), the authors observed a 150 mmol.kg^{-1} dw difference in resting glycogen storage

131   between males (550 mmol.kg^{-1} dw) and females (400 mmol.kg^{-1} dw). It was suggested that

132   such differences may be due to the combination of greater prior glycogen depletion and a higher

133   absolute CHO intake in males (8 g/kg body mass equating to 610 g CHO) compared with

134   females (6 g/kg body mass equating to 370 g CHO). Indeed, the same group later demonstrated

135   that when females complete a 4 day CHO loading protocol whereby a higher relative (9 g/kg

136   body mass) and absolute CHO intake was consumed (540 g CHO), no differences in glycogen

137   concentration (>700 mmol.kg^{-1} dw) was apparent when compared with males who consumed

138   a comparable absolute dose (600 g CHO equating to 8 g·kg^{-1} body mass) (Tarnopolsky et al.,

139   2001). Furthermore, (James et al., 2001) reported equivalent glycogen storage in male and

140   female endurance-trained females on oral contraceptives (OC) (878 and 839 mmol.kg^{-1} dw as

141   assessed pre- and post-menses, respectively) (796 mmol.kg^{-1} dw) after 3 days of 12 g CHO·kg^{-1}

142   fat-free mass per day. Collectively these data suggest that the capacity to "load" muscle

143   glycogen is not sex dependent provided a sufficient CHO intake is met (i.e. 8-12 g·kg^{-1}, as

144   recommended (Thomas et al., 2016).

145   A pertinent practical question is whether the capacity to store glycogen is altered during

146   specific phases of the menstrual cycle. A preliminary study in recreationally active non-OC

147   users reported that resting glycogen concentration was marginally but statistically greater in

148   the mid-luteal (ML) phase when compared with the mid-follicular (MF) phase (443 and 391

149   mmol.kg^{-1} dw, respectively) (Hackney, 1990). In response to a 3-day sub-optimal CHO feeding

150   protocol (4 g·kg^{-1}·d^{-1}) consumed after prior glycogen depleting exercise, (Nicklas et al., 1989)

151   also reported in moderately trained non-OC users that glycogen storage was greater in the ML

152   phase compared with the MF phase (383 and 313 mmol.kg^{-1} dw, respectively). (McLay et al.,

153   2007) similarly observed that the lowest muscle glycogen concentration occurred during the

154   MF phase under normal (5.2 g·kg^{-1}·d^{-1}) dietary CHO conditions (575 mmol·kg^{-1} dw) when

155   compared with the MF phase under CHO loaded (8.4 g·kg^{-1}·d^{-1}) conditions (728 mmol.kg^{-1} dw)

156   or the ML phase in either normal (761 mmol.kg^{-1} dw) or CHO loaded conditions (756 mmol.kg^{-1}

157   dw). Considering that exercise performance may be trivially impaired in the early follicular

158   phase (McNulty et al., 2020) coupled with females reporting negative physical symptoms at

159   the onset or during menses (Findlay et al., 2020), these data suggest female athletes should pay

160   attention to CHO availability during the follicular phase of the menstrual cycle, especially in

161   competitive or training scenarios where absolute glycogen availability may be limiting to

162   performance. Given that glycogen is stored within distinct sub-cellular pools in both type I and

163   type II muscle fibres, future studies on female participants should also assess the effects of

164   CHO loading on glycogen storage (and subsequent exercise-induced utilisation) within the

165   subsarcolemmal, intramyofibrillar and intermyofibrillar pools.

166

167   *3.2. Daily CHO Availability*

168   The most meaningful practical challenge is matching the CHO cost of a sport to that of

169   actual CHO availability according to training demands/goals. Surprisingly, little is known

170   regarding the glycogen requirements of "real world" competitive events and/or training

171   sessions that are typically completed by both amateur and elite athletes. To address this

172   shortcoming, we recently subjected a cohort of male and female (OC users) recreationally

173   active runners to three outdoor training sessions: 1) a 10-mile road run (10-mile) at lactate

174   threshold, 2) 8 x 800 m track intervals (8 x 800 m) at $\dot{V}O_{2max}$ velocity and 3) 3 x 10 minute

175   track intervals (3 x 10 min) at lactate turn point (Impey et al., 2020). Each training session was

176   commenced after a standardised training session and 2 days of controlled diet (6 g CHO.kg^{-1}

177   body mass per day) with females studied during the MF phase. In accordance with previous

178   studies utilising moderate daily CHO intakes (Tarnopolsky et al., 1995), we observed that

179   resting glycogen concentration prior to all training sessions was reduced in the gastrocnemius

180   muscle of females versus males (~400 and ~500 mmol.kg^{-1} dw, respectively) (Impey et al.,

181   2020). Nonetheless, such differences in absolute glycogen concentration were of no functional

182   relevance considering that all female participants were able to maintain the desired intensities

183   and workload associated with each training session. As such, we deemed it unlikely that such

184 differences in glycogen storage (and subsequent utilisation patterns) would necessitate sex-

185 specific practical recommendations, at least when considering the training protocols and

186 training status of the participants under investigation. It is acknowledged, however, that future

187 studies are required to further evaluate the glycogen requirements associated with other sport-

188 specific training and competition scenarios.

189

190 ### 3.3. CHO Feeding During Exercise

191 It is well accepted that CHO feeding during exercise is ergogenic to performance

192 (Stellingwerff & Cox, 2014), likely underpinned by liver glycogen sparing (Gonzalez et al.,

193 2015), maintenance of plasma glucose and elevated CHO oxidation rates (Coyle et al., 1986)

194 and direct effects upon the central nervous system (CNS) (Carter et al., 2004). Contemporary

195 guidelines for athletic populations (Thomas et al., 2016) currently recommend CHO mouth

196 rinsing when exercise duration is <60 minutes, CHO intake at a rate of 30-60 $g \cdot h^{-1}$ (from single

197 sources such as glucose or maltodextrin) during 1-2.5 h of endurance exercise and finally, up

198 to 90 $g \cdot h^{-1}$ of multi-transportable CHO (glucose:fructose blends) when exercise duration is

199 >2.5 h.

