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Abstract

**Introduction:** A diet relying more heavily on plant-based food products and less on animal-based products has been promoted to feed a growing world population in a sustainable fashion. One concern with such a dietary pattern is iron bioavailability, as plant-based sources of iron contain phytic acid, a compound that binds to cations such as iron, limiting absorption.

**Objectives:** The objectives of this research are to examine the efficacy of a biofortified pea protein (bred to have low phytic acid, and therefore high iron bioavailability) on nutritional intake and iron status in vulnerable athlete populations.

**Methods:** In study one, a pilot trial evaluated the acceptability of an experimental high-protein supplement (made from field peas with high iron bioavailability) and the feasibility of data collection measures in a group of female runners. Before and after an 8-week supplementation protocol, participants were assessed for iron status, body composition, and exercise performance. Participants were randomized to consume either a protein concentrate derived from regular peas, a protein concentrate derived from low phytic acid peas, or an isocaloric control (i.e., maltodextrin). Participants also consumed 500 mg of vitamin C daily throughout the trial to enhance iron absorption. The incidence of adverse effects thought to be related to the supplement (eg. Bloating, constipation, cramping) were assessed at weeks two, four, six, and eight. With feasibility confirmed, in study two, 28 female runners underwent the same measures as outlined in study one (iron status, body composition, and exercise performance) before and after being randomly assigned to consume either maltodextrin, regular pea protein, or low phytic acid pea protein along with 375 mg of vitamin C for eight weeks. In study three, the habitual dietary intake of macronutrients and key micronutrients (including iron) of 31 elite para cyclists was determined using a food frequency questionnaire. Risk of inadequacy was assessed using the
estimated average requirement cut-point method and intakes were compared to daily recommended intake values. Lastly, in study four, four individuals with spinal cord injuries that were recreationally active were assessed for bowel function, iron status, body composition, and exercise performance before and after an 8-week intervention in which they supplemented with 40 g of low phytic acid pea concentrate and 125 mg vitamin C twice daily.

**Results:** In study one a high degree of compliance was observed for all supplements (maltodextrin= 99.2 ±0.9% compliant; regular pea= 96.3 ±2.1% compliant; low phytic acid= 97.8 ±2.3% compliant). No differences in the incidence of adverse effects between the maltodextrin and pea groups were evident (p=0.53,) nor were any symptoms severe enough to cause the individual to withdraw from the study. Study two found a modest, though statistically non-significant, increase in ferritin was observed for the two pea groups (regular pea= 14.4% increase from baseline; low phytic acid= 5.1% increase from baseline), compared to the maltodextrin group who had a decrease in ferritin (2.2%). Protein (p=0.03) and iron (p=0.01) intakes were significantly higher in the regular pea group compared to the maltodextrin group. No other differences in dietary intake were observed (p>0.05). In study three, athletes consumed most nutrients in excess of the daily recommended intakes with the exception of iodine (males=87% recommended dietary allowance; females=62% recommended dietary allowance) and fibre for men (84% adequate intake). The predicted risk of inadequacy was noted for iodine (89% and 83% risk of inadequacy for females and males, respectively) and vitamin D (84% and 83% risk of inadequacy for females and males, respectively). In study four, no differences were apparent in exercise performance or markers of iron (ferritin and hemoglobin); however, iron intakes increased from baseline to week six (p=0.047) and protein intakes trended toward
increased from baseline to week eight (p=0.07). Scores of bowel dysfunction were largely unaltered in three participants, but one individual had a marked improvement.

**Conclusion:** Biofortified peas are well tolerated in both female runners and individuals with spinal cord injuries. Consumption of biofortified peas did not have any negative effects on nutrient intake, iron status, or exercise performance in these populations of active individuals. Thus, they have the potential to improve nutrient quality and health measures in vulnerable athlete populations. Larger doses or longer trials may be required to induce meaningful changes to iron status.
Research Published or Submitted During PhD Program and Co-Authors

Studies included in whole or part in this thesis:

Used in part in Chapter 1 (pg 1-3) and Chapter 2 (section pg 11-36):


Chapter 3:


Chapter 4:


Chapter 5:

Other articles published or accepted for publication:


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Dedication

To Mom and Dad, for telling me I could do whatever I set my mind to from the very beginning.

And to Aaron and Dallas, for always keeping me humble in the way only brothers can.
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List of Abbreviations

PB= Plant based
ID= Iron deficiency
RDA= Recommended dietary allowance
DEXA= dual energy x-ray absorptiometry
TT= Time trial
VO$_2$max= Maximal oxygen consumption
RED-S= Relative energy deficiency in sport
SCI= Spinal cord injury
lpa= Low phytic acid
RER= Respiratory exchange ratio
Reg= Regular pea
Malto= Maltodextrin
DRI= Dietary reference intake
AI= Adequate intake
EAR= Estimated average requirement
FFQ= Food frequency questionnaire
FO= Food only
F+S= Food plus supplement
RAE= Retinol activity equivalents
NBD= Neurogenic bowel dysfunction
VO$_2$peak= Peak oxygen consumption
Chapter 1-Introduction

Plant-based (PB) diets have become increasingly popular in recent years, particularly in developed countries that have traditionally consumed a large amount of meat and other animal products as part of their regular intake. A recent panel of experts have called for a “Great Food Transformation”, in which the food system is capable of feeding a growing population while minimizing the environmental impact of food production and distribution (Willett et al., 2019). This panel imagined a reference diet that includes little to no meat or animal products, refined grains, added sugar, or starchy vegetables and instead is rich in fruits, vegetables, whole grains, legumes, nuts, and unsaturated oils. Such recommendations are also becoming popular amongst the general population. In a review of internet search trends from different countries in the world between January 1, 2001 to July 18 of 2019, PB diets were the most searched dietary pattern on Google trends in the United States, much of South America, Australia, Russia, and much of Europe (Kamiński et al., 2020). While a PB diet is not adequately defined in the literature in terms of nutrient and food content, it broadly refers to a variety of dietary patterns that emphasize higher intakes of plant foods and are low in animal products (McEvoy et al. 2012). In a contribution to the Harvard Medical School Health Blog, McManus (2021) defines PB eating as “eating patterns focus[ed] on foods primarily from plants. This includes not only fruits and vegetables, but also nuts, seeds, oils, whole grains, legumes, and beans. It doesn’t mean that you are vegetarian or vegan and never eat meat or dairy. Rather, you are proportionately choosing more of your foods from plant sources.” Thus, these diets vary in their level of restrictiveness, from avoiding only red meat to the avoidance of animal products entirely (Clarys et al. 2014). An overview of common PB diets is presented in Table 1.1. For the purposes of this thesis, a PB diet will refer to a diet void of all animal products (i.e., vegan) unless otherwise specified.
Table 1.1 Classification of plant-based dietary patterns

<table>
<thead>
<tr>
<th>Type of diet</th>
<th>Definition</th>
<th>Animal Flesh</th>
<th>Fish</th>
<th>Dairy</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-vegetarian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omnivorous</td>
<td>Consumes all animal flesh and animal products (dairy, eggs, honey, gelatin, etc.)</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td><strong>Plant-Based/Vegetarian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pescatarian</td>
<td>Consumes fish, dairy, and milk but not red meat, pork, or poultry</td>
<td>X</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Lacto-Ovo vegetarian</td>
<td>Consumes eggs and dairy but no animal flesh</td>
<td>X</td>
<td>X</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Lacto-vegetarian</td>
<td>Consumes dairy but no animal flesh or eggs</td>
<td>X</td>
<td>X</td>
<td>✔️</td>
<td>X</td>
</tr>
<tr>
<td>Ovo-vegetarian</td>
<td>Consumes eggs but no animal flesh or dairy</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>✔️</td>
</tr>
<tr>
<td>Vegan</td>
<td>Avoids all products derived from animals</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

*Adapted from Le & Sabaté, 2014, as cited in Shaw et al. (2022)

Rationales for following a PB diet are plentiful. Common motivations include religious practices, health benefits, ethical and moral concerns (solicitude around animal welfare and treatment), or environmental concerns (reduced greenhouse gas emissions, less land, water, and energy usage compared to animal farming; Mensink et al. 2016). Research suggests that the adoption of a well-planned PB diet in general or clinical populations (cardiovascular disease, cancer, etc.) is nutritionally adequate and potentially superior to a traditional omnivorous diet for reducing the risk of chronic diseases, such as cardiovascular disease, type 2 diabetes, obesity, metabolic syndrome, hypertension, and some cancers (Kim et al. 2018, 2019; Tuso et al. 2013).
Despite the evidence for a PB diet's role in promoting good health, some doubt exists concerning a PB diet in supporting optimal athletic training and performance (Lynch et al. 2018). While the inclusion of dairy and eggs into a PB diet (i.e., lacto-ovo vegetarian) generally ensures an adequate supply of nearly all nutrients required for optimal human health and performance, a diet void of other animal products could be lacking micronutrients, such as iron, calcium, vitamin B12, and omega-3 fatty acids, which are crucial to not only health but also athletic performance (Kreider et al. 2010; Lukaski 2004). Therefore, proper dietary planning and consumption of a variety of PB foods are necessary to meet the nutritional requirements for all stages of life and fitness goals (Nebl et al. 2019; Venderley & Campbell 2006).

While a general consensus exists surrounding the beneficial health effects of a PB diet when implemented appropriately (Fehér et al., 2020; Kahleova et al., 2017), micronutrient deficiency remains a dilemma globally, particularly in developing countries that rely heavily on a PB diet. Such deficiencies are often attributed to a lack of diversity in the dietary intake of these individuals due to socioeconomic constraints (Akhtar et al., 2013). Several strategies to combat such deficiencies by improving nutrient bioavailability have been studied, including the addition of enzymes (either through genetic modification or through supplementation), additional processing designed to break down chelating agents, or plant breeding designed to yield crops with low antinutritional compounds (Bangar et al., 2017). At the University of Saskatchewan Crop Development Centre, a crop breeding process called chemical mutagenesis was utilized to produce field peas with reduced levels of phytic acid, an antinutrient that chelates to divalent irons in pulses and other PB foods (Warkentin et al., 2012). These plants have shown a decrease in phytic acid by 60% and increased bioavailability of iron by 1.9 times compared to the parent plant (non-modified) while maintaining similar agronomic performance, with a produced yield of
approximately 89% compared to the parent crops (Bangar et al., 2017). These fortified peas may pave the way for such breeding practices to enhance nutrient bioavailability in other staple food items, potentially providing a sustainable solution to micronutrient deficiencies.

Malnutrition, as it relates to nutritional deficiencies, has traditionally been considered a disease of impoverished individuals (Siddiqui et al., 2020). However, micronutrient deficiencies are becoming increasingly common in developed countries such as the United States and Canada (Health Canada, 2012; Millen et al., 2016). When considering nutritional inadequacies, populations of interest typically include those in rapid periods of growth such as children, adolescents, and pregnant women (Akhtar et al., 2013; Ofori et al., 2022) or older adults (Norman et al., 2021). Athletes may not be a population that comes to mind when considering those vulnerable to nutritional deficiencies, but the sport science literature suggests that micronutrient deficiencies may be increasingly common in some athletic populations. For example, in endurance athletes who compete in sports where low weight is considered beneficial for performance, relative deficiencies in energy are common which may directly or indirectly impact the consumption and absorption of key nutrients such as iron and fat-soluble vitamins (Robertson & Mountjoy, 2018). Further, athletes with physical impairments (e.g., para athletes) may also be at risk for nutritional deficiencies due to increased need based on specific pathophysiology or altered gastrointestinal function which leads individuals to restrict intakes of certain foods (Ayas et al., 2006; Graham-Paulson et al., 2017; Scarmella et al. 2018).

In the following chapter, I will summarize and discuss the current literature on the impact of a PB diet on health and performance as well as strategies to optimize bioavailability of nutrients present in PB foods (particularly pulses) through biofortification. I will also discuss how such biofortification may benefit certain athletic populations to improve health and performance.
Throughout this thesis, optimal training and performance will refer to the training and recovery supported by adequate nutrition to sustain and progress one’s fitness and exercise or sport performance. Particular attention will be paid to protein and iron, as is a focus of the research included in this thesis.

1.1 Purpose Statement

The overall goal of this thesis is to elucidate the role of biofortification of a PB protein source and how such a product might be used in an athletic population to maximize health and performance. The goal of the first study in this series was to create a PB protein product that could feasibly deliver a large amount of bioavailable micronutrients (with a focus on iron). The goal of the second study was to assess how this PB protein might influence the health and performance parameters of female runners. The third study aimed to assess the dietary intake of a group of cyclists with physical impairments and highlight any shortcomings to better understand how the product developed in study one might apply to athletes with impairments. The final study was to assess the application of the PB protein developed in study one in a population of athletes with physical impairments.

1.2 References


Kamiński, M., Skonieczna-Żydecka, K., Nowak, J. K., & Stachowska, E. (2020). Global and local diet popularity rankings, their secular trends, and seasonal variation in Google


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https://doi.org/10.1016/S0140-6736(18)31788-4
Chapter 2. Literature Review

Plant based (PB) diets are referenced throughout a variety of religious texts and ancient philosophers. For example, ancient philosophers Pythagoras and Aristotle are believed to have had strong beliefs for or against the PB dietary regime, respectively (Hardinge, 1951). However, despite this rich history, it has been reported that the first thoughtful academic investigation into PB diets arose from the doctoral thesis of Mervyn G. Hardinge in the early 1950s (Hardinge & Johnston, 1999; Le & Sabaté, 2014). Hardinge felt that the literature on the topic to date was lacking and that which was available was prejudiced against vegetarians (Hardinge & Johnston, 1999). As a lifelong vegetarian himself, Hardinge set out to assess the adequacy of a vegetarian diet in different populations (Hardinge & Stare, 1954a; Hardinge et al., 1966) as well as health benefits of such a diet (Hardinge & Stare, 1954b; Hardinge et al., 1958a, 1958b, 1962). In the 50 years between this seminal work to the turn of the 21st century, many challenged the adequacy and morality of PB diets (Anonymous, 1980; George, 1990; Varner, 1994; Zamir, 1970). However, in the two decades since, PB diets- when planned appropriately- have been widely accepted as adequate and potentially even superior to diets containing large amounts of animal products for minimizing the risk of non-communicable diseases, which currently account for almost three-quarters of global deaths each year (Melina et al., 2016; Tuso et al., 2013; World Health Organization, 2022).

The acknowledgment of the adequacy of PB diets from the scientific and dietetic communities has led to PB diets becoming increasingly popular among a variety of different populations for a myriad of reasons such as ethical and moral concerns, religious practices, or health benefits of such a dietary pattern (Mensink et al., 2016). The purpose of the first section of this chapter is to broadly discuss the health implications of a PB diet, including benefits and potential areas of the
diet that require specific attention to plan such a diet appropriately. I will then discuss how a PB diet might play a role in exercise and sport performance, both from a physiological perspective as well as what the literature suggests regarding the impact on outcome variables of performance.

2.1 The Role of PB Diets in Health and Chronic Disease

Consumption of some variety of a PB diet has increased in contemporary society, a trend Feher et al. (2020) have attributed to an increased health consciousness in the general population. While statistics surrounding the prevalence of such a dietary pattern are lacking across many developed countries, research suggests general intakes of PB diets tend to be between 5-10% of the population, though such numbers characterize any form of PB diet (Table 1.1). The proportion of the population abstaining entirely from all animal product intake (i.e., vegan) tends to be lower at approximately 2% (Nebl et al., 2019a). While there are many motives for choosing a PB diet, the health-promoting effects of such a pattern of intake appear to be dominant in the rationale for choosing to abstain from or limit animal products (Fehér et al., 2020; Pohjolainen et al., 2015). However, when appraising the health benefits of a PB diet, one must consider the source of the data. Much of the literature supporting the health benefits of a PB diet relies on observational cohort studies, mostly composed of Seventh-Day Adventists and Buddhist monks, which may bias and confound the data due to the overall healthy lifestyle behaviours observed in these communities (Kim et al., 2018; 2019). Further, any cohort study in the area is likely to be recruiting generally health-conscious individuals. Such populations are likely to practice other health-promoting behaviours such as moderate alcohol consumption, regular physical activity, and abstention from smoking. Socioeconomic class could be a further confounding variable, as lower socioeconomic status has been associated with decreased consumption of a healthful PB
diet (Gonzalgo et al., 2022). Therefore, more clinical trials are warranted to assess the role of a PB diet on health markers and the prevention and treatment of non-communicable diseases.