200 Although much of the foundation for CHO feeding guidelines are primarily based from

201 research studies conducted on male participants, there is no conclusive evidence to suggest that

202 practical strategies should be different for female athletes. For example, the metabolic

203 responses to CHO feeding during exercise (90 g/h of a 10.9% glucose solution) are similar in

204 trained males and females (MF) during a 2 h cycling protocol completed at 67% VO_{2max} with

205 no effects of sex on the relative contribution of fat, exogenous CHO, liver derived glucose or

206 muscle glycogen oxidation to total energy expenditure during the final 60 minutes of exercise

207 (Wallis et al., 2006). Moreover, peak exogenous CHO oxidation rates were not significantly

208 different between males and females (0.7 and 0.65 $g \cdot min^{-1}$, respectively). The ergogenic effect

209   of CHO ingestion during exercise (67 $g \cdot h^{-1}$ during 2 h cycling at 70% VO_{2peak} followed by a 4

210   kJ/kg time trial) is also apparent in both the MF and ML phase of the menstrual cycle in

211   endurance trained non-OC users with no differences in rate of glucose disappearance, plasma

212   glucose oxidation or total CHO oxidation between phases (Campbell et al., 2001). In contrast

213   to single source CHO solutions, it is not yet clear whether females retain the capacity to achieve

214   superior exogenous CHO oxidation rates when consuming dual source blends. Although this

215   has not been comprehensively examined within the same study, it is noteworthy that peak

216   exogenous CHO oxidation rates have been reported at 1.03 g/min in females (O'Hara et al.,

217   2019) and 1.42 $g \cdot min^{-1}$ in males (O'Hara et al., 2017) in response to consuming 1.8 g/min of

218   CHO (2:1 glucose/fructose ratio) during 2 h cycling (55% W_{max}). It is, of course, difficult to

219   directly compare between studies and it is noteworthy that the phase of menstrual cycle and

220   prevalence of contraceptive use was not specified in the former study.

221   Using a cohort of highly trained male and female cross country skiers, (Pettersson et

222   al., 2019) recently assessed the effects of an 18% maltodextrin and fructose solution (1:0.8

223   ratio with additional alginate and pectin) administered at a rate of 2.2 g/min during a 2 h sub-

224   maximal (70% VO_{2max}) roller skiing protocol. While CHO ingestion suppressed endogenous

225   CHO utilisation by a similar magnitude (~18%), peak rates of exogenous CHO oxidation

226   tended (P=0.064) to be less in females (1.2 g/min) than males (1.5 g/min). However, the

227   authors acknowledge several limitations in their design, namely they did not control for phase

228   of menstrual cycle and three out of six females were contraceptive users. Nonetheless, these

229   data suggest that highly trained female athletes are able to tolerate high doses of CHO feeding

230   during exercise (albeit in cold ambient conditions) without experiencing gastrointestinal

231   symptoms that limit performance.

232

233   *3.4. Post-Exercise Muscle Glycogen Resynthesis*

234   For athletes who compete in multi-day sporting events (e.g. cycling tours), undertake a

235   congested competition schedule (e.g. soccer competitions), and/or undertake a high volume of

236   training with multiple sessions in a 24-h period (e.g. distance runners, rowers, swimmers), the

237   replenishment of endogenous glycogen stores after such events or between specific training

238   sessions is of upmost importance to promote performance in the subsequent bout of exercise.

239   It is well established that CHO ingestion rates of 1.2 $g \cdot kg^{-1} \cdot h^{-1}$ are considered optimal during

240   the short-term (0-4 hours) recovery from exercise in males (Burke et al., 2017) and there is no

241   convincing evidence to support sex-specific differences during similar recovery durations. For

242   example, (Tarnopolsky et al., (1997) reported similar rates of muscle glycogen resynthesis (35-

243   40 $mmol.kg\ dw.h^{-1}$) in moderately trained males and females (MF with 38% OC users) when

244   ingesting 1 $g \cdot kg^{-1}$ of CHO immediately and 1 h post completion of a 90 minute cycling

245   protocol. More recently, Flynn et al., (2020) also reported similar rates of muscle glycogen

246   resynthesis in recreationally active males and females (OC users but without menstrual phase

247   standardization) when ingesting 1.6 $g \cdot kg^{-1}$ CHO immediately and 2 h post-completion of a 90

248   minute cycling protocol. To the authors' knowledge, however, no researchers have yet tested

249   the effects of menstrual cycle phase on rates of muscle glycogen resynthesis during the early

250   post-exercise recovery period.

251

252   ***3.5. CHO Periodisation***

253   In male participants, a growing body of literature demonstrates that deliberately

254   commencing and/or recovering from training sessions with reduced CHO availability (the so-

255   called *train low* paradigm) potentiates the activation of cell signalling pathways with regulatory

256   roles in training adaptation (Impey et al., 2018). Accordingly, several weeks of train low

257   protocols (e.g. twice per day training, fasted training, sleep-low:train-low) increases oxidative

258   enzyme activity and protein content (Morton et al., 2009; Yeo et al., 2008), whole body (Yeo

259 et al., 2008) and intramuscular lipid oxidation (Hulston et al., 2010) and may also improve

260 exercise capacity (Hansen et al., 2005) and performance (Marquet et al., 2016). These data

261 have been translated practically according to the "fuel for the work required" model whereby

262 CHO availability is adjusted day-by-day and meal-by-meal according to the metabolic

263 demands and personalised goals of the upcoming session (Impey et al., 2018). In relation to

264 females, fasted training did not induce superior mitochondrial adaptations in skeletal muscle

265 of obese females when compared with fed training (Gillen et al., 2013), though it is noteworthy

266 that no comparable studies have yet been conducted in healthy females. As such, it is currently

267 unclear if CHO restriction is beneficial, neutral, or potentially maladaptive for female athletic

268 populations, the latter of which is especially relevant when considering the potential effects of

269 reduced CHO availability on overall energy availability and the modulation of symptoms

270 associated with RED-S (see Heikura et al. This Issue). Despite 54% of female athletes recently

271 reporting they engage in "fasted" training (Rothschild et al., 2020), it is clear that the efficacy

272 of train-low strategies in female athletes should be a targeted area for further research.

273

274 **4. Protein requirements**

275 Dietary protein provides the requisite building blocks to help repair and rebuild body

276 and, especially, muscle protein after exercise, positioning it as a vital nutrient for active

277 populations. It is generally accepted that protein requirements exceed the recommended dietary

278 allowance of ~0.8 $g \cdot kg^{-1} \cdot d^{-1}$, which reduces the risk of protein malnutrition. Current

279 recommendations suggest a broad range for athletes (i.e. 1.2-2.0 $g \cdot kg^{-1} \cdot d^{-1}$) (Thomas et al.,

280 2016) that may not adequately reflect the different needs amongst athletes of varying

281 disciplines and physiological requirements (**Figure 1**). Compounding the challenge of

282 providing specific recommendations for female athletes is the relative dearth of research

283   performed in this population as well as the potential impact of menstrual status or contraceptive

284   use (for review, see (Mercer et al., 2020).

285

286   *4.1. Daily protein requirements*

287   Muscle growth with resistance training must be supported by sufficient protein and,

288   arguably, energy intake (Slater et al., 2019). It has recently been demonstrated using stable

289   isotope methodology that whole body protein synthesis and net protein balance (a surrogate

290   marker for acute lean tissue 'growth') after resistance exercise is maximized at an estimated

291   average requirement (EAR) of ~1.5 $g \cdot kg^{-1} \cdot d^{-1}$ in trained females (Malowany et al., 2019). This

292   tracer-derived EAR is similar to the ~1.6 $g \cdot kg^{-1} \cdot d^{-1}$ that has been reported to optimize training-

293   induced gains in fat-free mass in a mixed-sex meta-analysis (Morton et al., 2018). Accounting

294   for a standard 12% variance (i.e. 1.24 x EAR), these daily protein estimates would translate

295   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

296   current consensus recommendations for this macronutrient (Thomas et al., 2016). However, it

297   is important to note that strength trained athletes typically consume >1.9 $g \cdot kg^{-1} \cdot d^{-1}$ (Malowany

298   et al., 2018), which may increase the metabolic requirement for protein irrespective of the true

299   requirement for muscle growth (Tinline-Goodfellow et al., 2020). Thus, it may be more

300   relevant to consider the amount and pattern of meal protein intake (see below) to arrive at an

301   optimal daily protein target.

302   Protein requirements in endurance athletes have been known to be elevated above the

303   current RDA of 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ since Phillips et al., (1993) demonstrated that female

304   recreationally trained athletes were not able to maintain nitrogen balance on this intake.

305   Subsequent short term nitrogen balance studies in trained female cyclists and triathletes support

306   this finding by showing that the EAR for nitrogen equilibrium is 1.3-1.6 $g \cdot kg^{-1} \cdot d^{-1}$ in the

307   follicular phase (Houltham & Rowlands, 2014; Rowlands & Wadsworth, 2011), which is

308　within the range previously suggested for endurance athletes based on nitrogen balance studies

309　in males (i.e. 1.2-1.4 g protein/kg/d; (Tarnopolsky, 2004). However, recent estimates for male

310　endurance athletes during training suggest an EAR of ~1.6 g protein/kg/d and an RDI of ~1.8

311　g protein/kg/d is required to maximize whole body protein synthesis (Kato et al., 2016), which

312　is arguably more physiologically relevant for athletes than the 'black box' approach of nitrogen

313　balance studies (i.e. nitrogen in minus nitrogen out). This elevated requirement, which is ~1.7-

314　fold greater than the EAR for non-exercising males using identical methodology (Humayun et

315　al., 2007), is primarily related to the need to replenish the exercise-induced oxidative loss of

316　the branched chain amino acids (Kato et al., 2018). This is notable as estrogen has been shown

317　to attenuate amino acid (and specifically leucine) oxidation during exercise at the expense of

318　greater lipolysis and fatty acid oxidation (Hamadeh et al., 2005; Phillips et al., 1993). In

319　contrast, a low estrogen:progesterone (E:P) ratio that is characteristic of the luteal phase can

320　increase protein catabolism, amino acid oxidation, and, in exercising females, nitrogen

321　excretion (Lamont et al., 1987; Lariviere et al., 1994). Thus, the greater estogen:progesterone

322　(P:E) ratio of the follicular phase may have a 'protein-sparing' effect whereas the lower P:E

323　ratio of the luteal phase may align female athlete protein requirements more closely to their

324　male counterparts. For example, the EAR to maximize whole body protein synthesis (~1.4 vs.

325　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

326　$g \cdot kg^{-1} \cdot d^{-1}$)(Mazzulla et al., 2018) in female athletes during the mid-luteal phase performing a

327　variable intensity, stop-and-go 'team sport' type exercise is broadly similar to active males,

328　respectively. A final consideration for female athletes, perhaps more so during the luteal phase,

329　is that low CHO availability training (reviewed above) may modestly increase daily protein

330　requirements by ~12% to replenish a greater exercise-induced amino acid oxidative loss (Gillen

331　et al., 2019).

332

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

358 mitochondrial proteins (Churchward-Venne et al., 2020). Whereas the rate of mitochondrial

359 protein synthesis does not appear to be regulated by dietary protein, MyoPS is stimulated in a

360 dose-dependent manner up to a plateau of ~0.5 g protein/kg after 90 min of cycling

361 (60%VO2peak) in male athletes (Churchward-Venne et al., 2020). Interestingly, endurance

362 exercise is known to mobilize amino acids from the breakdown of muscle (primarily

363 myofibrillar) protein and attenuate muscle protein synthesis, which based on leg phenylalanine

364 kinetics could translate into an acute loss of ~0.1-0.2 g muscle protein/kg/h (Howarth et al.,

365 2010). Given that the rate of leucine oxidation (as a marker of protein oxidation) during

366 endurance exercise (i.e. running at ~70%VO_{2peak}) is of a similar magnitude as this muscle

367 protein mobilization (i.e. ~0.1 g body protein·kg^{-1}·h^{-1})(Mazzulla et al., 2017), the relative

368 difference in the maximal effective protein dose for MyoPS between resistance and endurance

369 exercise may represent in part the need to replenish these amino acid oxidative losses (Moore,

370 2020). Thus, inasmuch as these oxidative losses influence the per meal protein requirement to

371 maximize muscle, and primarily MyoPS, it is possible that female endurance athletes may

372 require a slightly lower acute protein intake in the follicular phase when the E:P ratio is highest

373 and amino oxidation is lowest. For exercise that incorporates both aerobic and resistive

374 components, such as team sports characterised by high-intensity intermittent exercise, female

375 athletes may wish to err on the side of caution with a post-exercise protein target of ~0.4 g

376 protein·kg^{-1}.

377

4.3. Protein frequency and pattern

379 While there does not appear to be a defined 'window of opportunity' for post-exercise

380 protein ingestion, female athletes should aim to consume a source of high quality protein

381 immediately after exercise to replenish any exercise-induced amino acid oxidative losses and,

382 more importantly, to initiate muscle protein remodelling and repair, a key aspect of the

383   recovery process that can only be maximally supported by exogenous amino acids. Often to

384   meet the high energy requirements of training, female athletes commonly consume 4-5 meals

385   per day (Burke et al., 2003). Incidentally, MyoPS rates in male athletes are greatest over 12 h

386   of recovery when consuming four moderate protein meals ($\sim$0.25 g protein·kg^{-1}) every 3 h as

387   compared to the same quantity of protein in two large or 8 small meals (Areta et al., 2013). In

388   addition, pre-sleep protein ingestion has also been shown to enhance overnight rates of MyoPS

389   (Snijders et al., 2019), highlighting this as an opportunistic meal time. Therefore, in contrast to

390   the typical skewed daily distribution (Gillen et al., 2017), female athletes should focus on

391   consuming moderate ($\sim$0.3 g protein·kg^{-1}) protein-containing meals (perhaps with the

392   exception of slightly larger intake immediately after endurance exercise) every 3-4 h to

393   maximize muscle protein repair and remodelling and to minimize amino acid oxidation during

394   the prolonged (>24 h) recovery period. Incidentally, using this 'muscle-centric' approach to

395   optimized meal protein intake and pattern would provide a daily intake of 1.5-1.7 g protein·kg^{-}

396   ^{1}·d^{-1} with 5 feeding occasions, which is similar to the daily EAR for resistance and endurance

397   athletes discussed above.