While a PB diet is often considered a health-promoting diet at face value, it, like any diet, requires consideration and planning to maximize health benefits. While a PB diet rich in unprocessed whole grains, fruits, vegetables, and legumes is beneficial for improving cardiometabolic health (Satija & Hu 2018), a PB diet high in processed and refined grains, potatoes, and sugar-sweetened beverages is likely to do the opposite (Satija et al., 2017; Satija & Hu, 2018). In fact, research has shown that most PB diets rely heavily on cereal grains rather than protein-rich sources such legumes, nuts and seeds (Marinangeli et al., 2021). Such a pattern of eating severely limits the protein quality of the diet, impacting the potential health impacts. Therefore, proper planning and attention must be given to the types of foods being consumed to ensure a nutritionally adequate diet that meets the individual’s requirements for health, as well as fitness goals, as is the case with any pattern of eating.

In a systematic review by Schwingshackl et al. (2017), it was concluded that a diet rich in whole grains, vegetables, fruits, nuts, legumes, and fish and subsequently a reduced intake of red and processed meat (not the avoidance thereof) and sugar-sweetened beverages led to a decreased risk of premature death by ~ 80% when compared to intakes from the highest risk category. Similarly, Boeing et al. (2012) suggested that increased intakes of fruits and vegetables independently decrease the risk for various diseases due to polyphenolic and antioxidant properties, which influence inflammatory, cellular redox, endothelial, and metabolic processes, all of which are involved in the pathogenesis of various diseases. Similar findings were reported in an umbrella review by Angelino et al. (2019), who concluded that increased intakes of fruits and vegetables are likely to have protective effects against an array of diseases with varying levels of evidence.
The high fibre content of a PB diet is also thought to have many beneficial effects on health through its impact on gut microbiota (Makki et al. 2018). Therefore, replacing a portion of one’s animal-product intake with PB sources of food may enhance the quality of the diet by increasing the intake of polyphenols, antioxidants, some vitamins and minerals, and fibre while decreasing the intake of energy and less desirable fatty acids (Craddock et al. 2016).

Although few randomized controlled trials have investigated the effects of a strict PB diet, many epidemiological studies have determined the effect of replacing a portion of animal products with PB foods. Such practices have shown benefits for the prevention, morbidity, and mortality related to some chronic diseases (Alwarith et al. 2020; Ashaye et al. 2011; Cheung et al. 2014; Dauchet et al. 2006; Joshi et al. 2020; Sakkas et al. 2020; Qian et al. 2019; Yadav et al., 2016). However, much of the research for clinical conditions has involved the inclusion of more fruits, vegetables, and whole grains in the diet and not necessarily the avoidance of animal products. Further, an abundance of evidence for a PB diet comes from epidemiological research, and therefore a cause–effect relationship cannot be inferred.

2.2 Nutrient Absorption in a Plant-Based Diet

Although a PB diet is adequate for various ages and nutrition goals (Fuhrman & Ferreri 2010), a poorly planned diet may predispose an individual to deficiencies in energy and some nutrients due to decreased amounts or bioavailability in PB sources (Rogerson 2017). PB foods such as pulses and legumes (including field peas), grains, oilseeds, and nuts, may contain anti-nutritional compounds, such as phytates, tannins, and fibre. While these compounds have some beneficial effects on health, they also decrease the absorption of some nutrients (Shubham et al. 2020).
2.2.1 Tannins

Tannin is a polyphenolic compound that is particularly astringent (i.e., pungent; bitter; drying) and found predominantly in grains, fruits, beans, pulses, nuts, and beverages, such as coffee, wine, and tea (Brglez Mojzer et al. 2016). Tannins create complexes with proteins, starch, and digestive enzymes in the gastrointestinal tract decreasing the absorption and utilization of nutrients, such as vitamins and minerals (Brglez Mojzer et al. 2016; Chung et al. 1998; Frutos et al. 2004; Shubham et al. 2020). However, despite decreasing the nutritional value of food, the consumption of tannins also has anti-inflammatory, anti-oxidative, anti-microbial, and anti-carcinogenic effects on human health (Brglez Mojzer et al. 2016).

2.2.2 Phytic Acid

Phytic acid, or myo-inositol-1,2,3,4,5,6-hexakisphosphate (C$_6$H$_{18}$O$_{24}$P$_6$), is a phosphorus-based molecule that predominantly acts as a storage form of phosphorus, with 60 to 80% of total phosphorus being stored as phytic acid in pulses and cereal grains (Raboy et al., 2017; Warkentin et al. 2012). In cereal grains such as rice, barley, and wheat, phytic acid is stored in the aleurone layer and bran, while in corn it is stored in the embryo and legumes in the cotyledon (Pramitha et al., 2021). Phytic acid is generally believed to be synthesized during seed development, after which it binds to cations such as potassium, magnesium, zinc, and iron (Raboy, 2017), creating a salt. These salts are collectively referred to as “phytates” (Feizollahi et al., 2021). As phytic acids and their salts typically occur simultaneously, many researchers do not distinguish between the two terms, rather choosing to collectively refer to phytic acid or phytate.

Phytic acid limits intestinal absorption of divalent cations by chelating minerals, such as calcium, magnesium, zinc, and iron, forming an insoluble complex that is ultimately excreted.
before the mineral can be absorbed (Bangar et al. 2017; Zhang et al. 2020). Despite the decrease in nutrient absorption, phytate also has health-promoting roles such as the prevention of diabetes, as an anti-carcinogenic agent, as an antioxidant, in the prevention of kidney stones, and the prevention of cardiovascular disease (Kumar et al. 2021).

2.2.3 Fibre

Dietary fibres are a complex group of carbohydrates and lignin that cannot be digested by the small intestine. Dietary fibres have many beneficial impacts on human health, such as decreasing the risk of cardiovascular disease (Soliman 2019), enhancing the diversity of the gut microbiome (Makki et al. 2018), and regulating blood glucose (Vithana Pathirannehelage & Joye 2020). However, they also decrease nutrient absorption and increase satiety, and, therefore, the athlete with high energy demands should be aware to not overconsume fibre (Soliman 2019). Many physicochemical aspects of fibre, such as fermentation, bulking ability, binding ability, viscosity and gel formation, water-holding capacity, and solubility cause fibre to impact the absorption of some nutrients (Adams et al. 2018). While slowed digestion and increased satiety due to increased fibre intake may be beneficial for overall health in the general Westernized population (He et al. 2022; McRorie & McKeown 2017; Soliman 2019), it could make meeting the energy requirements of elite sport challenging (Melin et al. 2016). However, research from our lab implementing a pulse-based diet for four weeks in soccer players did not impact body mass or performance, suggesting energy intake was not affected (Mizelman et al., 2020). That said, if an athlete is looking to decrease body mass, fibre may assist in managing hunger and meeting energy goals.
2.3 Dietary Considerations for the Plant-Based Athlete

Despite the evidence for the beneficial effects of a PB diet for general health and wellness, some continue to question its suitability for optimizing exercise performance, specifically around concerns of protein, iron, vitamin B12, and creatine (Kaviani et al. 2020a). Conversely, others believe that a PB diet may be superior for exercise performance due to the high quantities of carbohydrates, phytochemicals, and antioxidants (Figure 2.1; Craddock et al. 2016). The following paragraphs will discuss some of the nutritional considerations that many athletes may be concerned about when considering following a PB diet. Particular attention will be paid to iron and protein, as these have historically been major concerns in athletes contemplating a PB diet and will be a key aspect of the current thesis.

![Figure 2.1 Potential positive and negative implications of a plant-based diet on exercise performance (from Shaw et al., 2022).](image)

2.3.1 Energy
A well-planned, healthful PB diet is high in fibre and often includes high-volume, low-energy foods (Cialdella-Kam et al. 2016; Lis et al. 2019). As such, a PB diet is very satiating, leading to a decreased total energy intake. Decreased total energy intake is often attributed to a PB diet's beneficial health effects in some clinical populations (e.g., diabetes; Lynch et al. 2018; Newby 2009), but can be a concern for athletes with high energy requirements. If an athlete is not attentive to energy intake, they risk slipping into a state of low energy availability, leading to decrements in various measures of physical and mental health as well as performance (Mountjoy et al. 2018). Also, worth noting is that some athletes may adopt a PB diet to mask a restrictive eating disorder (Lis et al. 2019), so the integrated sport team (i.e., coaches, dietitians, and mental performance consultants) must be aware of the specific rationale for following a PB diet and monitor fluctuations in the athlete’s weight. To ensure adequate energy is consumed, the athlete should monitor their weight and be aware of unnecessary or sizable degrees of weight loss. While consuming adequate energy on such a diet may not be problematic for all athletes (Mizelman et al., 2020), if an individual finds consuming adequate energy while following a PB diet challenging, eating five to eight meals and snacks per day and choosing energy-dense food choices, such as nuts and seeds, nut and seed butter, avocados, dried fruit, trail mix, hummus, and granola may be beneficial (Lis et al. 2019).

2.3.2 Protein

Although protein is found in abundance in PB sources (Table 2.1), the amino acid profile in such sources is often suboptimal compared to animal-based sources (Lynch et al. 2018). In particular, PB sources of protein rarely match animal proteins for the branched-chain amino acids, especially leucine. Leucine plays a key role in muscle protein synthesis through its role in
the stimulation of the mammalian target of rapamycin (i.e., mTOR), a protein kinase that regulates protein synthesis and cellular growth (Devries et al. 2018; Hartman et al. 2007; Xu & Ren 2014). Decreased circulating branched-chain amino acids in both lacto-ovo vegetarians as well as vegans compared to their omnivorous peers has been observed (Wang & Babitt, 2019), thus substantiating concerns regarding protein quality in PB protein sources. However, the Academy of Nutrition and Dietetics has reported in their 2016 position that consuming a wide variety of PB sources of protein and consuming adequate energy are likely sufficient to meet the required intakes of all indispensable amino acids (Melina et al. 2016).

While concerns around consuming a PB protein and its role in supporting optimal exercise performance remains in some individuals, it is widely accepted in the literature that protein recommendations in developed countries can be met through the consumption of a variety of PB sources, sufficiently delivering all indispensable amino acids when caloric intake is adequate (Melina et al. 2016; Craig et al. 2009; Zello 2006). However, some athletes may find it challenging to meet their protein needs from PB foods if their caloric requirements are exceptionally high. Due to the high fibre content, a PB diet tends to be very filling and, therefore, individuals may have a concern with consuming enough protein from whole food sources to support optimal muscle protein synthesis. If an athlete struggles to meet protein recommendations, a protein supplement may be a viable strategy. Multiple PB protein supplements have been studied and shown to be effective in enhancing hypertrophic response to exercise and/or exercise performance, such as soy (Candow et al. 2006), mung bean (Bartholomae et al. 2019), oat (Xia et al. 2018), rice (Joy et al. 2013), pea (Banaszek et al. 2019), and hemp (Kaviani et al. 2016b). A PB protein supplement formulated with a variety of protein sources may show benefits relative to supplementing with a single source to ensure all
indispensable amino acids are consumed (Zello 2006). A supplement containing soy protein may be of particular interest due to its high leucine content (Rogerson 2017).

**Table 2.1 Plant-based sources with significant amounts of key nutrients**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant-Based Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein</strong></td>
<td>Beans, peas, lentils, chickpeas, soybeans, nuts</td>
</tr>
<tr>
<td><strong>Omega-3 Fatty Acids</strong>*</td>
<td>Walnuts, chia, camelina, hemp, flaxseed, soybeans</td>
</tr>
<tr>
<td><strong>Omega-6 Fatty Acids</strong>*</td>
<td>Corn, tortilla and potato chips, tofu, tempeh, nuts and seeds, vegetable oils, peanut butter</td>
</tr>
<tr>
<td><strong>Monounsaturated fatty acids</strong></td>
<td>Canola oil, olive oil, peanut oil, avocado, nuts</td>
</tr>
<tr>
<td><strong>Iron</strong></td>
<td>Beans, peas, lentils, chickpeas, nuts, seeds, whole grains, fortified bread and cereals</td>
</tr>
<tr>
<td><strong>Zinc</strong></td>
<td>Beans, peas, lentils, chickpeas, edamame, nuts, seeds</td>
</tr>
<tr>
<td><strong>Calcium</strong></td>
<td>Chinese cabbage, kale, texturized vegetable proteins, tofu, nuts, seeds, beans</td>
</tr>
<tr>
<td><strong>Vitamin D</strong></td>
<td>Fortified cereals, margarine, and plant-based “milk” (almond, soy, oat, pea, etc.)</td>
</tr>
<tr>
<td><strong>Riboflavin</strong></td>
<td>Nutritional yeast, quinoa, muesli, fortified cereals and plant-based “milk” (almond, soy, oat, pea, etc.), avocado, wild rice, and mushrooms</td>
</tr>
<tr>
<td><strong>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</strong></td>
<td>Nutritional yeast, fortified plant-based “milk” (almond, soy, oat, pea, etc.), fortified meat analogs, shiitake mushrooms</td>
</tr>
</tbody>
</table>

*Adapted from Lis et al., 2019; as cited in Shaw et al. (2022) *Polyunsaturated fat
2.3.3 Carbohydrates

The high concentration of carbohydrates found in a PB diet may offer performance advantages by assisting athletes in meeting recommendations for their activity levels, ensuring optimal glycogen concentrations, and preventing early fatigue (Barnard et al. 2019). Burke et al. (2017) observed not only improved race performance in trained race walkers but also improved exercise economy at a given pace in those consuming chronic high carbohydrate diets compared to those consuming a high fat, low carbohydrate (i.e., “keto”) diet. As such, the International Society for Sport Nutrition recommends athletes engaging in “moderate amounts of intense exercise” should consume 5–8 g/kg body weight carbohydrate daily to maintain liver and muscle glycogen (Kerksick et al. 2018). Given that muscle glycogen levels are directly correlated with time to fatigue in moderate (60–80% VO2 max) intensity exercise, optimizing glycogen levels may delay fatigue in endurance and team sports (Hermansen et al. 1967; Kaviani et al. 2020b).

Healthful PB diets also tend to be rich in foods with a low glycemic index, which limits the increase of blood glucose following the ingestion of carbohydrates. A reduced blood glucose response would subsequently result in a decreased insulin concentration, facilitating lipolysis during exercise (Gao & Chilibeck 2020). Kaviani et al. (2020b) observed an improvement in sport-specific performance as well as a decreased reliance on carbohydrates in recreational soccer players that consumed a low glycemic index sport bar made from lentils two hours before a simulated soccer game and at halftime compared to those that consumed a high glycemic index sport bar. However, Mizelman et al. (2020) observed no differences in distance covered during a soccer match in those consuming a pulse-based diet for four weeks compared to their regular (i.e., mixed) diet. Further, lentils, a low-glycemic index food, consumed before exercise tended to better preserve muscle glycogen during simulated soccer performance than a calorie- and macronutrient-matched meal containing potatoes and eggs (Little et al. 2010).
As noted above, athletes consuming a PB diet should be conscious not to overconsume fibre, which is abundant in such a diet. Fibre intake has been correlated with gastrointestinal disturbances during competition, especially in endurance running (Jeukendrup 2017). High intakes of fibre may also impact protein digestibility. While the exact mechanisms by which fibre impacts digestibility remain unclear, high fibre diets linearly decrease protein digestibility (Adams et al. 2018).

2.3.4 Fatty Acids

PB diets are typically lower in fat compared to the standard Western diet, a characteristic that has been attributed to many of its health benefits (Tuso et al. 2013). However, the type of fat and not the amount is more likely the source of the benefits found in following a PB diet. Mono- and polyunsaturated fatty acids play an important role in human health, especially in preventing and managing various diseases and inflammatory conditions while promoting the absorption of some vitamins (Gómez Candela et al. 2011; Moreno and Salvadó 2000). Omega-3 fatty acids may be of particular interest for PB athletes, as nutritional interventions involving omega-3s show promise in reducing the likelihood of enduring a sport-related concussion following a traumatic head contact and enhancing recovery following a head injury (Lust et al., 2020). A healthful PB diet is adequate in most omega-6 fatty acids (linolenic acid, arachidonic acid, gamma linoleic), though conjugated linoleic acid is less abundant. One must be aware not to overindulge in omega-6 fatty acids, however, as such dietary patterns have been linked to inflammation, vasoconstriction, and platelet aggregation (Saini & Keum 2018). Though rich in omega-6 fatty acids, PB diets lack the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid most often found in fatty fish while containing limited sources of the omega-3 fatty acid α-
linolenic acid (Table 2.1). Although α-linolenic acid can be converted endogenously to eicosapentaenoic acid and docosahexaenoic acid, it is a rather inefficient conversion and highly variable depending on factors, such as sex, dietary composition, health status, and age (Melina et al. 2016). Further, high intakes of linoleic acid may suppress the conversion of α-linolenic acid (Plourde & Cunnane 2007; Rogerson 2017). To maximize conversion, intake ratios of 4:1 (linolenic acid to α-linolenic acid) are suggested (Melina et al. 2016). Philpott et al. (2019) reported supplementation with omega-3 fatty acids may be effective in improving muscle adaptation, energy metabolism, muscle recovery, and injury prevention, regardless of the dietary patterns followed. Therefore, athletes looking to optimize their performance may want to pay close attention to ensure they are achieving optimal intake levels.