398

399   *4.4. Protein type*

400   Consuming a protein source that is rapidly digested and enriched in the essential amino

401   acid leucine is generally regarded as the most effective means to 'turn on' and support maximal

402   rates of muscle protein remodelling immediately after exercise (Stokes et al., 2018). However,

403   the remodelling of skeletal muscle and replenishment of body protein stores can occur for up

404   to 24 h after exercise (West et al., 2012), which may reduce the importance of this immediate

405   post-exercise window if there is sufficient time between training bouts for recovery and

406   refuelling (e.g. CHO replenishment; see above). Certainly within this prolonged recovery

407   window athletes should prioritize the consumption of nutrient dense whole foods, which are a

408 relatively understudied aspect of sports nutrition in comparison to protein supplements. In

409 some cases, the food matrix of a protein source may be more anabolic than the sum of its parts

410 (for review, see: (Burd et al., 2019)).

411

412 **5. Summary**

413 On the basis of current evidence, we consider it premature to substantiate that female athletes

414 require sex-specific guidelines in relation to CHO or protein requirements provided energy

415 needs are met. Rather, there is a definitive need for further research using sport-specific

416 competition and training related exercise protocols that rigorously control for prior exercise,

417 CHO/energy intake, contraceptive use and phase of menstrual cycle. Until such data exists, it

418 remains prudent for female athletes to therefore adhere to previously published best practice

419 guidelines that are generalised to athletic populations. However, our overarching

420 recommendation is to adopt an individualised approach that takes into account athlete specific

421 training and competition goals whilst also considering personal symptoms associated with the

422 menstrual cycle.

423

424 **Acknowledgements**

425 None to report.

426

427 **Declaration of interest statement**

432

433

434 **References**

435 Areta, J. L., Burke, L. M., Camera, D. M., West, D. W., Crawshay, S., Moore, D. R., . . . Coffey,
436   V. G. (2014). Reduced resting skeletal muscle protein synthesis is rescued by
437   resistance exercise and protein ingestion following short-term energy deficit. *Am J*
438   *Physiol Endocrinol Metab, 306*(8), E989-997. doi: 10.1152/ajpendo.00590.2013
439 Areta, J. L., Burke, L. M., Ross, M. L., Camera, D. M., West, D. W., Broad, E. M., . . . Coffey, V.
440   G. (2013). Timing and distribution of protein ingestion during prolonged recovery
441   from resistance exercise alters myofibrillar protein synthesis. *J Physiol, 591*(9), 2319-
442   2331. doi: 10.1113/jphysiol.2012.244897
443 Binzen, C. A., Swan, P. D., & Manore, M. M. (2001). Postexercise oxygen consumption and
444   substrate use after resistance exercise in women. *Med Sci Sports Exerc, 33*(6), 932-
445   938. doi: 10.1097/00005768-200106000-00012
446 Biolo, G., Tipton, K. D., Klein, S., & Wolfe, R. R. (1997). An abundant supply of amino acids
447   enhances the metabolic effect of exercise on muscle protein. *Am J Physiol, 273*(1 Pt
448   1), E122-129. doi: 10.1152/ajpendo.1997.273.1.E122
449 Burd, N. A., Beals, J. W., Martinez, I. G., Salvador, A. F., & Skinner, S. K. (2019). Food-First
450   Approach to Enhance the Regulation of Post-exercise Skeletal Muscle Protein
451   Synthesis and Remodeling. *Sports Med, 49*(Suppl 1), 59-68. doi: 10.1007/s40279-
452   018-1009-y
453 Burke, L. M., Hawley, J. A., Jeukendrup, A., Morton, J. P., Stellingwerff, T., & Maughan, R. J.
454   (2018). Toward a Common Understanding of Diet-Exercise Strategies to Manipulate
455   Fuel Availability for Training and Competition Preparation in Endurance Sport. *Int J*
456   *Sport Nutr Exerc Metab, 28*(5), 451-463. doi: 10.1123/ijsnem.2018-0289
457 Burke, L. M., Slater, G., Broad, E. M., Haukka, J., Modulon, S., & Hopkins, W. G. (2003).
458   Eating patterns and meal frequency of elite Australian athletes. *Int J Sport Nutr Exerc*
459   *Metab, 13*(4), 521-538
460 Burke, L. M., van Loon, L. J. C., & Hawley, J. A. (2017). Postexercise muscle glycogen
461   resynthesis in humans. *J Appl Physiol (1985), 122*(5), 1055-1067. doi:
462   10.1152/japplphysiol.00860.2016
463 Campbell, S. E., Angus, D. J., & Febbraio, M. A. (2001). Glucose kinetics and exercise
464   performance during phases of the menstrual cycle: effect of glucose ingestion. *Am J*
465   *Physiol Endocrinol Metab, 281*(4), E817-825. doi: 10.1152/ajpendo.2001.281.4.E817
466 Capling, L., Beck, K. L., Gifford, J. A., Slater, G., Flood, V. M., & O'Connor, H. (2017). Validity
467   of Dietary Assessment in Athletes: A Systematic Review. *Nutrients, 9*(12). doi:
468   10.3390/nu9121313
469 Carter, J. M., Jeukendrup, A. E., & Jones, D. A. (2004). The effect of carbohydrate mouth
470   rinse on 1-h cycle time trial performance. *Med Sci Sports Exerc, 36*(12), 2107-2111.
471   doi: 10.1249/01.mss.0000147585.65709.6f
472 Churchward-Venne, T. A., Pinckaers, P. J. M., Smeets, J. S. J., Betz, M. W., Senden, J. M.,
473   Goessens, J. P. B., . . . van Loon, L. J. C. (2020). Dose-response effects of dietary
474   protein on muscle protein synthesis during recovery from endurance exercise in
475   young men: a double-blind randomized trial. *Am J Clin Nutr, 112*(2), 303-317. doi:
476   10.1093/ajcn/nqaa073
477 Costello, J. T., Bieuzen, F., & Bleakley, C. M. (2014). Where are all the female participants in
478   Sports and Exercise Medicine research? *Eur J Sport Sci, 14*(8), 847-851. doi:
479   10.1080/17461391.2014.911354