2.3.5 Micronutrients

Although all micronutrients except vitamin B12 can be found in adequate amounts in a PB diet, some must be paid particular attention. Supplementation may be desirable to prevent or treat nutrition deficiencies regardless of diet, such as iron, vitamin D, calcium, and vitamin B12, and should be informed by a registered dietitian (Maughan et al. 2018; Larson-Meyer et al. 2018). For more detailed information on micronutrients often requiring supplementation in athletes, the interested reader is directed to Maughan et al. (2018).

Iron. Iron is an essential nutrient involved in oxygen transport, energy production, cell proliferation, and erythropoiesis (Sim et al. 2019). Although iron is found abundantly in a PB diet (Table 2.1), the bioavailability of PB sources (non-heme iron) is less than that found in animal sources (heme iron). Non-heme iron must be converted from its ferric form (Fe^{3+}) to a ferrous
form (Fe\textsuperscript{2+}) before being absorbed, making it a more complex and less efficient physiological process compared to the absorption of heme iron, decreasing bioavailability (Figure 2.2; Zimmermann and Hurrell 2007). Additionally, bioavailability may be decreased by the anti-nutritional factors discussed above (Section 2.2). Consequently, despite similar or greater iron intake, ferritin levels in PB individuals tend to be lower compared to omnivorous individuals (Śliwińska et al., 2018).

Despite lower ferritin levels in PB individuals, ferritin values typically fall within the normal range, potentially due to an adaptive response to a long-term PB diet that allows the individual to absorb non-heme iron more effectively (Melina et al. 2016). Further, some actions can enhance the body's absorption, including pairing non-heme iron with vitamin C or heme sources (if not entirely a PB diet) and avoiding foods rich in phytates, polyphenols, and calcium as well as exercise within two hours of consumption (Alaunyte et al. 2015; Zimmermann and Hurrell 2007).

Athletes tend to be at greater risk for iron deficiency due to increased inflammation from training inhibiting absorption, increased iron losses through sweat, and repeated foot contact on the ground damaging red blood cells (DellaValle and Haas 2012). Females may be at further risk due to iron losses through menstruation (Badenhorst et al. 2021). Further, increased iron intake may be required to support increased erythropoiesis (Pedlar et al. 2018). With increased iron needs in athletes and the high prevalence of iron deficiency across all populations, individuals may opt to supplement iron to maximize performance, especially endurance athletes (Nabhan et al. 2020; Sim et al. 2019). Iron is important for endurance athletes because iron makes up the “heme” portion of hemoglobin, which binds oxygen and carries it throughout the body (Dutt et al., 2022). Athletes in a state of iron deficiency tend to have decreased aerobic power due to a reduction in oxygen-carrying capacity and, therefore, an increased reliance on anaerobic metabolism, with the degree of impairment positively correlated with the degree of deficiency (Sim et al. 2019). While
those with iron deficiency anemia (iron deficiency with decreased hemoglobin mass) experience performance improvements when hemoglobin levels return to normal following iron supplementation (Pedlar et al. 2018), whether the same holds true for those with iron deficiency without anemia (iron deficiency with normal hemoglobin mass) remains equivocal (Pedlar et al. 2018; Sim et al. 2019).

**Figure 2.2** Iron absorption in the duodenum. *HCP=*Heme–iron transporter protein, 

*Dcytb=*duodenal cytochrome B (reducing agent), 

*DMT1=*divalent metal transporter 1, 

*H=*hydrogen; from Shaw et al. (2022)

**Zinc.** Zinc is the second-most prevalent trace element in the human body after iron and plays an important role in various metabolic and immune processes (Hernández-Camacho et al. 2020; Saunders et al. 2013). Although zinc deficiency in developed countries is rare, PB individuals tend to have lower plasma zinc levels than omnivores due to its decreased bioavailability from
PB sources through the binding of phytate, which diminishes absorption (Melina et al. 2016). Despite this decreased bioavailability, PB individuals tend to have zinc levels within the normal range, possibly due to an adaptive homeostatic mechanism that enhances zinc absorption from PB sources (Saunders et al. 2013). Some food preparation techniques, such as soaking and sprouting beans, grains, nuts, and seeds and leavening bread, can decrease the binding of anti-nutritional factors to zinc, as well as iron, and increase bioavailability (Melina et al. 2016).

Athletes may want to pay particular attention to zinc bioavailability in their diet due to the role of zinc in energy metabolism, immunity, and muscular contraction, especially given the reduced levels of zinc typically observed in an active population compared to sedentary individuals (Chu et al. 2018; McClung, 2019). In particular, zinc plays an important role in regulating ion channels at the neuromuscular junction, leading to activation or inhibition of the corresponding ion currents, depending on zinc concentration and binding location (Hernández-Camacho et al. 2020). Athletes and sedentary individuals alike consuming a PB diet may benefit from consuming more zinc to counteract decreased bioavailability from PB sources and increased excretion of the mineral secondary to physical activity (McClung, 2019). Limited research has investigated the use of zinc as an ergogenic aid, but that which is available is equivocal; therefore, supplementation as a method to improve performance is not warranted (Heffernan et al. 2019). However, research has reported that individuals with insufficient dietary zinc intake have impaired exercise performance, aerobic power, and exercise metabolism (Lukaski 2005; Van Loan et al. 1999). Taken together, depletion of zinc in the body may negatively impact exercise performance but supplementing above the current recommended dietary allowance (RDA; 11 mg for men, 8 mg for women) is not indicated.
Calcium. Calcium is the most abundant mineral in the human body and is important for maximizing bone mineral accrual, blood clotting, nerve transmission, muscle contraction, and metabolism (Kreider et al. 2010). A common misconception is that individuals not consuming dairy products do not get adequate calcium, but a well-planned PB diet provides sufficient levels to meet the RDA (Tuso et al. 2013). In addition to a variety of PB sources of calcium (Table 2.1), many PB foods are fortified with calcium to assist in meeting recommendations (Melina et al. 2016). However, the bioavailability of calcium from PB sources is often decreased due to high oxalate levels and, to a lesser degree, phytic acid in various PB food options. While consuming slightly higher levels of calcium may offset the decreased absorption of calcium from PB sources, simply consuming a variety of PB sources of calcium has been suggested to ensure adequate absorption (Hever, 2016).

Vitamin D. Vitamin D is an imperative vitamin not only for bone health, immune function, and inflammatory modulation, but also is involved in muscular function through its actions as an anabolic hormone (Antoniak and Greig 2017; Larson-Meyer and Willis 2010). Despite its crucial role in human health and performance, 77% of Americans have insufficient levels of vitamin D, regardless of diet (Ginde et al. 2009). Athletes may be at risk for low levels of vitamin D if they participate in indoor sports, use excessive sun protection (i.e., sunscreen, clothing), if they live at high latitudes, or if they have more skin pigmentation (Larson-Meyer & Willis 2010; Melina et al. 2016). Those following a PB diet may be at greater risk for inadequate vitamin D levels due to limited PB sources of vitamin D (Table 2.1), particularly if they do not achieve adequate sun exposure and/or have darker skin (Rogerson 2017). Those who receive sufficient sun exposure with adequate (but not excessive) sun protection may achieve optimal vitamin D levels through endogenously produced vitamin D in the skin. However, if maintaining adequate vitamin D
levels is difficult and a synthetic supplement is not desired, PB vitamin D supplements derived from lichen, a composite fungal-algae organism, are commercially available (Rogerson 2017).

*B vitamins.* Although all of the B vitamins are crucial for optimal health and performance, only vitamins B<sub>2</sub> and B<sub>12</sub> will be discussed here due to their scarcity in a PB diet. Riboflavin (vitamin B<sub>2</sub>) is an important nutrient involved in energy metabolism and participates in redox reactions in various metabolic pathways with derivatives of the nutrient acting as electron carriers (Peechakara and Gupta 2021). Given its role in ATP generation, a riboflavin deficiency is likely to cause a disturbance at the intermediate steps of metabolism and, therefore, impact energy availability during aerobic exercise (Kreider et al. 2010). Riboflavin is found predominantly in organ meats and eggs, however, PB sources do provide amounts adequate in meeting the RDA (Table 2.1). Riboflavin is often found fortified in PB foods (e.g., cereal, bread), assisting those consuming such products in meeting recommendations. Exercise may increase the requirements for riboflavin intake, but the additional intake needed to cover increased needs is small and easily achievable through nutritious food choices (Manore 2000). Despite riboflavin’s key role in various metabolic processes, research is inconclusive on whether supplementation with riboflavin has the potential to improve performance and any current research available varies in quality (Gonçalves and Portari 2021).

Vitamin B<sub>12</sub> (cobalamin) is an essential nutrient necessary for normal nervous system function, homocysteine metabolism, and DNA synthesis and is important for red blood cell formation and transmission of neural signals, aspects that may be of particular interest for athletes (Krzywański et al. 2020). Cobalamin is found solely in animal products and, therefore, requires supplementation. Marginal amounts of cobalamin may be available through nutritional yeast and fortified cereals and PB “milk” (e.g., soy, almond, pea, oat), but such sources will not provide
sufficient intake (Rogerson 2017). In addition to detrimental health effects, cobalamin deficiency is associated with impaired methionine production, leading to lower creatine biosynthesis and potentially impaired physical performance (Mahmood 2014).

2.3.6 Ergogenic Aids

Use of ergogenic aids is highly prevalent in athletes of all levels (Maughan et al., 2018). However, despite the vast array of supplements available to the general public, only a handful have sufficient scientific evidence to suggest safety and marginal performance gains to date, including caffeine, creatine, nitrate, sodium bicarbonate, and beta-alanine (Maughan et al., 2018). Below I discuss supplements that may be of particular interest in a PB individual with goals of optimizing performance.

Creatine. Creatine is a component of phosphocreatine, which is an important fuel source during high-intensity, short-duration exercise. In an omnivorous diet, approximately 1 g per day of creatine is consumed through meat and fish, and an additional 1 g per day is synthesized endogenously through arginine, glycine, and methionine metabolism, which appears to be sufficient to maintain creatine stores (Kaviani et al. 2020a; Rogerson 2017). However, creatine is limited in a lacto-ovo vegetarian diet and devoid in a strict PB diet, leading to decreased creatine concentrations in serum, plasma, red blood cells, and muscle, but not in the brain of those following a PB diet. This may result in suboptimal fuel available for alactic anaerobic metabolism and subsequent decreases in performance (Solis et al. 2015; Wallimann et al. 2011). Therefore, supplementation of creatine might be considered to optimize short-duration, high-intensity exercise performance in those following a PB diet. PB individuals can increase their
phosphocreatine levels with creatine supplementation to a greater extent than omnivores due to a “super-compensation” effect, despite no differences in the muscle creatine transporter (Watt et al. 2004). Some studies indicate that vegetarians can enhance their physical performance (i.e., work output during a repeated-contraction isokinetic test) compared to omnivores following creatine supplementation (Burke et al. 2003), while others show no greater benefit for enhancement in exercise performance in vegetarians compared to omnivores when supplementing with creatine (Watt et al. 2004). Although a minimum of 1 g of supplemental creatine per day is necessary to match creatine intakes consumed in the average omnivorous diet (Kaviani et al. 2020a), supplementation with 5 g/day is associated with optimal tissue saturation (Maughan et al. 2018).

In addition to providing fuel for short duration, high-intensity exercise, creatine supplementation may also have beneficial effects on cognitive function and concussion recovery (Roschel et al. 2021). As such, athletes that participate in team skill-based sports may benefit from creatine supplementation. Some have suggested that vegetarians and vegans may experience greater enhancements in cognition following creatine supplementation compared to their omnivorous peers (Benton and Donohoe 2011; Rae et al. 2003), however, due to methodological concerns, more research is required. Athletes in activities at increased risk for concussions may also seek to maximize their creatine stores through supplementation as a precautionary strategy to improve their recovery should a concussion be sustained (Antonio et al. 2021).

Beta-alanine. Beta-alanine is the rate-limiting precursor to carnosine, an intracellular buffering agent (Derave et al. 2007; Peeling et al. 2018). Beta-alanine is found in meat and poultry, thus not consumed by those avoiding such products. Although beta-alanine can be created endogenously, individuals consuming PB diets have lower muscle carnosine concentrations than omnivores due to decreased intakes of beta-alanine (Creighton et al., 2022; Everaert et al., 2011).
Carnosine is an important molecule for exercise performance, as it is an intracellular buffer, acting as the immediate defense against proton accumulation in the contracting musculature during exercise, delaying fatigue (Maughan et al. 2018). Therefore, decreased levels may negatively impact performance (Rogerson 2017). Maughan et al. (2018) showed that supplementing with ~ 65 mg beta-alanine per kilogram over multiple doses per day for 10–12 weeks had a small but meaningful benefit in exercise lasting 30 s–10 min.

**Taurine.** Taurine is a conditionally indispensable amino acid concentrated in skeletal muscle and plays a role in osmoregulation and neurotransmission (Yatabe et al. 2009). Taurine is produced endogenously in the liver but can also be consumed through intakes of meat, seafood, and dairy products. PB individuals may consume negligible amounts, leading to potentially low or deficient levels (Fuhrman and Ferreri 2010; Rogerson 2017). Taurine is thought to play a role in exercise metabolism, as decreased taurine concentrations have been observed in rat muscle following exhaustive exercise (Yatabe et al. 2009). Taurine has a role in glucose tolerance, insulin sensitivity, and substrate uptake, storage, and oxidation in skeletal and cardiac muscle, all of which may impact exercise performance (Galloway et al. 2008). In humans, Rutherford et al. (2010) found that supplementation with 1.66 g of taurine one hour prior to a bout of cycling had no effect on time trial (TT) performance but did increase fat oxidation. da Silva et al. (2014) found that ingestion of 50 mg/kg/day of taurine for 14 days before a bout of eccentric exercise and seven days after resulted in improved performance and decreased markers of muscle damage and oxidative stress. While taurine is not included in the list of evidence-based supplements to enhance performance in the general population, such physiological effects have led some researchers to suggest that PB athletes should supplement with 500 mg of taurine twice daily to
maximize performance (Fuhrman and Ferreri 2010), although this type of supplementation protocol has not been reported in the literature.

2.4 Impact of Plant-Based Diets on Exercise Performance

Several hypotheses have been presented to suggest a PB diet may be either superior or inferior to a meat-containing diet for exercise performance (Figure 2.1). While some have suggested the high prevalence of carbohydrates and polyphenols may lead a PB diet to be superior for performance than one containing animal products, others have questioned the appropriateness of such eating patterns, with particular concern around protein quality and creatine, particularly when considering muscle protein synthesis. The following sections will examine the factors thought to enhance performance as well as the literature on performance outcomes in those following a PB versus omnivorous diet.

2.4.1 Antioxidants and Polyphenols

A PB diet may enhance endurance exercise performance through the high levels of polyphenols and antioxidants, compounds that may help reduce the amount of oxidative stress and inflammation accrued during exercise, enhancing recovery and overall health and wellness (Bowtell and Kelly 2019). Further, such compounds may play a role in augmenting vessel diameter and enhancing blood flow, thus enhancing the delivery of oxygen and nutrients to working muscles while removing metabolic by-products (Potì et al. 2019; Roelofs et al. 2017). A paucity of high-quality research makes drawing firm conclusions difficult (Higgins et al. 2020). A review by Merry and Ristow (2016) reported no evidence to support the use of supplemental antioxidants for exercise performance. While research supports the use of
supplemental antioxidants to improve performance in those with known deficiencies (Paschalis et al. 2016, 2018), research suggests that unnecessary supplementation may blunt the adaptations to exercise through reductions in mitochondrial biosynthesis, insulin sensitivity, and muscular hypertrophy in those consuming antioxidant supplements (Gomez-Cabrera et al., 2008; Higgins et al., 2020; Morrison et al., 2015; Paulsen et al., 2014). However, such research utilizes supplemental antioxidants, not whole food products alone. When considering dietary sources of antioxidants, antioxidant compounds that are found naturally in PB food products may assist in recovery from exercise and enhancing health and wellness (Bowtell & Kelly, 2019).

2.4.2 Body Composition

An abundance of evidence exists in support of a PB diet for leaner body composition and decreased body mass (Chen et al. 2019; Yadav et al. 2016; Sabaté and Wien 2010), characteristics that athletes may strive for, especially if they compete in weight class or esthetic sports or sports where weight has a direct impact on performance (i.e., cycling). Phillips et al. (2004) found that adherence to a PB diet decreased skinfold thickness and waist-to-height ratio, independent of weight loss. Further, consuming a post-exercise meal consisting of lentils has favorable effects on fat oxidation compared to a post-exercise meal consisting of potatoes and eggs; this was attributable to the low glycemic index of lentils and other PB sources of protein (Kaviani et al. 2016a). This enhanced fat oxidation, over time, may contribute to improved body composition. A PB diet may be beneficial for athletes interested in decreasing their body mass and/or adiposity through the consumption of nutrient-rich foods with low energy density, typical of a healthy PB diet. Such characteristics help with weight management through increased satiation at a lower caloric intake compared to foods with a lower volume-to-energy ratio (Boeing
et al. 2012). Additionally, a PB diet's high fibre content may help decrease energy intake by slowing gastric emptying and energy/nutrient absorption, increasing satiety, and influencing fat oxidation and storage (Slavin 2005).