480 Coyle, E. F., Coggan, A. R., Hemmert, M. K., & Ivy, J. L. (1986). Muscle glycogen utilization
481   during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol (1985),*
482   *61*(1), 165-172. doi: 10.1152/jappl.1986.61.1.165
483 Ebine, N., Feng, J. Y., Homma, M., Saitoh, S., & Jones, P. J. (2000). Total energy expenditure
484   of elite synchronized swimmers measured by the doubly labeled water method. *Eur J*
485   *Appl Physiol, 83*(1), 1-6. doi: 10.1007/s004210000253
486 Edwards, J. E., Lindeman, A. K., Mikesky, A. E., & Stager, J. M. (1993). Energy balance in
487   highly trained female endurance runners. *Med Sci Sports Exerc, 25*(12), 1398-1404
488 Findlay, R. J., Macrae, E. H. R., Whyte, I. Y., Easton, C., & Forrest Née Whyte, L. J. (2020).
489   How the menstrual cycle and menstruation affect sporting performance: experiences
490   and perceptions of elite female rugby players. *Br J Sports Med, 54*(18), 1108-1113.
491   doi: 10.1136/bjsports-2019-101486
492 Flynn, S., Rosales, A., Hailes, W., & Ruby, B. (2020). Males and females exhibit similar muscle
493   glycogen recovery with varied recovery food sources. *Eur J Appl Physiol, 120*(5),
494   1131-1142. doi: 10.1007/s00421-020-04352-2
495 Gillen, J. B., Percival, M. E., Ludzki, A., Tarnopolsky, M. A., & Gibala, M. J. (2013). Interval
496   training in the fed or fasted state improves body composition and muscle oxidative
497   capacity in overweight women. *Obesity (Silver Spring), 21*(11), 2249-2255. doi:
498   10.1002/oby.20379
499 Gillen, J. B., Trommelen, J., Wardenaar, F. C., Brinkmans, N. Y., Versteegen, J. J., Jonvik, K. L.,
500   . . . van Loon, L. J. (2017). Dietary Protein Intake and Distribution Patterns of Well-
501   Trained Dutch Athletes. *Int J Sport Nutr Exerc Metab, 27*(2), 105-114. doi:
502   10.1123/ijsnem.2016-0154
503 Gillen, J. B., West, D. W. D., Williamson, E. P., Fung, H. J. W., & Moore, D. R. (2019). Low-
504   Carbohydrate Training Increases Protein Requirements of Endurance Athletes. *Med*
505   *Sci Sports Exerc, 51*(11), 2294-2301. doi: 10.1249/mss.0000000000002036
506 Gonzalez, J. T., Fuchs, C. J., Smith, F. E., Thelwall, P. E., Taylor, R., Stevenson, E. J., . . . van
507   Loon, L. J. (2015). Ingestion of glucose or sucrose prevents liver but not muscle
508   glycogen depletion during prolonged endurance-type exercise in trained cyclists. *Am*
509   *J Physiol Endocrinol Metab, 309*(12), E1032-1039. doi: 10.1152/ajpendo.00376.2015
510 Hackney, A. C. (1990). Effects of the menstrual cycle on resting muscle glycogen content.
511   *Horm Metab Res, 22*(12), 647. doi: 10.1055/s-2007-1004994
512 Hamadeh, M. J., Devries, M. C., & Tarnopolsky, M. A. (2005). Estrogen supplementation
513   reduces whole body leucine and carbohydrate oxidation and increases lipid
514   oxidation in men during endurance exercise. *J Clin Endocrinol Metab, 90*(6), 3592-
515   3599. doi: 10.1210/jc.2004-1743
516 Hansen, A. K., Fischer, C. P., Plomgaard, P., Andersen, J. L., Saltin, B., & Pedersen, B. K.
517   (2005). Skeletal muscle adaptation: training twice every second day vs. training once
518   daily. *J Appl Physiol (1985), 98*(1), 93-99. doi: 10.1152/japplphysiol.00163.2004
519 Hill, R. J., & Davies, P. S. (1999). The validity of a four day weighed food record for
520   measuring energy intake in female classical ballet dancers. *Eur J Clin Nutr, 53*(9), 752-
521   753. doi: 10.1038/sj.ejcn.1600836
522 Hill, R. J., & Davies, P. S. (2002). Energy intake and energy expenditure in elite lightweight
523   female rowers. *Med Sci Sports Exerc, 34*(11), 1823-1829. doi: 10.1097/00005768-
524   200211000-00020

Houltham, S. D., & Rowlands, D. S. (2014). A snapshot of nitrogen balance in endurance-trained women. *Appl Physiol Nutr Metab, 39*(2), 219-225. doi: 10.1139/apnm-2013-0182

Howarth, K. R., Phillips, S. M., MacDonald, M. J., Richards, D., Moreau, N. A., & Gibala, M. J. (2010). Effect of glycogen availability on human skeletal muscle protein turnover during exercise and recovery. *J Appl Physiol (1985), 109*(2), 431-438. doi: 10.1152/japplphysiol.00108.2009

Hulston, C. J., Venables, M. C., Mann, C. H., Martin, C., Philp, A., Baar, K., & Jeukendrup, A. E. (2010). Training with low muscle glycogen enhances fat metabolism in well-trained cyclists. *Med Sci Sports Exerc, 42*(11), 2046-2055. doi: 10.1249/MSS.0b013e3181dd5070

Humayun, M. A., Elango, R., Ball, R. O., & Pencharz, P. B. (2007). Reevaluation of the protein requirement in young men with the indicator amino acid oxidation technique. *Am J Clin Nutr, 86*(4), 995-1002. doi: 10.1093/ajcn/86.4.995

Impey, S. G., Hearris, M. A., Hammond, K. M., Bartlett, J. D., Louis, J., Close, G. L., & Morton, J. P. (2018). Fuel for the Work Required: A Theoretical Framework for Carbohydrate Periodization and the Glycogen Threshold Hypothesis. *Sports Med, 48*(5), 1031-1048. doi: 10.1007/s40279-018-0867-7

Impey, S. G., Jevons, E., Mees, G., Cocks, M., Strauss, J., Chester, N., . . . Morton, J. P. (2020). Glycogen Utilization during Running: Intensity, Sex, and Muscle-Specific Responses. *Med Sci Sports Exerc, 52*(9), 1966-1975. doi: 10.1249/mss.0000000000002332