When considering the accrual of lean tissue, the evidence is much more equivocal. While a meta-analysis by Messina et al. (2018) reported no differences between soy and animal protein supplementation for strength or lean mass gains in response to resistance training, others have reported superior muscle protein synthesis and lean tissue mass accretion with milk-based proteins compared to soy (Hartman et al. 2007; Wilkinson et al. 2007). Hevia-Larraín et al. (2021) concluded that both soy and animal protein are effective for gains in lean body mass and strength and that neither is superior. Though the evidence surrounding PB compared to animal-based proteins remains ambivalent, some have suggested increasing the amount of protein consumed at each meal as well as throughout the day when relying PB protein and mixing specific/complementary sources of PB protein to create a more balanced amino acid profile to maximize the anabolic response (Berrazaga et al. 2019; Pinckaers et al. 2021).

2.4.3 Impact on Exercise Performance

Numerous concerns around the adequacy of a PB diet for athletes relate to performance in strength or power-based sports, in particular protein quality and quantity, as well as creatine intake. However, limited evidence is available characterizing the impact of a PB diet on such types of exercise performance. If the intake of key nutrients required for the performance or recovery of strength and power exercise, such as creatine, carnosine, and protein, is inadequate, performance and recovery may be limited (Lynch et al. 2018). As discussed above, although these are real concerns, adequate consumption of all three of these may be obtained either
through dietary, including PB, or supplemental sources. The limited evidence available suggests that PB diets may produce similar gains in strength compared to omnivorous diets when matched for protein (Hevia-Larraín et al. 2021; Messina et al. 2018).

Many of the potential benefits of PB diets, such as improved blood flow, reduced oxidative stress and inflammation, and improved glycogen storage, may directly impact endurance exercise performance (Barnard et al. 2019). However, despite the wide variety of potential mechanisms that may support a PB diet as beneficial for exercise performance, current literature suggests that neither a PB nor an omnivorous diet is superior for aerobic exercise performance (Craddock et al. 2016; Nebl et al. 2019b; Venderley and Campbell 2006). While some research suggests PB athletes may have higher aerobic power (VO$_2$ max) scores compared to omnivores (Boutros et al. 2020), the same findings do not hold when nutrient intake, training status, body weight, and other confounders are controlled for (Nieman 1999; Venderly and Campbell 2006). Limited evidence is available characterizing the impact of a PB diet on anaerobic, strength, or power performance; however, if the above-noted considerations are taken into account and supplementation occurs as indicated, there is no reason to suggest that such exercise should be impaired by a PB diet.

2.5 Dietary Iron Intakes of Vulnerable Populations

While a PB diet can be nutritionally adequate in both general and athletic populations, micronutrient deficiencies continue to be a concern globally, particularly in certain vulnerable populations. Further, increased production and consumption of PB foods are likely to be necessary in the coming years to feed a growing population sustainably; a shift that requires advancement in the research and development of augmenting the nutritional profiles of PB foods to support optimal health. While much of the current research designed to combat micronutrient
deficiencies places a large emphasis on those in developing and impoverished countries (Akhtar et al., 2013; Ofori et al., 2022; Yunus et al., 2021), various populations in developed countries are also at risk, particularly of deficiencies in iron, which is a focus of the current thesis. Iron was chosen as the micronutrient of interest due to the prevalence of deficiency as well as the degree of disability that can arise if needs are not met. The following paragraphs will discuss what is known regarding dietary intakes and dietary adequacies in two vulnerable athletic populations: female athletes and athletes with physical impairments. Such information will justify the research conducted in this thesis.

2.5.1 Pathophysiology of Iron

Iron is one of the essential trace minerals in the human body and is a necessary part of various enzymes and oxygen transport proteins, as discussed above (section 2.3.5). Notably, iron is an important component of aerobic energy production in the mitochondria as well as in the oxygen-transporting molecules, hemoglobin and myoglobin (Dev & Babitt, 2017), as well as in processes such as DNA synthesis, the maintenance of myelin, and function of the immune system (McKay et al., 2019). However, despite the crucial role that iron plays in human physiology no excretory process exists if the iron level rise above that which the body requires. Iron is ideally suited for electron transfer reactions which can lead to the production of toxic oxygen species and ultimately damage bodily cells and tissues (Anderson & Frazer, 2017; Anderson & Wang, 2012). As such, iron in the body presents a paradox: An essential component of human physiology that becomes toxic when in excess. Therefore, a sophisticated array of processes is employed within the body to keep body iron within an optimal range of 4 to 5 g in adults (Powers & Buchanan, 2019).
Although relatively rare, excessive levels of iron in the body can lead to organ damage through the creation of reactive oxygen species (Kohgo et al., 2008). While consumption and absorption of excessive iron through dietary intakes are highly unlikely, iron toxicity due to supplementation may occur, especially if supplementation is delivered through intravenous or intramuscular injections (Oliveira, et al., 2014; Drozd et al., 2017). Contrarily, an imbalance between the absorption of iron from food and bodily losses of iron leading to a net loss of iron will eventually lead to iron deficiency (ID), the most prevalent nutrient deficiency in the world (Gedfie et al., 2022). In North America, ID with anemia (reduced hemoglobin mass) affects approximately 10% of women of childbearing age, while ID without anemia affects 16% of young women (World Health Organization, 2021). Although 2% of men are also affected by ID, women are at greater risk due to a tendency for inadequate dietary iron intake as well as iron losses through menstruation. Active women are further at risk for ID compared to their sedentary counterparts, with 30% of active women affected by ID without anemia (Dellavalle & Haas, 2012). Less developed parts of the world experience a greater prevalence of iron deficiency, with many reporting >40% prevalence of iron deficiency anemia in women of childbearing age (Figure 2.3; World Health Organization, 2021).
Figure 2.3 Prevalence of iron deficiency anemia in women of childbearing age (15-49 years) according to the World Health Organization (2021).

While clinical ID is defined as having serum ferritin levels <12 μg/L (DellaValle, 2013), a categorization based on severity has been proposed (Peeling et al., 2007; Table 2.2). Three main strategies are often employed to combat ID in developed countries: 1) education and dietary alterations or diversification, together or alone, 2) increase iron bioavailability in the body, or 3) supplementation and fortification (Zimmermann & Hurrell, 2007). Education and changes in dietary habits are often more sustainable; however, people often find such habits quite challenging and iron-rich foods (such as red meat) can be expensive, which may be a barrier for some. Iron supplementation can be very successful and affordable, provided the patient is compliant with their prescribed regime. However, such a treatment plan is often accompanied by
undesirable symptoms such as GI discomfort, bloating, nausea, and constipation, limiting compliance (Zimmermann & Hurrell., 2007). Dietary interventions involve increasing the amount of dietary iron consumed in the diet (Burke et al. 2012; Ishizaki et al. 2006) or strategies to improve iron bioavailability in the diet (Anschuetz et al., 2010). Although some research shows increased ferritin after a four-week dietary intervention of 15 mg/day (Ishizaki et al. 2006), others show no change after a dietary intervention (Burke et al. 2012; Alaunyte et al. 2014). Such inconsistencies could be due to differences in dietary behaviours outside of the experimental food, thus impacting the bioavailability of iron, as Anschuetz et al. (2010) demonstrated that meal composition might influence the amount of iron available for absorption and maintaining iron status over time.

Table 2.2 Categorization of iron deficiency severity

<table>
<thead>
<tr>
<th>Stage 1-Iron Depletion</th>
<th>Ferritin (μg/L)</th>
<th>Hemoglobin (g/L)</th>
<th>Transferrin Saturation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 2-Non-anemic Iron</td>
<td>&lt;35</td>
<td>&gt;115</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Stage 3- Iron Deficiency Anemia</td>
<td>&lt;20</td>
<td>&gt;115</td>
<td>&lt;16</td>
</tr>
</tbody>
</table>

Active individuals are at greater risk for ID relative to their sedentary peers due to increased iron losses through perspiration, frequent use of non-steroidal anti-inflammatory drugs causing gastrointestinal damage and bleeding, inflammation secondary to exercise, hormonal changes from training impairing iron absorption, increased body temperature during training affecting iron stores (ferritin), and iron loss in the GI tract due to the shunting of blood away from that
system during exercise (Bjarnason et al., 2018; Coates et al., 2017; DellaValle, 2013). Runners are also affected by a phenomenon referred to as foot-strike hemolysis where red blood cells are damaged as they flow through the blood vessels of the feet during running (Sim et al., 2014).

Endurance athletes may also require greater levels of ferritin relative to their non-athletic peers to support increased erythropoiesis, although no specific recommendations for an athletic population have been made (Nabhan et al., 2020). A schematic representation of risk factors in female athletes is displayed in Figure 2.4.

![Diagram of risk factors in female athletes](image)

**Fig 2.4** Iron Considerations for Endurance-Trained Females. NSAID = non-steroid anti-inflammatory drugs

Although athletes can often restore their ferritin levels with pharmaceutical iron interventions, such supplements often lead to undesirable side effects such as gastric irritation, nausea, epigastric discomfort, and constipation, which may decrease compliance and long-term efficacy (Moretti et al., 2015). Moreover, unnecessary and/or unmonitored usage can result in iron overload, increasing oxygen radicals and cell deterioration (Chen et al., 2017). As depicted in Figure 2.4, two of the three variables that put females at higher risk for ID compared to their male
peers are related to dietary intake, namely higher prevalence of both low energy availability and dietary restrictions.

With the onset of menarche, females require a greater intake of dietary iron in order to counteract iron loss through menstruation (Institute of Medicine, 2001). The recommended intakes for males and females are once again equated with the onset of menopause, representing the end of menstruation (Table 2.3). Worth noting is the development of the RDA for iron differs from many other nutrients due to the requirement of iron for women being skewed, as blood loss between women varies greatly (Barr, 2006). Specifically, the recommended dietary allowance for most nutrients (those with requirements that are normally distributed) is calculated as two standard deviations above the estimated average requirement, while iron recommendations are derived using factorial modeling of all components of iron requirement namely basal losses, menstrual losses, and accretion (Institute of Medicine, 2001). Zimmermann and Hurell (2007) have reported males should absorb 0.8 mg/day of iron to maintain homeostasis while females should absorb 1.4 g/day. These differences in absorption requirements between males and females are reflected in the RDA for iron.

Table 2.3 Recommended Daily Intake of Iron by Sex Across the Lifespan

<table>
<thead>
<tr>
<th>Age</th>
<th>Males (mg/day)</th>
<th>Females (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 months</td>
<td>0.27*</td>
<td>0.27*</td>
</tr>
<tr>
<td>7-12 months</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>1-3 years</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>4-8 years</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age Group</td>
<td>Adequate Intake</td>
<td>Recommended Dietary Allowance</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>9-13 years</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>14-18 years</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>19-50 years</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>&gt;50 years</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

From Institute of Medicine (2001); *Adequate intake based on normally developing infants consuming primarily breast milk. All other values recommended dietary allowance.

2.5.2. Dietary Intake of Iron in Female Athletes

Early research on female athletes suggests that female collegiate athletes fail to consume adequate iron through their regular diet to offset losses (Hinton, 2014). However, most reports of dietary intake in this population are from the early 2000s with minimal updated reports to inform whether such a trend still exists two decades later. What we know from the limited literature from the last ten years in this regard is that the trend for low dietary intake in female athletes is likely unchanged. Kokubo et al. (2016) reported intakes of ~6 mg per day through diet only in female gymnasts, Beerman et al. (2020) reported greater intakes but still suboptimal in collegiate distance runners (15 mg/day), and Alaunyte et al. (2014) reported intakes of ~11 mg in female runners. Of note is athletes studied by Beerman and colleagues corrected the dietary insufficiency through supplementation, bringing total intakes up to approximately 44 mg/day.

Though the literature suggests a largely insufficient intake of iron in female athletes, the amount of iron consumed is only one aspect of dietary intake concerning bodily iron status. Another factor that may impact iron status in female athletes is the overall amount of energy consumed. Relative energy deficiency in sport (RED-S) describes a syndrome with the etiology attributed to inadequate energy intake relative to energy expenditure. Specifically, Mountjoy et
al. (2014) describe relative RED-S as “an inadequacy of energy to support the range of body functions involved in optimal health and performance”. This inadequacy leads to disruption in a variety of bodily systems including decreased immunity, poor bone health, disrupted endocrine function, stunted growth and development, altered metabolic function, and more (Mountjoy et al., 2014). The overall prevalence of RED-S is largely unknown due to the relatively recent definition of the syndrome (Dipla et al., 2021). However, one cohort study has suggested a prevalence of 80% of participants presenting with at least one symptom consistent with RED-S in elite and pre-elite female athletes (Rogers et al., 2021), while a similar study conducted in female recreational exercisers reported 45% of participants being at risk of low energy availability (Slater et al., 2016). Interestingly, Slater and colleagues (2016) observed the odds of being at risk of low energy availability increased by 1.13x for every hour of exercise performed during the week. Based on this literature athletes across the spectrum of performance levels (recreational to elite) are at risk for RED-S.

RED-S is a critical consideration when attempting to improve iron status in athletes, as it has been suggested that low iron may contribute to low energy availability or its clinical indicators and also that low energy availability may contribute to low iron in athletes (McKay et al., 2020). Research in military personnel (Pasiakos et al., 2016) and endurance athletes (Ishibashi et al., 2020) has observed that increased levels of hepcidin, an iron absorption regulator, independent of inflammatory markers were associated with reductions in energy intake, suggesting a reduction in duodenal iron absorption with intakes below energy balance. On the other side of this bidirectional relationship, iron deficiency appears to play a role in energy deficiency. One hypothesis behind this relationship stems from basic metabolism. With oxidative metabolism in the electron transport chain involving heme and non-heme iron-containing cytochromes, a deficiency of iron leads to less efficient production of ATP, causing the body to shift from
aerobic to anaerobic metabolism at a lower intensity, increasing the energy demand for a given workload (Beard & Tobin, 2000; McKay et al., 2020). Given this intricate link between iron status and energy intake, all supplements and comparators used in the randomised controlled trials that are included in this thesis are isoenergetic.

2.5.3. Dietary Intake of Athletes with Physical Impairments

While enough literature is available to investigate specific nutrient deficiencies and insufficiencies in female athletes, the same cannot be said about athletes with physical impairments (i.e., para athletes). Seminal research in the dietary intakes of para athletes came from Canada in 1996, assessing the dietary intake of elite marathoners with varying levels of spinal cord injury (SCI; Potvin et al., 1996). The authors had athletes complete a three-day food record, collected on three consecutive days including one weekend day. Analysis displayed generally an adequate intake of most nutrients except for zinc and vitamin E, for which average intakes fell below the recommended nutrient intakes (11.3 mg/day compared to recommended intakes of 12 mg/day for zinc; 6.4 mg/day compared to recommended intakes of 9 mg/day for vitamin E). However, the authors highlight that, despite average values falling within the normal range, three out of the ten participants studied had intakes below 2/3 of the recommended intake for vitamins A, D, and B6.

Following the publication of this fundamental research, a gap of approximately 15 years occurs before further research assessed the intakes in a para athlete population again. Though one can only hypothesize why such a gap persisted despite the increasing numbers of individuals with disabilities participating in elite sport (Gold & Gold, 2007), many barriers exist that might lead researchers to shy away from studying this unique athlete population. One such obstacle is the
broad scope of physical impairments that are classifiable under the Paralympic umbrella (Shaw et al., 2021). Such heterogeneity within this group of athletes makes recruitment and strong internal validity within a study difficult, as pathophysiology and requirements vary greatly from one impairment to the next. Add the heterogeneity of athletes to the heterogeneity of requirements within different sports, the conducting a study with tight internal validity becomes extremely challenging. This is portrayed in the literature with very limited research reporting the dietary intake of this unique group of elite athletes. Madden et al. (2017) reported nutrient intake gathered using three-day food intakes of 42 athletes across nine sports. These sports included those that are limited to only tetraplegics (wheelchair rugby), athletes with visual impairment but no physical impairment (goalball, blind soccer), and sports involving a variety of impairment types (wheelchair basketball, sit skiing, para bobsleigh, sitting volleyball, wheelchair curling, para cycling). This research reported micronutrient intakes meeting or exceeding the RDA for most micronutrients, though suboptimal intakes were reported for vitamin D, vitamin E, and magnesium for both sexes. Sex-specific differences were also observed, with males consuming less than the RDA for folate and vitamin A, while females consumed less than the RDA for iron and calcium. Eskici and Ersoy (2016), on the other hand, had a much more homogeneous population, reporting on 22 wheelchair basketball players with limb deficiency exclusively. The authors collected a 24-hour food recall and found insufficient intakes of vitamin B₁, folic acid, magnesium, iron, and fibre. The authors also reported a low percentage of total energy from carbohydrates (42.7%) and a high percentage coming from fats (44%).