James, A. P., Lorraine, M., Cullen, D., Goodman, C., Dawson, B., Palmer, T. N., & Fournier, P. A. (2001). Muscle glycogen supercompensation: absence of a gender-related difference. *Eur J Appl Physiol, 85*(6), 533-538. doi: 10.1007/s004210100499

Kammoun, I., Ben Saâda, W., Sifaou, A., Haouat, E., Kandara, H., Ben Salem, L., & Ben Slama, C. (2017). Change in women's eating habits during the menstrual cycle. *Ann Endocrinol (Paris), 78*(1), 33-37. doi: 10.1016/j.ando.2016.07.001

Kato, H., Suzuki, K., Bannai, M., & Moore, D. R. (2016). Protein Requirements Are Elevated in Endurance Athletes after Exercise as Determined by the Indicator Amino Acid Oxidation Method. *PLoS One, 11*(6), e0157406. doi: 10.1371/journal.pone.0157406

Kato, H., Suzuki, K., Bannai, M., & Moore, D. R. (2018). Branched-Chain Amino Acids Are the Primary Limiting Amino Acids in the Diets of Endurance-Trained Men after a Bout of Prolonged Exercise. *J Nutr, 148*(6), 925-931. doi: 10.1093/jn/nxy048

Krishnan, S., Tryon, R. R., Horn, W. F., Welch, L., & Keim, N. L. (2016). Estradiol, SHBG and leptin interplay with food craving and intake across the menstrual cycle. *Physiol Behav, 165*, 304-312. doi: 10.1016/j.physbeh.2016.08.010

Lamont, L. S., Lemon, P. W., & Bruot, B. C. (1987). Menstrual cycle and exercise effects on protein catabolism. *Med Sci Sports Exerc, 19*(2), 106-110

Lariviere, F., Moussalli, R., & Garrel, D. R. (1994). Increased leucine flux and leucine oxidation during the luteal phase of the menstrual cycle in women. *Am J Physiol, 267*(3 Pt 1), E422-428. doi: 10.1152/ajpendo.1994.267.3.E422

Malowany, J. M., West, D. W. D., Williamson, E., Volterman, K. A., Abou Sawan, S., Mazzulla, M., & Moore, D. R. (2018). Protein to Maximize Whole-Body Anabolism in Resistance-trained Females after Exercise. *Med Sci Sports Exerc*. doi: 10.1249/mss.0000000000001832

Malowany, J. M., West, D. W. D., Williamson, E., Volterman, K. A., Abou Sawan, S., Mazzulla, M., & Moore, D. R. (2019). Protein to Maximize Whole-Body Anabolism in

572   Resistance-trained Females after Exercise. *Med Sci Sports Exerc, 51*(4), 798-804. doi:
573   10.1249/mss.0000000000001832
574 Mara, J. K., Thompson, K. G., & Pumpa, K. L. (2015). Assessing the Energy Expenditure of
575   Elite Female Soccer Players: A Preliminary Study. *J Strength Cond Res, 29*(10), 2780-
576   2786. doi: 10.1519/jsc.0000000000000952
577 Marquet, L. A., Brisswalter, J., Louis, J., Tiollier, E., Burke, L. M., Hawley, J. A., & Hausswirth,
578   C. (2016). Enhanced Endurance Performance by Periodization of Carbohydrate
579   Intake: "Sleep Low" Strategy. *Med Sci Sports Exerc, 48*(4), 663-672. doi:
580   10.1249/mss.0000000000000823
581 Mazzulla, M., Parel, J. T., Beals, J. W., S, V. A. N. V., Abou Sawan, S., West, D. W. D., . . . Burd,
582   N. A. (2017). Endurance Exercise Attenuates Postprandial Whole-Body Leucine
583   Balance in Trained Men. *Med Sci Sports Exerc, 49*(12), 2585-2592. doi:
584   10.1249/mss.0000000000001394
585 Mazzulla, M., Volterman, K. A., Packer, J. E., Wooding, D. J., Brooks, J. C., Kato, H., & Moore,
586   D. R. (2018). Whole-body net protein balance plateaus in response to increasing
587   protein intakes during post-exercise recovery in adults and adolescents. *Nutr Metab*
588   *(Lond), 15*, 62. doi: 10.1186/s12986-018-0301-z
589 McLay, R. T., Thomson, C. D., Williams, S. M., & Rehrer, N. J. (2007). Carbohydrate loading
590   and female endurance athletes: effect of menstrual-cycle phase. *Int J Sport Nutr*
591   *Exerc Metab, 17*(2), 189-205. doi: 10.1123/ijsnem.17.2.189
592 McNulty, K. L., Elliott-Sale, K. J., Dolan, E., Swinton, P. A., Ansdell, P., Goodall, S., . . . Hicks, K.
593   M. (2020). The Effects of Menstrual Cycle Phase on Exercise Performance in
594   Eumenorrheic Women: A Systematic Review and Meta-Analysis. *Sports Med, 50*(10),
595   1813-1827. doi: 10.1007/s40279-020-01319-3
596 Mercer, D., Convit, L., Condo, D., Carr, A. J., Hamilton, D. L., Slater, G., & Snipe, R. M. J.
597   (2020). Protein Requirements of Pre-Menopausal Female Athletes: Systematic
598   Literature Review. *Nutrients, 12*(11). doi: 10.3390/nu12113527
599 Moore, D. R. (2019). Maximizing Post-exercise Anabolism: The Case for Relative Protein
600   Intakes. *Front Nutr, 6*, 147. doi: 10.3389/fnut.2019.00147
601 Moore, D. R. (2020). One size doesn't fit all: postexercise protein requirements for the
602   endurance athlete. *Am J Clin Nutr, 112*(2), 249-250. doi: 10.1093/ajcn/nqaa144
603 Morton, J. P., Croft, L., Bartlett, J. D., Maclaren, D. P., Reilly, T., Evans, L., . . . Drust, B. (2009).
604   Reduced carbohydrate availability does not modulate training-induced heat shock
605   protein adaptations but does upregulate oxidative enzyme activity in human skeletal
606   muscle. *J Appl Physiol (1985), 106*(5), 1513-1521. doi:
607   10.1152/japplphysiol.00003.2009
608 Morton, R. W., Murphy, K. T., McKellar, S. R., Schoenfeld, B. J., Henselmans, M., Helms, E., . .
609   . Phillips, S. M. (2018). A systematic review, meta-analysis and meta-regression of
610   the effect of protein supplementation on resistance training-induced gains in muscle
611   mass and strength in healthy adults. *Br J Sports Med, 52*(6), 376-384. doi:
612   10.1136/bjsports-2017-097608
613 Mountjoy, M., Sundgot-Borgen, J., Burke, L., Ackerman, K. E., Blauwet, C., Constantini, N., . .
614   . Budgett, R. (2018). International Olympic Committee (IOC) Consensus Statement on
615   Relative Energy Deficiency in Sport (RED-S): 2018 Update. *Int J Sport Nutr Exerc*
616   *Metab, 28*(4), 316-331. doi: 10.1123/ijsnem.2018-0136