Similar to the population studied by Madden et al. (2017), research by Sasaki et al. (2021) investigated athletes with any classifiable impairment participating in any sport. The final sample included athletes from 13 different Paralympic disciplines and while the impairments studied were not specifically identified, one can assume a vast range of impairments based on the sports
represented (archery, athletics, badminton, wheelchair basketball, boccia, equestrian, football for cerebral palsy, powerlifting, sailing, swimming, wheelchair rugby, wheelchair tennis, sitting volleyball). This study is unique to others in the field because of its large sample size (n=101) and multiple 3-day food records in both home and training camp settings, strengthening the research. Inadequate vitamin D intake in almost 100% of participants, and average intakes in the group were below estimated average requirements for all bone-related micronutrients (i.e., vitamin D, calcium, magnesium). This is particularly noteworthy, as many para athletes are likely at elevated risk for poor bone health due to limited weight-bearing activities or drug-nutrient interactions due to medications consumed to assist in managing their impairment (Scarmella et al., 2018). A relatively high prevalence of insufficient intakes of vitamin A (>50% inadequate) and vitamin C (>33% inadequate) was observed in this group, while females specifically reported insufficient iron intakes (29.5% inadequate).

Goosey-Tolfrey & Crosland (2010) investigated the intakes of wheelchair basketball and tennis players, including those with chronic arthritis, spinal cord injury, brittle bones, dystrophic dysplasia, multiple sclerosis, and lower limb nerve damage. Data were collected using a 7-day food log. Authors reported intakes of most micronutrients to be in excess of the reference nutrient intake (the UK equivalent to the RDA in North America), though mean intakes of iron in the female participants was only 89% of the recommended nutrient intake.

Krempien and Barr (2011) assessed the risk of nutrient inadequacy in 32 athletes with a spinal cord injury from wheelchair basketball, wheelchair rugby, and athletics. The authors reported that all participants had fibre intakes below the adequate intake of 14 g fibre/1000 kcal, with men consuming 9.4 g/1000 kcal and women consuming 9.5 g/1000 kcal. Almost all athletes consumed inadequate amounts of vitamin D, with a high prevalence of inadequacy for (males 50% inadequate; females 25% inadequate), calcium (males 54% inadequate; females 38% inadequate),
magnesium (males 42% inadequate; females 12% inadequate), and zinc (males 38% inadequate; females 25% inadequate), and zinc (males=38% inadequate; females=25% inadequate). Contrary to many of the reports summarized above, Krempien and Barr (2011) did not observe especially high rates of iron insufficiencies, with ~4% of males and 16% of females being insufficient. That said, average intakes for females were below the RDA of 18mg for females at 15.2 g/day. Gordon et al. (2022) studied a similar population of para cyclists with spinal cord injuries and observed low carbohydrate and high fat intake compared to suggested intakes for para athletes by Flueck (2021), Flueck and Parnell (2021), Islamoglu et al. (2019), and Scaramella et al. (2018). Notably, these suggested intakes are opinions of the authors noted above, not based on clinical trials. Males were reported to consume inadequate folic acid, vitamin D, calcium, and potassium, while females did not consume adequate amounts of folic acid, specific B vitamins (B5, B2, B1, B6), calcium, iron, magnesium, potassium, selenium, and zinc compared to dietary reference intakes.

While intakes in the above studies were compared to the government-recommended intakes, such recommendations have not been validated in a population of people with physical impairments. The dietary needs of these individuals cannot be inferred directly from research conducted on an able-bodied population, as different impairments may present with differences in body physiology and composition, impacting their needs (Scarmella et al., 2018). Para athletes have been reported to have decreased energy intakes, inadequate micronutrient consumption, and are at greater risk for micronutrient deficiencies, including iron (Eskici and Ersoy, 2016; Madden et al., 2017; Scaramella et al., 2018). Further, many conditions that would be classifiable for a para sport often present with chronic, low-grade inflammation that may increase hepcidin levels, decreasing iron absorption (Allison & Ditor, 2015; Mayer et al., 2013). Para athletes, especially those with SCI, may also be affected by neurogenic bowel dysfunction. This may lead to fecal incontinence, difficulty evacuating, abdominal distention, and constipation (Ayas et al., 2006).
To control such symptoms, individuals often restrict foods that may lead to worsened symptoms, such as red meat and fibre, impacting the consumption of some micronutrients, including iron.

The heterogeneity of research in this area informed our third study (Chapter 6), *Dietary Intakes in Elite Para cyclists*. The goal of this research was to assess dietary intake in a group of elite para cyclists and assess the risk for inadequacy, specifically for iron (i.e., focus nutrient in this thesis). Para cycling is a unique population, given the wide range of classifiable impairments included, which are then subcategorized into different classes involving different varieties and severities of impairment (Flueck, 2021; Shaw et al., 2021). While different varieties of impairment may be present within the same category, levels of function are similar and, therefore, likely that broad nutritional requirements (energy, macronutrient) are similar.

The paucity of literature in this area as well as differences in study design, analysis, and population make drawing conclusions on the general intakes of para athletes incredibly challenging. Such information is important for athlete support personnel to better guide athletes in optimal fueling to maximize their health and achieve their best performances. Unclear from Madden et al. (2017) or Goosey-Tolfrey and Crosland (2010) was if dietary supplements were taken into account when assessing micronutrient intake. However, Sasaki and colleagues (2021) along with Krempien and Barr (2011) specified that dietary supplements were included in the dietary assessment. That said, Krempien and Barr (2011) reported that the use of dietary supplements did not impact the prevalence of inadequacy. When discussing diet, it is very important to consider whether or not dietary supplements are used, as the use of dietary supplements has been reported in over half the adult population (Bailey et al., 2010). Research supports high intakes of supplements in para athletes as well, with over 80% of athletes reporting using a supplement at least one time in the past three months (Madden et al., 2017; 2018). Currently no scientific evidence is available to provide best practice protocols for sports.
supplements in particular in para athletes. Research is emerging, but much of the literature has been completed in a population with spinal cord injury, one of many classifiable impairments. Such scarcity in this topic led us to conduct a review of the literature assessing the efficacy of supplements to enhance sport performance in a para athlete population (Shaw et al., 2021). We concluded that evidence for supplement use in a population of para athletes remains inconclusive, largely due to the lack of literature for any given supplement and differences in the varieties of impairments studied. However, some notable differences between requirements for para athletes relative to the able-bodied recommendations were observed, particularly in supplementation protocols for caffeine and creatine. Clear though is that supplements in able-bodied athletes cannot be blindly or directly applied to athletes with impairments due to differences in physiology, body composition, and/or drug-nutrient interactions (Scaramella et al., 2018; Shaw et al., 2021).

2.6 Biofortification of Plant Foods

Dietary supplementation may be an efficacious approach to improve nutrient status in individuals presenting with one or more nutrient deficiencies. However, the American Dietetic Association cautions against the use of dietary supplements, due to the risk of intakes exceeding the tolerable upper intake of some nutrients (Marra & Boyar, 2009). Further, supplementing single nutrients may have unintended consequences in some parts of the world, particularly when considering iron (Schümann & Solomons, 2013). In the sport community, a food-first approach is also supported, though experts still acknowledge the role of specific evidence-based sport supplements for optimizing exercise performance (Close et al., 2022). Pulses, the edible seed from a legume plant, are an interesting vehicle to consider for improving nutritional status in
various populations due to their rich nutrient profile, low cost, and ability to fix nitrogen in the soil, a property which assists in maintaining agricultural sustainability (Rasskazova & Kirse-Ozolina, 2020). Field peas (*pisum sativum*) were chosen as the pulse of interest in the current thesis due to their rich micronutrient profile, slowly digestible carbohydrates, and high-quality proteins (Jha & Warkentin, 2020). Field peas are rich in the amino acids leucine and lysine compared to cereals (Han et al., 2020). Pulses, therefore, can help to optimize the amino acid composition of a PB diet when consumed in conjunction with cereal grains, which are lacking in leucine and lysine but rich in the sulphur-containing amino acids (methionine and cysteine) (Han et al., 2019). Further, research suggests that pulses such as field peas may promote a healthy and diverse gut microbiome due to the high amounts of resistant starch (Kadyan et al., 2023). A healthy gut microbiome has the potential to improve both health and performance (Clauss et al., 2021). In addition to the health benefits noted above, field peas are a cost-effective protein source and therefore are staples in traditional diets worldwide, making them accessible to individuals globally, particularly those from low-income countries who may benefit most from improved nutrient delivery through the diet. The following sections of this chapter will discuss specific agricultural practices utilized to maximize nutrient bioavailability, specifically in PB protein sources.

Increasing one’s intake of PB food products can be beneficial for health when implemented appropriately (Kahleova et al., 2017; Medawar et al., 2019; Satija & Hu, 2018). However, many PB foods contain “antinutrients”, some of which were described above (section 2.2). These compounds should not be discounted when considering the health implications of such foods. Compounds such as tannins, fibre, and phytates have innate health-promoting effects though they also chelate different nutrients and limit their absorption, increasing the risk of nutrient deficiency in those with high intakes such as those following a PB diet (Shubham et al., 2020,
Zhang et al. 2020). Yet, a significant reduction in many antinutrients can occur through traditional processing methods such as dehulling, soaking, roasting, or boiling (Samtiya et al., 2020). While such culinary practices decrease some of the negative effects of antinutrients (López-Moreno et al., 2022), food technology has also advanced rapidly in recent years to develop food biofortification techniques to further maximize the bioavailability of nutrients present in various PB foods. Such practices are important to avoid micronutrient deficiencies, a condition referred to as “hidden hunger” that affects one in three individuals globally (Han et al., 2022). Those most vulnerable to micronutrient deficiencies tend to be pregnant women and children, particularly those of lower socioeconomic status or in developing countries (Akhtar et al., 2013; Ofori et al., 2022). Common micronutrient deficiencies include iron, vitamin A, iodine, and folate, and can lead to moderate or severe conditions such as microcytic anemia, preventable blindness and severe infection, and preventable brain damage and neural tube defects, respectively (Czeizel et al., 2013; Han et al., 2022).

Pulses and legumes may be a sustainable solution not only to micronutrient deficiencies but also to the dilemma of feeding a quickly increasing global population sustainably (Jha & Warkentin, 2020; Meyer et al., 2020). Such food products are grown globally and are cost-efficient, making these attractive to all groups of people (Jha & Warkentin, 2020). Strategies such as increased food production, supplementation, food fortification, and biofortification have been employed to make pulses and legumes as efficacious as possible in combating micronutrient deficiency worldwide, (Akhtar et al., 2013). Of these, biofortification may be of particular interest from an economic standpoint, as a one-time investment in plant breeding can yield nutrient-rich crops for years to come. This may be preferable compared to the continuous financial input that is required for supplementation and/or agronomic fortification techniques such as fertilization, either through the foliar application or to the soil directly (Bouis et al., 2011;
Saltzman et al., 2013). Biofortification is a primary focus of the current thesis as a strategy to combat nutrient deficiencies in vulnerable populations.

Biofortification refers to improving the concentration of nutrients in a crop through selective breeding practices, transgenic techniques, or agronomic practices (Bouis & Saltzman, 2017; Jha & Warkentin, 2020; Saltzman et al., 2013). These practices represent a paradigm shift in the agricultural system, from goals revolving around increased crop yields and productivity to a focus on the role of the agricultural system in the improvement of human health (Guindon et al., 2021). Field peas and other pulses are attractive targets for biofortification because they are inexpensive and provide a rich source of dietary proteins, complex carbohydrates, vitamins, and minerals while also being staples in traditional diets around the world (Jha & Warkentin, 2020).

2.6.1 Chemical Mutagenesis

Chemical mutagenesis is a selective breeding process by which plant seeds are exposed to a chemical mutagen in order to produce genetic mutation in plants. These mutated alleles increase genetic diversity within the seeds (Oladosu et al., 2015), allowing the breeder to select desirable traits to introduce into a breeding program. This differs from what would be considered “genetic modification”, in which a foreign gene is introduced into the plant to achieve a desired trait. Treated seeds are then planted, harvested, and the seeds assessed for the desired trait. At this point, those seeds from the second generation are considered “putative mutants” or “false mutants”, requiring confirmation of the desired trait in further planting and harvesting cycles (Figure 2.5; Shu et al., 2012). The Crop Development Centre at the University of Saskatchewan has used this process to develop field pea lines that are low in phytic acid (lpa) (Warkentin et al., 2012). Specifically, the authors exposed 1 kg of seed to sodium azide (1 mM) for 4 h, and a
second 1 kg lot to 1.5% (v/v) ethyl methane sulfonate (EMS) for 4 h (M1 seeds). Treated seeds were planted and subsequently harvested, producing M2 seeds. These M2 seeds were assessed for the \textit{lpa} trait. The phytic acid levels of these putative mutants were confirmed prior to being planted and harvested, resulting in the M3 generation which was again planted to produce the M4 generation. Two lines of this M4 generation were identified as having the greatest inorganic phosphate (and therefore the lowest phytic acid) concentration and were used in three of the studies included in this research. These \textit{lpa} lines have shown a decrease in phytic acid by 60% and increased bioavailability of iron by 1.9 times compared to the parent plant (non-modified) when assessed using a CaCo-2 cell culture assay. The plants also show good agronomic performance, with a produced yield of 86% and 92% compared to the parent crops (Bangar et al., 2017; Lindsay et al., 2021; Warkentin et al., 2012).

\textbf{Figure 2.5} Generalized protocol for plant mutation breeding
While such biofortified peas show promise in improving hemoglobin status when consumed by chickens (Warkentin et al., 2020), high levels of fibre and protein and a low glycemic index lead such foods to be very satiating (Li et al., 2014). While this may be beneficial in a sedentary population, it can be a source of concern in active individuals if they are not meeting their energy goals (Lis et al., 2019). Therefore, the goal of the first study of this thesis was to create a product using these lpa peas that could feasibly be used in an athletic population without significantly interrupting their habitual dietary intake or causing gastrointestinal distress (chapter 3). By creating protein concentrate from the peas, the product becomes more attractive to an active population by assisting in meeting their heightened protein needs without the bulk and satiety that would occur if the same amount of protein was consumed in the form of whole peas. Preliminary unpublished data from the Crop Development Centre at the University of Saskatchewan also suggest that iron concentration is preserved with the removal of the pea hulls and starch, allowing protein concentrates to contain a higher percentage of iron than the whole seed. A comparison of nutritional data of a fine fraction pea concentrate compared to the same amount of whole or dehulled peas is displayed in Table 2.4.

**Table 2.4 Nutritional Information of 100g of each whole cooked pea and high protein pea concentrate**

<table>
<thead>
<tr>
<th></th>
<th>Whole Peas</th>
<th>High Protein Pea Concentrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (kcal)</strong></td>
<td>118</td>
<td>397</td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
<td>21</td>
<td>39</td>
</tr>
<tr>
<td><strong>Fibre (g)</strong></td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>
### Protein (g)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Value1</th>
<th>Value2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>0.60</td>
<td>3.53</td>
</tr>
<tr>
<td>Valine</td>
<td>0.39</td>
<td>2.33</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.34</td>
<td>2.04</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.59</td>
<td>3.46</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.09</td>
<td>0.52</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.38</td>
<td>2.27</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.30</td>
<td>1.75</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.09</td>
<td>0.55</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.60</td>
<td>3.55</td>
</tr>
</tbody>
</table>

### Fat (g)

<table>
<thead>
<tr>
<th>Fat (g)</th>
<th>Value1</th>
<th>Value2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

### Iron (mg)

<table>
<thead>
<tr>
<th>Iron (mg)</th>
<th>Value1</th>
<th>Value2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3</td>
<td>5.4</td>
<td></td>
</tr>
</tbody>
</table>

#### 2.7 Objectives and Hypotheses

Study 1 - The purpose of study 1 was to produce a pea product to deliver high amounts of bioavailable iron and conduct a small pilot trial to assess the acceptability of such a product and the feasibility of data collection measures in a group of female runners. We hypothesized that some gastrointestinal discomfort will be present at the beginning of consumption but that these discomforts will diminish over time. We also hypothesized that the powder will be a tolerable medium to provide a significant amount of iron and that the methodology chosen will be feasible to assess our variables of interest (i.e., assessments of ferritin, assessment of lactate while running).
Study 2- Based on the results of study 1, a second study was completed to address the question of a lpa pea product in female runners. After the product was deemed intolerable by the participants, further processing and testing was scheduled to ensue. After tolerability was confirmed in study 1, the purpose of study 2 was to scale up the methodology from study 1 into a more well-powered randomized control trial to assess the efficacy of a high protein powder made from lpa peas on the iron status, body composition, and exercise performance in female runners of reproductive age. We hypothesized that those consuming the lpa pea powder would experience a greater improvement in their ferritin compared to those consuming regular peas or a non-pea control which may improve their exercise performance.

Study 3- The purpose of study 3 was to assess the dietary intake of another group of vulnerable athletes, para cyclists, to assess whether the product developed in study 1 may be beneficial in improving dietary intake. We hypothesized that this population will be consuming suboptimal amounts of some micronutrients, including iron, which could be improved through supplementation with the product developed in study 1.

Study 4- The purpose of study 4 was to assess the efficacy of a high protein pea concentrate with high iron bioavailability on the iron status in active individuals with a spinal cord injury. We hypothesized that consumption of such a supplement will improve iron status nutrient intake, potentially improving exercise performance.

2.8 References


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Chapter 3- Study 1- The Feasibility of a Pea Protein with High Iron Bioavailability for Female Runners
3.1 Abstract

Objective: Iron deficiency is a global dilemma, with a high prevalence among females from both low- and high-income countries. Athletic females, especially those who engage in running, are at a particularly high risk of developing iron deficiency due to blood losses through menstruation coupled with decreased iron absorption secondary to exercise. Field peas, along with other plant-based protein sources, are rich in iron and inexpensive, making them an attractive option for those wishing to increase their iron intake without consuming more animal products. However, field peas are high in phytic acid, an inherent compound that binds to cations such as iron, forming a salt (phytate), and limiting absorption in the small intestine. The goal of the current research was to conduct a feasibility study to assess the acceptability of a high protein powder made from low phytic acid field peas in female runners.

Methods: Eight recreational female runners (age: 32.7 ±13.5 years; VO₂max: 46.9±5.4 mL/kg/min) were assessed for iron status, body composition, and exercise performance before and after an 8-week intervention period, during which they supplemented with either a powder derived from regular peas, a powder derived from peas with high iron bioavailability (low phytic acid), or maltodextrin.

Results: A high degree of compliance was observed, with all groups reporting >90% compliance (maltodextrin= 99.2 ±0.9%; regular pea= 96.3 ±2.1%; low phytic acid pea= 97.8 ±2.3).

Supplementing with a high-protein pea powder derived from low phytic acid peas is feasible and is deserving of future research.

Conclusion: A larger trial is warranted to investigate the role these peas might have in combating iron deficiency in athletes.
3.2 Introduction

Iron is an essential mineral that can be consumed through plant or animal sources and is involved in oxygen transport, energy production, and cell proliferation (Shoemaker et al., 2019). Iron deficiency is the most prevalent micronutrient deficiency worldwide (Low et al., 2016; Saka et al., 2019), impacting approximately 16% of premenopausal women and 2% of adult men (Coates et al., 2017). Iron deficiency is also the principle cause of anemia (Mirza et al., 2018), a condition the World Health Organization has set a target of reducing by 50% in women of childbearing age by 2025 (World Health Organization, 2017). Athletic individuals tend to be disproportionately affected by iron deficiency: 15%-35% of female athletes and 3-11% of male athletes, with some reports describing a prevalence of >50% in females and up to 30% in males (Sim et al., 2019). Females are prone to iron deficiencies relative to males due to blood loss through menstruation (Pasricha et al., 2014). Athletes, especially those involved in running, tend to be at greater risk than their sedentary peers due to foot strike hemolysis, ischemia to the viscera, frequent use of anti-inflammatory drugs, or increased losses through urine or sweat (Coates et al., 2017).

While pharmaceutical interventions (i.e., iron supplements) appear to be beneficial for improving serum ferritin levels (Low et al., 2016), the use of pharmaceuticals often cause gastrointestinal disturbances (Sim et al., 2019). Further, such approaches may not be accessible to those at the greatest risk, including the most vulnerable, poorest, and least educated groups (Tembhare et al., 2015). As such, dietary approaches may be a preferable strategy to address and prevent iron deficiency globally. Many dietary approaches include increasing the consumption of red meat due to the increased bioavailability of heme iron compared to non-heme iron (Lim et
al., 2013) but such practices may not be practical for many individuals due to ethical, cultural, or economic factors (Dobersek et al., 2021; Schenk et al., 2018).

Plant-based sources of iron may be a feasible strategy to improve dietary iron intake and enhance iron status. Iron is found abundantly in a variety of plant-based foods. Vegetarians often consume more iron than omnivores (Śliwińska et al., 2018). In particular, field peas are a rich source of macro- and micro-nutrients, including iron (Bangar et al., 2017). A 100 g sample of raw split peas contains 4.82 mg of iron: 60% and 27% of the recommended dietary allowance for men and women, respectively (Deeks et al., 2017). However, peas (and other crop seeds) can also contain high levels of phytic acid, an anti-nutritional compound that binds to cations such as iron and zinc, forming a non-soluble salt (phytate) that is excreted, decreasing the amount of mineral available for absorption by the gut. While food fortification processes appear to be beneficial for improving the iron concentration in the bloodstream (Shubham et al., 2020), to our knowledge no research currently exists investigating field peas bred to achieve optimal absorption of the nutrients naturally available.

The Crop Development Centre at the University of Saskatchewan developed two lines of lpa peas through chemical mutagenesis (Warkentin et al., 2012). These lpa lines show reductions of phytic acid by ~60% while performing similarly to the parent line in terms of agronomic performance (Lindsay et al., 2021). Although having similar iron levels as control peas, these mutant lines have been reported to have 1.4- and 1.9-times higher iron bioavailability compared to the parent lines (Liu et al., 2015). Pea lines (or breeding lines) derived from crosses between cultivars and these lpa lines, then, have the potential to be used in a plant-based dietary approach to combat iron deficiency in afflicted individuals. However, given the large quantity of peas that would need to be consumed to produce a significant effect, concerns over tolerability and
gastrointestinal side effects such as bloating and flatulence exist (Dahl et al., 2014). Gastrointestinal disturbances tend to occur in a large percentage of runners and include complaints such as flatulence, bloating, acid reflux, abdominal pain, and others (Parnell et al., 2020). Further, Parnell and colleagues (2020) found that 23% of runners avoided high fibre foods in order to minimize their symptoms. As a result, we were unsure how a pea powder containing high amounts of fibre compared to other supplements would be accepted by the running community.

Given that peas have independently been reported to produce symptoms similar to those experienced by runners (Dahl et al., 2014; Parnell et al., 2020), we believed it prudent to be cautious and to conduct this feasibility study prior to conducting an adequately powered trial. The primary objective of the current feasibility study was to assess the acceptability of a high-protein powder made of peas in female runners and assess any differences in tolerability between regular peas and those with lpa. Our secondary objective was to assess the protocol for an intervention to determine the advantage of differing pea supplements for enhancing iron status and exercise performance. We hypothesized that the pea powders would be well-tolerated, no difference would be apparent in the tolerability of powders made from regular peas or from peas with lpa, and the study protocol would be appropriate for future investigations.

3.3 Methods

3.3.1 Supplement Development

Development of a field pea line with low levels of phytic acid began with an assessment of six different pea varieties grown in Saskatchewan, Canada. Of these, the CDC Bronco cultivar was chosen to be used in the development of a lpa pea due to favorable agronomic and
nutritional qualities. Seeds underwent chemical mutagenesis and tested for the lpa trait. For further information on the development of the lpa pea, see Warkentin et al. (2012). Viable lpa lines were then assessed for iron absorption using an in vitro digestion Caco-2 cell culture bioassay. This assessment revealed that, while containing similar amounts of total iron, the seeds with lpa had 1.4 to 1.9x increased iron bioavailability (Liu et al., 2015).

In vivo application of these peas for measures of iron began with a broiler chicken (Gallus gallus) model for dietary iron absorption. The birds consuming feed with lpa peas had moderately improved iron status as well as gut microbiota and duodenal brush border membrane functionality (Warkentin et al., 2020). With such promising results, we aimed to investigate the impact these peas might have in a human population likely to be afflicted with iron deficiency, i.e., female runners.

The pea powder development was completed in conjunction with a local food product development center (Saskatchewan Food Industry Development Centre Inc., Saskatoon, Canada). Powders were designed to be low in compounds known to reduce iron absorption through chelating the mineral in the small intestine (polyphenols, tannins; Milman et al., 2020) while maximizing the amount of iron delivered to recipients. All products were tested for acceptability by a small panel of individuals.

Due to poor acceptability of sampled products and the bulk of food that would need to be consumed in order to reach the intake goals of dietary iron, it was decided to deliver the peas in the form of a high-protein concentrate (Pelgrom et al., 2013). Through dehulling, grinding, and air classifying the peas, we were able to remove much of the bulk while maintaining similar iron concentrations as is found in the whole pea. The resultant powder consisted of 49% protein, 7% ash, 4.7% lipids, 39.3% carbohydrate, and 59 µg/g iron.
To create a shelf-stable product for human consumption, the protein concentrate was cooked to kill microbes, denature the protein, and gelatinize the starch (Appendix B). Water and powder were combined at a ratio of 4:1 and heated to 90°C, where it was held for 30 minutes while stirring continuously. After the cooking process, the product was transferred to sheet pans and dried in a tray oven at 70°C for 16-20 hours, until moisture was below 14%. The product was then milled using a hammer mill in three steps, with progressively smaller mesh to produce the desired consistency. After processing, the pea powders were stored in plastic bags in a cool room out of direct sunlight. The non-pea control, maltodextrin powder, was purchased commercially (Univar Solutions, Illinois, United States).

Participants consumed ~75 g per day split over three doses. The regular pea powder was found to have slightly higher iron concentration compared to the lpa pea powder, so to match the pea powders for iron those consuming the lpa pea powder consumed slightly more than those consuming the regular peas. The maltodextrin dose was chosen in order to equate energy intake. Participants were provided with 500 mg vitamin C tablets as a reducing agent to optimize absorption of the iron (Timoshnikov et al., 2020), which they were instructed to consume with their first dose of supplement daily. Nutritional value of supplements is displayed in Table 3.1.

**Table 3.1** Nutritional content delivered daily for each supplement.

<table>
<thead>
<tr>
<th></th>
<th>Regular Pea</th>
<th>Low Phytic Acid Pea</th>
<th>Maltodextrin</th>
</tr>
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<tbody>
<tr>
<td>Daily amount consumed (g)</td>
<td>72</td>
<td>78</td>
<td>75</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>286</td>
<td>309</td>
<td>300</td>
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</table>
### Carbohydrate (g)

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<tbody>
<tr>
<td>Carbohydrate</td>
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<td></td>
<td>75</td>
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### Protein (g)

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<tr>
<td></td>
<td>35.3</td>
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### Fat (g)

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<td>3.7</td>
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### Iron (mg)

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<th></th>
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<tr>
<td></td>
<td>4.3</td>
<td>4.6</td>
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<tr>
<td></td>
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### 3.3.2 Participants and Intervention Study

In this study, we utilized a randomised, double-blind, placebo-controlled parallel groups design. Twelve women were recruited to participate. Inclusionary criteria included: Being a regular runner (≥2 days per week, ≥ 20 minutes each time), not having supplemented with pharmaceutical iron in the past 6-weeks, and having a regular intake of dietary iron below the recommended dietary allowance (18 mg per day). Participants were excluded if they were deemed to be anemic based on baseline hemoglobin analysis (< 115 g/L) and were advised to speak to their healthcare practitioner. After hemoglobin measurements were completed, participants were randomly assigned to one of three conditions: Maltodextrin, *lpa* pea powder, or regular phytic acid pea powder. Randomization was completed in blocks of three by a researcher not involved in any other part of recruitment, data collection, or analyses for a double-blind trial (participants and researchers involved in conducting the trial were blinded). Allocation schedule was concealed from the researcher recruiting participants. The trial was registered at clinicaltrials.gov (NCT04872140).

### 3.3.3 Compliance to the supplement

Participants were provided with a tracking sheet to report their supplement intake over the 8-week intervention period. The tracking sheet was returned to the researcher responsible for data
collection after the supplementation period was completed in order to assess compliance. Participants also returned the previous bag of supplement when each new bag was delivered and any powder left was weighed to confirm compliance.

3.3.4 3-Day Food Diary
Prior to enrollment in the study, participants completed a 3-day food diary to assess their regular intake of dietary iron. Participants were instructed to record their food and beverage intake on 3 consecutive days and provided an example of a properly completed entry. They were reminded to provide brands of specific food choices where applicable for maximum tracking accuracy. Three-day food intakes were assessed using the online food tracker app Cronometer (https://cronometer.com). All individuals had an average intake of iron below the recommended dietary allowance of 18 mg/day for adult females (Institute of Medicine, 2001) and therefore were invited to complete baseline testing.

3.3.5 Measurement Protocol
Baseline testing occurred over three separate visits. The first visit included a measurement of body composition using dual energy X-ray absorptiometry (DEXA), as well as a venous blood draw for assessment of ferritin. The second visit involved measurement of height and weight, hemoglobin, maximal oxygen uptake, and a familiarization trial of a 5 km time trial (TT). The third visit involved a 5 km TT, which was used as our performance indicator. After all baseline testing was completed, participants were randomly assigned to consume maltodextrin, a powder made from regular peas, or a powder made from peas bred to have lpa, for eight weeks. After the eight-week intervention period, participants underwent the same assessments as baseline, except
for familiarization of the TT. All post-intervention visits were completed at the same time of day as the corresponding baseline visit (±90 minutes) to account for any changes due to circadian rhythm.

3.3.6 Body Composition and Hematology

Participants were instructed to avoid any exercise in the 24 hours prior to coming to the lab for assessment of body composition and blood draw. Height and weight were assessed using a calibrated stadiometer and electronic weigh scale (Healthweigh, Rice Lake, United States), respectively. Body composition was assessed via DEXA (QDR Discovery Wi, Hologic, Inc., Bedford, Md.) using QDR software for Windows XP (QDR Discovery). All scans were completed by an individual trained in radiology. Blood draws for assessment of ferritin were obtained by a trained phlebotomist. Samples were left to rest at room temperature for 10-45 minutes and then centrifuged for 10 minutes at 3000g to separate the plasma, which was pipetted into microcentrifuge tubes and frozen at -80°C until analysis. Analyses were completed by the local health region using an electrochemiluminescence immunoassay (measuring range 0.5-2000 µg/L; CV 2.1-7.1%). Hemoglobin was assessed via fingerpick capillary sample using a portable haemoglobinometer (HemoCue, Ängelholm, Sweden).

3.3.7 Exercise Tests

Participants were instructed to follow their typical pre-exercise nutrition, hydration, and caffeine intake and avoid moderate to vigorous exercise in the 24 hours prior to each exercise session. Maximal oxygen consumption (VO$_{2\text{max}}$) was assessed using a progressive exercise test. Participants completed a standardized warmup of 3 minutes at what they considered a
comfortable walking speed and two minutes at what they considered to be a running pace at which they would reach exhaustion after 20 minutes if the incline of the treadmill were to stay at 0%. These speeds were recorded for future exercise tests. After warmup, participants rested for 5 minutes while a heart rate monitor (Polar; Kempele, Finland) and gas collection masks were fitted. After the five-minute rest period, participants ran at the speed chosen in the final two minutes of warmup with the grade of the treadmill increasing by two percent every two minutes until exhaustion. Expired gases were collected via indirect calorimetry (Max Series 29 Calorimeter, SensorMedics, USA). Expired gas and heart rate data were collected on a breath-by-breath basis. After the participant reached exhaustion, they underwent a 5-minute cool-down at a self-selected speed. After the baseline VO₂max test, the participant rested passively for 30 minutes and then completed a familiarization of the 5 km TT following the same protocol described below but without any data collection.

A minimum of 3 days after the VO₂max test, participants completed a 5 km TT on a motorized treadmill, which they were instructed to complete as quickly as possible. The test started with a standardized 500 m warmup, which was completed at the walking pace chosen during the warmup of the VO₂max test. As soon as the 500 m were complete, participants were given control of the treadmill speed and instructed to increase or decrease the speed as necessary in order to complete the 5 km as quickly as possible. The incline of the treadmill was set to 1% for the duration of the warmup and test. During the trial, fingertip blood samples were used to assess lactate every 5 minutes (Lactate Scout; EKF Diagnostics, Wales, United Kingdom) and heart rate and expired gas data were collected every breath. After the 5 km was completed, the participants completed a three-to-five-minute cooldown at a self-selected speed. All breath and heart rate data were averaged over 20 seconds for analyses.
3.3.8 Biweekly Check-ins

In order to assess dietary intake and physical activity throughout the trial, biweekly check-ins were completed. At weeks two, four, and six during the intervention and at the end of the intervention (eight weeks), participants were emailed a link to an online survey (www.surveymonkey.ca) in which they completed a 24-hour food record, the Godin Leisure Time Exercise Questionnaire (Godin & Shephard, 1985) and reported any symptoms/discomforts they were experiencing that they thought might be attributable to the supplement. Participants were provided with a list of possible symptoms one might expect (e.g., bloating, changes in bowel movements, etc.) but encouraged to report any symptoms they thought might be related to the intervention.