617 Nicklas, B. J., Hackney, A. C., & Sharp, R. L. (1989). The menstrual cycle and exercise:
618   performance, muscle glycogen, and substrate responses. *Int J Sports Med, 10*(4),
619   264-269. doi: 10.1055/s-2007-1024913

620 O'Hara, J. P., Duckworth, L., Black, A., Woods, D. R., Mellor, A., Boos, C., . . . King, R. (2019).
621   Fuel Use during Exercise at Altitude in Women with Glucose-Fructose Ingestion. *Med
622   Sci Sports Exerc, 51*(12), 2586-2594. doi: 10.1249/mss.0000000000002072

623 O'Hara, J. P., Woods, D. R., Mellor, A., Boos, C., Gallagher, L., Tsakirides, C., . . . King, R. F.
624   (2017). A comparison of substrate oxidation during prolonged exercise in men at
625   terrestrial altitude and normobaric normoxia following the coingestion of 13C
626   glucose and 13C fructose. *Physiol Rep, 5*(1). doi: 10.14814/phy2.13101

627 Packer, J. E., Wooding, D. J., Kato, H., Courtney-Martin, G., Pencharz, P. B., & Moore, D. R.
628   (2017). Variable-Intensity Simulated Team-Sport Exercise Increases Daily Protein
629   Requirements in Active Males. *Front Nutr, 4*, 64. doi: 10.3389/fnut.2017.00064

630 Pettersson, S., Edin, F., Bakkman, L., & McGawley, K. (2019). Effects of supplementing with
631   an 18% carbohydrate-hydrogel drink versus a placebo during whole-body exercise in
632   -5 °C with elite cross-country ski athletes: a crossover study. *J Int Soc Sports Nutr,
633   16*(1), 46. doi: 10.1186/s12970-019-0317-4

634 Phillips, S. M., Atkinson, S. A., Tarnopolsky, M. A., & MacDougall, J. D. (1993). Gender
635   differences in leucine kinetics and nitrogen balance in endurance athletes. *J Appl
636   Physiol (1985), 75*(5), 2134-2141. doi: 10.1152/jappl.1993.75.5.2134

637 Rothschild, J. A., Kilding, A. E., & Plews, D. J. (2020). Prevalence and Determinants of Fasted
638   Training in Endurance Athletes: A Survey Analysis. *Int J Sport Nutr Exerc Metab*, 1-12.
639   doi: 10.1123/ijsnem.2020-0109

640 Rowlands, D. S., & Wadsworth, D. P. (2011). Effect of high-protein feeding on performance
641   and nitrogen balance in female cyclists. *Med Sci Sports Exerc, 43*(1), 44-53. doi:
642   10.1249/MSS.0b013e3181e93316

643 Schulz, L. O., Alger, S., Harper, I., Wilmore, J. H., & Ravussin, E. (1992). Energy expenditure of
644   elite female runners measured by respiratory chamber and doubly labeled water. *J
645   Appl Physiol (1985), 72*(1), 23-28. doi: 10.1152/jappl.1992.72.1.23

646 Silva, A. M., Santos, D. A., Matias, C. N., Minderico, C. S., Schoeller, D. A., & Sardinha, L. B.
647   (2013). Total energy expenditure assessment in elite junior basketball players: a
648   validation study using doubly labeled water. *J Strength Cond Res, 27*(7), 1920-1927.
649   doi: 10.1519/JSC.0b013e31827361eb

650 Sjödin, A. M., Andersson, A. B., Högberg, J. M., & Westerterp, K. R. (1994). Energy balance in
651   cross-country skiers: a study using doubly labeled water. *Med Sci Sports Exerc, 26*(6),
652   720-724. doi: 10.1249/00005768-199406000-00011

653 Slater, G. J., Dieter, B. P., Marsh, D. J., Helms, E. R., Shaw, G., & Iraki, J. (2019). Is an Energy
654   Surplus Required to Maximize Skeletal Muscle Hypertrophy Associated With
655   Resistance Training. *Front Nutr, 6*, 131. doi: 10.3389/fnut.2019.00131

656 Snijders, T., Trommelen, J., Kouw, I. W. K., Holwerda, A. M., Verdijk, L. B., & van Loon, L. J. C.
657   (2019). The Impact of Pre-sleep Protein Ingestion on the Skeletal Muscle Adaptive
658   Response to Exercise in Humans: An Update. *Front Nutr, 6*, 17. doi:
659   10.3389/fnut.2019.00017

660 Stellingwerff, T., & Cox, G. R. (2014). Systematic review: Carbohydrate supplementation on
661   exercise performance or capacity of varying durations. *Appl Physiol Nutr Metab,
662   39*(9), 998-1011. doi: 10.1139/apnm-2014-0027

663 Stokes, T., Hector, A. J., Morton, R. W., McGlory, C., & Phillips, S. M. (2018). Recent
664   Perspectives Regarding the Role of Dietary Protein for the Promotion of Muscle
665   Hypertrophy with Resistance Exercise Training. *Nutrients, 10*(2). doi:
666   10.3390/nu10020180

667 Sutehall, S., Galloway, S. D. R., Bosch, A., & Pitsiladis, Y. (2020). Addition of an Alginate
668   Hydrogel to a Carbohydrate Beverage Enhances Gastric Emptying. *Med Sci Sports
669   Exerc, 52*(8), 1785-1792. doi: 10.1249/mss.0000000000002301

670 Tarnopolsky, M. (2004). Protein requirements for endurance athletes. *Nutrition, 20*(7-8),
671   662-668. doi: 10.1016/j.nut.2004.04.008

672 Tarnopolsky, M. A., Atkinson, S. A., Phillips, S. M., & MacDougall, J. D. (1995). Carbohydrate
673   loading and metabolism during exercise in men and women. *J Appl Physiol (1985),
674   78*(4), 1360-1368. doi: 10.1152/jappl.1995.78.4.1360