3.3.9 Statistics

Given this was a feasibility study with a small sample size, traditional statistics were not run. Rather, data are descriptive. The values are expressed as means ± standard deviations. Number of adverse events was compared between groups using Chi-squared tests. Differences between groups at baseline were assessed by ANOVA. All analyses were completed using JASP statistical software; version 0.15 (University of Amsterdam, 2021).

3.4 Results

Twelve individuals were recruited for the study. Three participants dropped out after randomization but before beginning the supplement. One participant completed the dietary intervention and submitted her compliance log but was lost to follow-up for post testing.
Therefore, eight individuals were included in analyses of body composition, iron status, and exercise. Demographic information for nine participants who took part in the dietary intervention is displayed in Table 3.2. No differences between groups were evident at baseline (p>0.05).

Table 3.2 Participant Demographic Information

<table>
<thead>
<tr>
<th></th>
<th>Maltodextrin (n=3)</th>
<th>Regular pea (n=3)</th>
<th>Low Phytic Acid Pea (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.7±25.6</td>
<td>24.7±2.1</td>
<td>36.3±4.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.0±4.7</td>
<td>161.8±0.6</td>
<td>167.4±4.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.0±10.8</td>
<td>60.4±5.4</td>
<td>76.2±23.5</td>
</tr>
<tr>
<td>VO\textsubscript{2}max (mL/kg/min)</td>
<td>42.5±4.4</td>
<td>49.9±4.7</td>
<td>45.4±7.8</td>
</tr>
<tr>
<td>5 km time (min)</td>
<td>33.7±8.7</td>
<td>26.4±2.0</td>
<td>35.4±10.0</td>
</tr>
<tr>
<td>Running Experience (years)</td>
<td>30.5±34.6</td>
<td>10.0±0</td>
<td>4.3±4.9</td>
</tr>
<tr>
<td>Average Weekly Running (min)</td>
<td>160.0±69.3</td>
<td>100.0±34.6</td>
<td>238.3±213.6</td>
</tr>
</tbody>
</table>

\textit{VO_2max} = maximal oxygen consumption; value are means ± SD

3.4.1 Compliance
A high compliance to the supplements was found for all conditions, with all participants reporting >90% compliance (range 94.1%-100%). Compliance rates by group are displayed in Table 3.3.
Table 3.3 Compliance to the supplement

<table>
<thead>
<tr>
<th>Condition</th>
<th>Compliance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td>99.2 ±0.9</td>
</tr>
<tr>
<td>Regular</td>
<td>96.3 ±2.1</td>
</tr>
<tr>
<td>Phytic Acid</td>
<td></td>
</tr>
<tr>
<td>Low Phytic</td>
<td>97.8 ±2.3</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation

3.4.2 Adverse Effects

All but one participant (from the regular pea group) reported a symptom they thought might be related to the supplement at some point during the intervention. In the early stages of the study, all but two participants reported no symptoms they thought might be related to the supplement. Of the two who did report symptoms (at week two of the intervention), one was in the *lpa* pea group, and one was in the maltodextrin group. Reported symptoms were highest in all groups at the second check-in (week four of the intervention), with two participants in both pea groups and one from the maltodextrin group reporting symptoms. Symptoms then seemed to decrease at the third check-in, with one individual from each the maltodextrin group and the regular pea group reporting symptoms and by the last check-in (week 8 of the intervention) symptoms were only reported in the regular pea group, with one individual assigned to consume the regular pea reporting symptoms. Most participants only reported symptoms at one check-in. A participant
from the maltodextrin group reported bloating at week 4 and week 6 check-in and one participant from the regular pea group reported gassiness and weeks 4, 6, and 8 check-ins.

Symptoms reported included one or more of bloating, increased flatulence, reduced appetite, changes to bowel movements (increased frequency, loose stools), and indigestion. By the third biweekly check-in (week six), symptoms had resolved in all but two participants and by the end of the trial (week eight on supplement) only one participant was still experiencing symptoms. There were no differences in the number of symptoms experienced between groups (p=0.53). No participant experienced symptoms to a severity that would cause them to drop out of the study.

3.4.3 Dietary Intake

Dietary intake at different time points for all three groups is displayed in Table 3.4. When assessing dietary intake, recorded biweekly, overall intakes were similar between groups for energy intake, carbohydrate, and fat. Intakes of iron and protein trended higher for the groups consuming the regular pea and the lpa pea supplement, which was expected given that the supplements in these groups were designed to provide extra iron and protein.

3.4.4 Iron Markers, Body Composition, and Time Trial Performance

Ferritin increased in all groups, with the greatest increase being observed in the lpa pea supplement group (+42.8 ug/L). All other outcome measures remained relatively stable from pre to post intervention (Table 3.5).
Table 3.4 Dietary Intake Throughout the Intervention

<table>
<thead>
<tr>
<th></th>
<th>Energy (kcal)</th>
<th>Carbohydrates (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Iron (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malto</td>
<td>1821±426</td>
<td>188.6±78.5</td>
<td>66.4±15.9</td>
<td>85.8±22.2</td>
<td>9.2±2.2</td>
</tr>
<tr>
<td>Reg</td>
<td>1537±213</td>
<td>189.3±45.5</td>
<td>80.0±20.0</td>
<td>53.5±10.4</td>
<td>9.6±3.0</td>
</tr>
<tr>
<td>lpa</td>
<td>1748±261</td>
<td>218.4±21.0</td>
<td>66.3±16.0</td>
<td>61.2±16.4</td>
<td>12.9±1.5</td>
</tr>
<tr>
<td><strong>2 Weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malto</td>
<td>1922±788</td>
<td>200.6±91.5</td>
<td>62.9±17.4</td>
<td>51.5±34.6</td>
<td>9.3±4.0</td>
</tr>
<tr>
<td>Reg</td>
<td>1524±110</td>
<td>190.7±74.4</td>
<td>83.9±39.0</td>
<td>46.4±25.5</td>
<td>15.8±3.5</td>
</tr>
<tr>
<td>lpa</td>
<td>1976±83</td>
<td>225.2±24.5</td>
<td>92.6±32.6</td>
<td>57.6±17.2</td>
<td>12.0±9.1</td>
</tr>
<tr>
<td><strong>4 Weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malto</td>
<td>1807±351</td>
<td>203.3±58.7</td>
<td>60.0±17.3</td>
<td>79.5±36.1</td>
<td>10.0±0.0</td>
</tr>
<tr>
<td>Reg</td>
<td>1666±68</td>
<td>193.7±12.0</td>
<td>88.8±9.2</td>
<td>52.9±5.0</td>
<td>14.3±2.8</td>
</tr>
<tr>
<td>lpa</td>
<td>2197±586</td>
<td>257.7±83.5</td>
<td>103.2±4.6</td>
<td>75.7±49.5</td>
<td>18.0±3.1</td>
</tr>
<tr>
<td><strong>6 Weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malto</td>
<td>1763±466</td>
<td>206.3±73.4</td>
<td>58.0±14.8</td>
<td>70.5±40.3</td>
<td>9.2±5.1</td>
</tr>
<tr>
<td>Reg</td>
<td>1922±582</td>
<td>239.2±94.8</td>
<td>83.8±30.4</td>
<td>66.9±7.8</td>
<td>15.8±5.0</td>
</tr>
<tr>
<td>lpa</td>
<td>1619±118</td>
<td>162.4±41.4</td>
<td>92.2±17.7</td>
<td>57.4±13.6</td>
<td>12.5±4.6</td>
</tr>
<tr>
<td><strong>8 Weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malto</td>
<td>1681±157</td>
<td>215.7±24.8</td>
<td>59.3±26.6</td>
<td>58.5±0.7</td>
<td>8.7±5.1</td>
</tr>
<tr>
<td>Reg</td>
<td>1613±151</td>
<td>167.7±36.1</td>
<td>90.8±5.0</td>
<td>54.9±10.6</td>
<td>13.3±1.4</td>
</tr>
<tr>
<td>lpa</td>
<td>2038.7±639</td>
<td>212.5±54.8</td>
<td>101.5±28.6</td>
<td>72.5±31.9</td>
<td>14.3±4.6</td>
</tr>
</tbody>
</table>

Data include regular intake + supplement; Data are expressed as mean ± SD; Malto = maltodextrin; Reg = regular pea; lpa = low phytic acid
Table 3.5 Baseline and post-intervention blood, performance, and body composition variables

<table>
<thead>
<tr>
<th>Measure</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malto (n=3)</td>
<td>Reg (n=3)</td>
</tr>
<tr>
<td>Ferritin (ug/L)</td>
<td>43.4±38.9</td>
<td>53.7±56.6</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>129±2</td>
<td>125±5</td>
</tr>
<tr>
<td>5 km TT (s)</td>
<td>2021±519.0</td>
<td>1587±121</td>
</tr>
<tr>
<td>VO₂max (mL/kg/min)</td>
<td>42.5±4.4</td>
<td>49.9±4.7</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>61.0±10.8</td>
<td>60.4±5.4</td>
</tr>
<tr>
<td>LBM (kg)*</td>
<td>41.9±1.7</td>
<td>42.7±3.8</td>
</tr>
<tr>
<td>Adipose (kg)*</td>
<td>16.3±9.8</td>
<td>15.0±1.7</td>
</tr>
<tr>
<td>% adipose</td>
<td>25.7±11.5</td>
<td>25.1±1.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD; Malto= maltodextrin; Reg= regular pea; lpa= low phytic acid pea; LBM= lean body mass; VO₂max= maximal oxygen consumption; TT= time trial

*one participant in the Low phytate group was lost to follow up and one was removed as an outlier from the data, leaving only one data point for Low Phytate group for LBM, FM, and weight

3.4.5 Heart Rate, Lactate and Expired Gases

No differences were evident between groups for heart rate or indicators of metabolism (lactate production, oxygen consumption, respiratory exchange ratio) during the 5 km treadmill run (Table 3.6)
Table 3.6 Heart rate and metabolic data collected during 5 km treadmill run

<table>
<thead>
<tr>
<th></th>
<th>Lactate (mmol/L)</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malto</td>
<td>Reg</td>
<td>lpa</td>
<td>Malto</td>
<td>Reg</td>
<td>lpa</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Rest</td>
<td>1.5±0.4</td>
<td>2.2±0.8</td>
<td>1.1±0</td>
<td>1.7±1.3</td>
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<td>1.1±0</td>
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<tr>
<td>Q1</td>
<td>6.7±1.2</td>
<td>7.2±1.2</td>
<td>6.6±1.9</td>
<td>4.1±1.9</td>
<td>5.6±1.2</td>
<td>6.2±2.7</td>
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<tr>
<td>Q2</td>
<td>5.4±0.8</td>
<td>6.9±4.7</td>
<td>5.9±2.5</td>
<td>4.5±2.6</td>
<td>7.2±1.5</td>
<td>7.7±1.8</td>
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<tr>
<td>Q3</td>
<td>7.1±1.8</td>
<td>10.5±1.6</td>
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<td>7.3±3.2</td>
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</tr>
<tr>
<td>Q4</td>
<td>5.8±2.6</td>
<td>13.1±1.1</td>
<td>8.1±4.7</td>
<td>5.8±2.6</td>
<td>10.8±3.8</td>
<td>10.2±3.5</td>
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<tr>
<td></td>
<td>VO₂ (mL/kg/min)</td>
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<tr>
<td>Q1</td>
<td>31.5±5.2</td>
<td>37.3±4.6</td>
<td>33.3±9.1</td>
<td>39.7±7.9</td>
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<td>36.3±17.5</td>
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<tr>
<td>Q2</td>
<td>36.0±6.3</td>
<td>42.1±5.1</td>
<td>36.9±12.0</td>
<td>41.9±10.8</td>
<td>43.0±13.5</td>
<td>48.8±10.2</td>
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<tr>
<td>Q3</td>
<td>36.5±6.9</td>
<td>43.5±4.7</td>
<td>36.4±11.7</td>
<td>42.4±12.0</td>
<td>45.8±11.6</td>
<td>49.3±13.8</td>
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<td>Q4</td>
<td>37.8±11.5</td>
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<td>37.8±11.5</td>
<td>46.4±13.0</td>
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<td>*HR (bpm)</td>
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<td>Q1</td>
<td>151±28</td>
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<tr>
<td>Q2</td>
<td>163±28</td>
<td>177±9</td>
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<td>152±29</td>
<td>173±21</td>
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<tr>
<td>Q3</td>
<td>166±23</td>
<td>165±27</td>
<td>185</td>
<td>157±23</td>
<td>176±23</td>
<td>179</td>
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<tr>
<td>Q4</td>
<td>171±18</td>
<td>176±12</td>
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<td>162±9</td>
<td>184±18</td>
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<tr>
<td>Q1</td>
<td>0.93±0.03</td>
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<td>0.98±0.04</td>
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<td>1.00±0.09</td>
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<tr>
<td>Q2</td>
<td>0.95±0.03</td>
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<tr>
<td>Q3</td>
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<td>1.04±0.09</td>
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<tr>
<td>Q4</td>
<td>0.952±0.05</td>
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<td>0.94±0.04</td>
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<td>1.04±0.09</td>
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</tbody>
</table>

Values are mean ± SD; Malto= maltodextrin; Reg= regular pea; lpa= low phytic acid pea; 

$VO_2$= oxygen consumption $HR$= heart rate; $RER$= respiratory exchange ratio; $Q1$= first quarter of 5km run; $Q2$= second quarter of 5 km run; $Q3$= third quarter of 5 km run; $Q4$= fourth quarter of 5 km run; *one participant in Low Phytate group was lost to follow up and one did not have valid HR data due to equipment malfunction during data collection

### 3.5 Discussion

The most important finding of the current research is that a high-protein powder made of field peas is well tolerated by female runners. A high degree of compliance was observed for both pea conditions. The incidence of adverse effects did not differ between the supplement conditions and the prevalence of symptoms appeared to decrease as time on the supplement progressed for those randomized to the pea supplements. Symptoms reported were similar to those observed following chickpea use (Dahl et al. 2014). However, Dahl et al. did not observe a decrease in symptoms as time went on, though their intervention only included three weeks of canned chickpea intake. The results of the current trial suggest that approximately four to six weeks of consumption are required in order for gastrointestinal symptoms to subside. The current results are supported by findings of Veenstra et al. (2010), who found mild effects on flatulence and gastrointestinal symptoms in healthy males consuming different types of pulses for 28 days, but nothing severe enough to warrant limiting or avoiding pulse consumption.

Our study indicated a noticeable increased intake of protein in those consuming the pea-based supplements. Given the increased protein requirements for athletes in order to optimize
performance (Phillips et al., 2011) and the relatively low energy intake relative to exercise energy expenditure (Logue et al., 2020; Melin et al., 2019) pea protein may be a strategy to assist athletes in reaching their protein requirements in order to maximize their exercise performance. Further, as pea protein does not contain the common allergens found in other protein supplements (dairy, wheat, soy, egg, nut), it may prove an attractive option for those limited by allergies or intolerances. Consumption of a pea-based protein supplement may also be attractive to environmentally conscious individuals due to its low carbon footprint compared to other protein supplements (Nette et al., 2016).

Protein supplements manufactured from regular peas (i.e., with normal phytic acid levels) have been a topic of interest in recent years. Some research has concluded that pea protein may be just as efficacious as whey for enhancing muscular strength and hypertrophy (Babault et al., 2015; Banaszek et al., 2019). Nieman et al. (2020) found whey to be significantly better than water in ameliorating muscle damage following a bout of damage-inducing eccentric exercise, but no differences were observed between pea protein and water or pea protein and whey protein. The currently available evidence focuses on resistance exercise training and adaptations such as muscular strength and hypertrophy, but to the authors’ knowledge no research has been conducted on a pea protein supplement in endurance athletes.

The literature on lpa peas for iron status in humans is scarce, however Warkentin et al. (2020) found that chickens fed a lpa pea diet had moderately improved hemoglobin levels, as well as improved markers of gut function. While the current research did not replicate these findings, the greatest increase in ferritin in our participants was in those supplemented with the lpa peas (Table 3.5). Interestingly, the lpa pea group had relatively high baseline iron intake compared to the maltodextrin and regular pea groups (Table 3.4), which may have attenuated the effect of
raising ferritin since the \textit{lpa} group would have been less responsive to a supplement designed to increase iron levels (Wang et al., 2019)

The techniques and protocol used in this pilot study proved to be acceptable and the outcomes obtained were representative and realistic. The running protocol used to assess exercise performance was doable by all participants with no concerns for participant safety or data validity. While the increase in ferritin observed in the \textit{lpa} group was unreasonably high, such a change is likely driven by one participant in the \textit{lpa} group whose ferritin increased by 70 ug/L. Such differences could be driven by increased inflammation, artificially inflating ferritin measurements (Ueda et al., 2018). Multiple technical trials of blood measurements can be carried out to improve confidence in the measure as well as measuring markers of inflammation alongside the iron measure to rule out any differences due to the presence of inflammation. All other measures (body composition, lactate, expired gasses, hemoglobin, TT performance, maximal oxygen consumption, heart rate) were within the expected ranges for the current population.