675 Tarnopolsky, M. A., Bosman, M., Macdonald, J. R., Vandeputte, D., Martin, J., & Roy, B. D.
676   (1997). Postexercise protein-carbohydrate and carbohydrate supplements increase
677   muscle glycogen in men and women. *J Appl Physiol (1985), 83*(6), 1877-1883. doi:
678   10.1152/jappl.1997.83.6.1877

679 Tarnopolsky, M. A., Zawada, C., Richmond, L. B., Carter, S., Shearer, J., Graham, T., & Phillips,
680   S. M. (2001). Gender differences in carbohydrate loading are related to energy
681   intake. *J Appl Physiol (1985), 91*(1), 225-230. doi: 10.1152/jappl.2001.91.1.225

682 Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). American College of Sports Medicine
683   Joint Position Statement. Nutrition and Athletic Performance. *Med Sci Sports Exerc,
684   48*(3), 543-568. doi: 10.1249/mss.0000000000000852

685 Tinline-Goodfellow, C. T., West, D. W. D., Malowany, J. M., Gillen, J. B., & Moore, D. R.
686   (2020). An Acute Reduction in Habitual Protein Intake Attenuates Post Exercise
687   Anabolism and May Bias Oxidation-Derived Protein Requirements in Resistance
688   Trained Men. *Front Nutr, 7*, 55. doi: 10.3389/fnut.2020.00055

689 Wallis, G. A., Dawson, R., Achten, J., Webber, J., & Jeukendrup, A. E. (2006). Metabolic
690   response to carbohydrate ingestion during exercise in males and females. *Am J
691   Physiol Endocrinol Metab, 290*(4), E708-715. doi: 10.1152/ajpendo.00357.2005

692 West, D. W., Burd, N. A., Churchward-Venne, T. A., Camera, D. M., Mitchell, C. J., Baker, S.
693   K., . . . Phillips, S. M. (2012). Sex-based comparisons of myofibrillar protein synthesis
694   after resistance exercise in the fed state. *J Appl Physiol (1985), 112*(11), 1805-1813.
695   doi: 10.1152/japplphysiol.00170.2012

696 Wooding, D. J., Packer, J. E., Kato, H., West, D. W. D., Courtney-Martin, G., Pencharz, P. B., &
697   Moore, D. R. (2017). Increased Protein Requirements in Female Athletes after
698   Variable-Intensity Exercise. *Med Sci Sports Exerc, 49*(11), 2297-2304. doi:
699   10.1249/mss.0000000000001366

700 Yeo, W. K., Paton, C. D., Garnham, A. P., Burke, L. M., Carey, A. L., & Hawley, J. A. (2008).
701   Skeletal muscle adaptation and performance responses to once a day versus twice
702   every second day endurance training regimens. *J Appl Physiol (1985), 105*(5), 1462-
703   1470. doi: 10.1152/japplphysiol.90882.2008

704

705

706 **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	$^2H^{18}O$	N/R	(Ebine et al., 2000)
	Cross-country skiing	Elite (national team)	4	54.4±5.1	~17.5	4373±525	$^2H^{18}O$	N/R	(Sjödin et al., 1994)
	Rowing, lightweight	Elite	7	60.9±2.3	22.8±5.1	3957±1219	$^2H^{18}O$	N/R	(Hill & Davies, 2002)
	Running, endurance	Elite	9	53±4	12±3	2826±315	$^2H^{18}O$	N/R	(Schulz et al., 1992)
	Running, endurance	Sub-elite (university)	9	55.3±6.2	13.0±3.2	2990±415	$^2H^{18}O$	N/R	(Edwards et al., 1993)
Mixed	Basketball	Sub-elite (junior national)	7	64.0±5.4	~20.3	2497±242	$^2H^{18}O$	N/R	(Silva et al., 2013)
	Dance, ballet	Sub-elite (university)	12	N/R	N/R	~3176	$^2H^{18}O$	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)

707 Mixed athletes = discipline requires both strength and endurance and typically features stop-and-go exercise. ACC = accelerometer; IC =
708 indirect calorimetry; N/R = not reported. Values are mean ± standard deviation. N = number of female athletes.
709
710

711 **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 ($\approx$ 1 h) and intensity	4-6 g/kg		
High: Longer duration ($\approx$ 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.	*Where relevant and possible, future studies should control for prior exercise, energy / CHO intake, menstrual cycle phase and contraceptive use.*
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	• Consider that glycogen storage may be reduced in the follicular phase	• Effects of menstrual cycle phase on training adherence and habitual nutritional intakes, food / taste preferences and gut health / function.
Pre-Exercise Meal: 1-4 h before training or competition.	1-4 g/kg	• Consider the phase of menstrual cycle in relation to appetite regulation, food cravings and potential effects on habitual absolute CHO intake.	• 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".

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.	*Where relevant and possible, future studies should control for prior exercise, energy / CHO intake, menstrual cycle phase and contraceptive use.*
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		• Effects of menstrual cycle phase on training adherence and habitual CHO intake preferences during exercise
Endurance exercise: > 2.5 h	90 g/h (dual source CHO blends)	• Consider that glycogen storage may be reduced in	

		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". |

712
713

714 **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

715 [1]Post-exercise refers to the first meal after exercise, preferably within 1h after training cessation to maximize muscle protein synthesis.
716 [2]Daily meals refer to all meals throughout the day with the exception of the post-exercise meal
717 [3]Endurance training refers to aerobic-based exercise of moderate-high intensity (e.g. $\geq 70\% VO_{2peak}$)
718 [4]Resistance training refers to high effort, externally loaded muscle contractions (e.g. weight lifting)

719 [5]Mixed training refers to mixed aerobic/anaerobic exercise typically with weight-bearing stop-and-go decelerations and accelerations, such as
720 that common to many team sports (e.g. soccer, rugby, ice hockey)
721

722

723   **Figure 1.** Daily habitual (mean±SD) and recommended (estimated average requirement±95% confidence interval) protein intakes in endurance,

724   mixed, and strength athletes. Mixed athletes refers to mixed aerobic/anaerobic exercise typically with weight-bearing stop-and-go decelerations

725   and accelerations, such as that common to many team sports (e.g. soccer, rugby, ice hockey). Habitual intakes for female athletes from (Gillen et

726   al., 2017). Recommended protein intakes determined to maximize whole body protein synthesis and anabolism during recovery as determined by

727   stable isotope methodology for female strength athletes (Malowany et al., 2019), female mixed athletes after a simulated soccer match (Wooding

728   et al., 2017), and male endurance athletes (Kato et al., 2016) given the lack of research in females. Dashed line represent the recommended dietary

729   allowance. Shaded area represents athlete nonspecific range according to current sports nutrition guidelines (Thomas et al., 2016).

730

731   Figure 1.

Figure 1.

732