Given the high tolerability and minor GI symptoms of consuming a pea protein supplement in a cohort of female runners, an investigation of the role that consumption of a \textit{lpa} pea powder on the health and performance of female athletes is prudent and feasible. Given the current global push for the reduction of animal product consumption (Iwasa-Madge et al., 2020; Meyer et al., 2020) in addition to the increasing cost of such products, \textit{lpa} peas may prove to be a viable strategy to increase protein intake and improve iron levels in female athletes, and other groups, leading to improved health and performance.
Competing interests
The authors declare that they have no competing interests.

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Authors' contributions
Conceptualization: KAS, GAZ, DLL, TDW, PDC; Methodology: KAS, GAZ, DLL, TDW, PDC; Data collection: KAS, JK; Data analysis: KAS, PDC; Manuscript Preparation: KAS; Manuscript Review and Editing: KAS, GAZ, DLL, TDW, PDC; Supervision: TDW, GAZ, PDC; Funding Acquisition: TDW, GAZ, PDC. All authors have read and agreed to the published version of the manuscript.

3.6 References


3.7 Next Steps

Given the high rates of compliance and tolerability of the pea supplements observed in study 1 of this thesis, a larger randomized control study was completed to assess the role of a *lpa* pea protein supplement in improving the iron status in a group of female runners.
Chapter 4- Study 2- Low phytic acid pea supplementation as an approach to combating iron deficiency in female runners: a randomized control trial
4.1 Abstract

**Background**: Iron deficiency is the most prevalent micronutrient deficiency in the world and the leading cause of anemia globally. Female athletes are at a disproportionate risk for iron deficiency due to blood loss through menstruation and decreased iron absorption secondary to exercise. Field peas are a rich source of iron but, similar to iron from other plant-based sources, the iron has limited bioavailability due to high levels of phytic acid, an inherent compound that binds to cations, creating a salt (phytate), which limits absorption during digestion. **Aim**: The purpose of our research was to investigate the effect of a field pea variety bred to have low levels of phytic acid on plasma ferritin, exercise performance, and body composition in female runners. **Methods**: Twenty-eight female runners (age: 34.6±9.7 years; weight: 65.1±8.1 kg; VO\textsubscript{2max}: 50.7±8.9 mL/kg/min) underwent measures of ferritin, exercise performance, and body composition before and after being randomly assigned to consume a powder derived from regular peas, low phytic acid peas, or a non-pea control (maltodextrin), plus vitamin C for eight weeks. **Results**: The regular pea and low phytic acid pea groups had a 14.4% and 5.1% increase in plasma ferritin, respectively, while the maltodextrin group had a decrease of 2.2%; however, the difference in changes between groups was not statistically significant. No differences between groups were evident in any of the other measures. **Conclusion**: Larger doses or longer duration of pea supplementation may be necessary to induce meaningful changes in iron status. This trial was registered at ClinicalTrials.gov (NCT04872140).
4.2 Introduction

Iron plays a crucial role in human physiology such as oxygen transport, energy production, and cell proliferation (Shoemaker et al., 2019). Despite the importance of iron, iron deficiency (ID) is the most prevalent micronutrient deficiency globally, with athletic individuals being impacted to a greater extent than their sedentary peers. Research suggests 15-35% of female athletes and 3-11% of male athletes present with ID, though a prevalence of >50% in females and up to 30% in males has been reported (Sim et al., 2019; Sinclair & Hinton, 2005). Increased prevalence of ID in females is related to blood loss through menstruation (Mirza et al., 2018; Pasricha et al., 2014), further exacerbated in athletes due to exercise-induced losses through foot strike hemolysis, ischemia to the viscera, frequent use of anti-inflammatory drugs, or increased losses through urine and sweat (Coates et al., 2017). Further, the requirement for iron may be increased due to increased erythropoiesis in response to aerobic exercise (Clénin et al., 2015).

In addition to the fatigue generally observed in ID, athletes with ID are likely to experience reduced performance, training tolerance, and recovery from training (McCormick et al., 2020). To maximize their iron levels and performance, athletes often turn to pharmaceutical interventions. Skorseth et al. (2020) reported that 42% of high school distance runners report taking an iron supplement, while Coates et al. (2017) reported significant intakes of supplemental iron in elite runners and triathletes. Pharmaceutical interventions appear beneficial for improving serum ferritin levels (Pasricha et al., 2021), though they are reported to cause gastrointestinal disturbances (Sim et al., 2019).

When considering dietary approaches to combat ID, the literature is limited and inconclusive. Some research has reported increases in serum ferritin after increasing dietary iron intake.
(Ishizaki et al., 2006) while others have found no change in ferritin levels with the inclusion of 255g/week of lean beef (Burke et al., 2012) or diets designed to deliver 18.5 mg of dietary iron daily (Alaunyte et al., 2014).

Field peas are a rich source of macro- and micro-nutrients, including iron (Bangar et al., 2017). However plant-based proteins are also high in phytic acid. This antinutritional compound binds to cations such as iron and zinc, forming a salt (phytate) that is then excreted, decreasing the bioavailability of the mineral (Warkentin et al., 2012). The Crop Development Centre at the University of Saskatchewan developed two lines of field peas with low phytic acid (lpa) through chemical mutagenesis (Warkentin et al., 2012), a cost-effective plant breeding technique used to optimize nutrient status (Bouis et al., 2011). These lpa lines have approximately 60% less phytic acid while performing similarly agronomically (Lindsay et al., 2021) and have 1.4- and 1.9 times higher iron bioavailability than the parent lines (Liu et al., 2015). These lpa lines may potentially be used in a plant-based dietary approach to combat ID in affected individuals. The current study aims to assess the effectiveness of a powder made from lpa peas for improving iron status and exercise performance in female runners. We hypothesized that the lpa peas would improve iron status (i.e. ferritin) to a greater extent than the regular-phytic acid peas or a non-pea control which may, in turn, improve exercise performance.

4.3 Methods

4.3.1 Participants and Study Design

This study utilized a randomized, double-blind, placebo-controlled parallel-groups design and was approved by the Biomedical Research Ethics Board at the University of Saskatchewan (BIO 1207). Sample size calculations were conducted using data from Anschuetz et al. (2010), who
assessed dietary iron bioavailability in runners by comparing two groups of runners with
different dietary iron bioavailability scores and a non-runner control group, assuming that our
pea product would change dietary iron bioavailability from low bioavailability to medium-high
bioavailability. Based on an effect size of 1.61 (Anschuetz et al. (2010) while using an alpha of
0.05 and 80% power, calculations suggested a sample size of nine participants per group to be
sufficient. A convenience sample of thirty-four women was recruited from the Saskatoon,
Saskatchewan area from September 2021- January 2022 to take part in the research. Inclusionary
criteria included being a regular runner (≥2 days per week, ≥ 20 minutes each time), not having
supplemented with pharmaceutical iron in the past six weeks, not postmenopausal, and having an
intake of dietary iron below the recommended dietary allowance (RDA) of 18 mg per day
(Institute of Medicine, 2001). Participants were excluded if they were deemed to be anemic
based on hemoglobin analysis (< 115 g/L). After providing written free and informed consent,
participants completed a 3-day food diary. If iron intakes were below the RDA, participants
underwent baseline testing sessions, followed by randomization to one of three dietary
supplements (described below). After the 8-week supplementation period, participants completed
post-testing, which was identical to baseline testing but without a familiarization time trial (TT).
The 8-week intervention period was chosen based on other research involving dietary approaches
to improve iron status in female athletes lasting 4-8 weeks (Alaunyte et al 2014; Burke et al
2012; Ishizaki et al. 2006). Randomization was completed in blocks of three by a researcher not
involved in any other part of the study. The allocation schedule was concealed from the
researcher recruiting participants. All baseline and post-testing measurements were conducted at
the same time of day (± 60 minutes). All powders had a similar appearance. This trial was
registered at ClinicalTrials.gov (NCT04872140).
4.3.2 Dietary Intervention

Participants were randomly assigned by a computer random-number generator to consume one of three powders for eight weeks: a powder made from regular peas, a powder made from peas bred to have \textit{lpa}, or a non-pea control (maltodextrin). Intervention length was based on reports of four to eight weeks of supplementation to produce an increase in serum ferritin (Blee et al., 1999), as well as intervention lengths in other food-based approaches to improve iron status of four to eight weeks (Alaunyte et al., 2014; Anschuetz et al., 2010; Burke et al., 2012). Participant randomization was sent to a third-party researcher with no other role in the study, who prepared the powder. Participants and the researcher responsible for data collection and analysis were blinded to intervention assignment. Nutritional information of dietary conditions is displayed in Table 4.1. Pea powders were equated for iron delivered through the supplement. The \textit{lpa} pea contained slightly more iron than the regular pea (51.4 mg/kg vs. 47.9 mg/kg), so those randomized to the regular pea group consumed slightly more supplement daily. The maltodextrin condition was approximately equated for calories. Participants consumed the daily amount broken up into three doses spread throughout the day in the way that best fit their lives and taste palettes but were instructed not to consume the supplement within two hours of any dairy or dairy alternatives to optimize iron absorption. Participants were given no other instruction on their dietary intake but were informed of the approximate caloric load of the supplement and instructed to adjust their diet in order to maintain their habitual energy intake. Participants were also provided 125 mg tablets of vitamin C, which they were instructed to consume alongside each dose of supplement in order to enhance iron absorption. Each participant recorded compliance in a log and leftover powder was collected and weighed to confirm. Upon
completion of the intervention, participants were asked which supplement they thought they consumed to assess the efficacy of blinding.

Table 4.1 Nutritional Information of Supplements

<table>
<thead>
<tr>
<th></th>
<th>Low Phytic Acid Pea</th>
<th>Regular Pea</th>
<th>Maltodextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daily amount</strong></td>
<td>120</td>
<td>130</td>
<td>125</td>
</tr>
<tr>
<td>(g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Energy (kcal)</strong></td>
<td>498</td>
<td>540</td>
<td>500</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td>53.2</td>
<td>57.6</td>
<td>125</td>
</tr>
<tr>
<td>(g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>58.8</td>
<td>63.7</td>
<td>0</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>5.6</td>
<td>6.1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Iron (mg)</strong></td>
<td>6.2</td>
<td>6.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

4.3.3 Three-Day Food Diary

Regular dietary intake was assessed using a 3-day food diary. Participants were instructed to record their food and beverage intake on three days, including one weekend day. Participants were instructed not to record on days they did not consider to be "normal" (they were ill, went to a celebration, etc.) and to provide as much detail for each food item as possible, including product brands where applicable. Dietary intakes from these three days were assessed using Cronometer (https://cronometer.com), with confirmation of nutritional data performed through cross-referencing with the Canadian Nutrient File (2015) where necessary. Inadequate dietary iron intakes relative to the RDA were used as a proxy for iron status, therefore individuals with
dietary iron consumption below the RDA of 18 mg/day were invited to complete baseline testing.

4.3.4 Body Composition

Participants arrived at the research facility in a normally fed and hydrated state and were instructed to avoid vigorous exercise in the 24 hours prior to the visit. Body composition was measured by DEXA (Hologic© Discovery Wi; Bedford, MA) using QDR software for Windows XP (QDR Discovery, Hologic, Inc.). Measurements were obtained by a certified radiology technologist. Weight and height were assessed using a calibrated weigh scale and stadiometer, respectively. Reproducibility of repeated measures of lean tissue mass and fat mass in our facility have coefficients of variation of 1% and 3%, respectively.

4.3.5 Hematology

Participants were asked how many days it had been since their last menstrual cycle such that timing could be replicated at post-testing. A venous blood sample was collected by a trained phlebotomist then left for 10-60 minutes at room temperature before being centrifuged at 3000g for 10 minutes at 10°C. Plasma was then separated into microcentrifuge tubes and stored at -80°C until analysis. When all plasma samples were collected (pre- and post-intervention), samples were transferred to a clinical laboratory (Royal University Hospital, Saskatoon) for analysis of ferritin and C-reactive protein, which were assessed using electrochemiluminescence immunoassay and particle enhanced immunoturbidimetric assay, respectively. The ferritin assay had a CV of 2.1-7.1% and the C-reactive protein assay had a CV of 1.0-8.4%. Two trials of all plasma measures were run and results averaged for data analyses. Hemoglobin was determined
by a fingertip using capillary samples and assessed by a point of care testing device (HemoCue, Ängelholm, Sweden). Participants with hemoglobin levels <115 g/L were excluded from the study and referred to their health care practitioner due to risk of anemia.

4.3.6 Maximal Oxygen Consumption (VO$_{2\text{max}}$)

Participants were instructed to follow their typical pre-exercise nutrition, hydration, and caffeine intake and arrive to the research facility having not engaged in moderate to vigorous exercise in the 24 hours prior. Upon arrival, participants rested for five minutes, after which heart rate and blood pressure measures were taken. Following resting measures, participants completed a standardized five-minute treadmill warmup, during which they walked for three minutes at a speed they considered a "comfortable" warmup walk and ran for two minutes at what they thought to be a pace they could hold for 20-minutes but not longer. These speeds were recorded and replicated for post-intervention assessments. Participants were then given a five-minute rest period while a heart rate monitor strap and gas collection mask were fitted. Participants underwent a progressive exercise test in which they ran at the speed chosen in the warmup and the grade of the treadmill increased by 2% every two minutes until volitional fatigue. Expired gases (Vmax Series 29 Calorimeter, SensorMedics, USA) and heart rate (Polar, Kempele, Finland) data were collected for the test duration and averaged over 20-seconds. Participants were considered to have reached VO$_{2\text{max}}$ if there was a plateau in oxygen consumption with increasing work rate and a respiratory exchange ratio of >1.1. If these criteria were not met, participants were considered to have reached VO$_{2\text{peak}}$. After an active cooldown of at least five minutes, participants rested passively for 30 minutes before completing a familiarization 5 km TT, as below.
4.3.7 5 km Time Trial (TT)

At least 36 hours following the VO\textsubscript{2}max test and familiarization TT, participants arrived at our facility having followed the same pre-test instructions as for the maximal oxygen uptake test. Participants completed a 500 m warmup walk at the pace chosen during the first 3 minutes of the warmup in the previous exercise session and at a 1% grade incline. Immediately upon completion of the 500 m, participants were given control of the treadmill speed and instructed to complete the 5 km as quickly as possible. Percent grade of the treadmill remained at 1% for the duration of the run. Participants were permitted to see the treadmill speed and distance covered but were blinded to the time elapsed. Expired gases and heart rate were collected continuously during the warmup and 5 km run. Lactate measurements were assessed using fingertip capillary samples (Lactate Scout; EKF Diagnostics, Wales, United Kingdom) at rest as well as every five minutes during the 5 km run. Because test durations differed between participants, lactate, heart rate, and expired gas data were normalized to six time points (i.e., warm-up (or rest for lactate), quintiles of the 5 km run).

4.3.8 Biweekly Check-Ins

Participants were instructed to maintain their habitual physical activity and dietary behaviours and were asked to try to maintain their pre-intervention energy intake during the intervention period (i.e., to replace a dessert or snack with the supplement so as to not increase caloric intake due to the supplement). Physical activity and dietary habits were monitored through questionnaires sent every two weeks. A link over email every two weeks to complete a questionnaire delivered using the online platform SurveyMonkey. In this questionnaire,
participants recorded a 24-hour food log and completed the Godin Leisure Time Exercise Questionnaire (Godin & Shephard, 1985). Participants were also given an opportunity to report any symptoms or side effects they were experiencing that they thought might be related to the supplement. Participants were provided with examples of symptoms they might experience (bloating, decreased appetite, increased flatulence, etc.), but encouraged to report any symptom they thought might be related to consumption of the supplement. Total dietary intakes (regular diet plus supplement) were assessed using an online assessment tool, Chronometer, as described above.

4.3.9. Statistics

All data were analyzed using JASP statistical software (version 16.2; 2021, University of Amsterdam). Data are expressed as mean ± standard deviation unless otherwise stated. Prior to analysis, data were assessed for normality through assessment of skewness and kurtosis and outliers were assessed using boxplots. Data were considered to be outliers if they exceeded 1.5 times the interquartile range of the boxplot. Body composition, 5 km TT performance, hemoglobin, ferritin, C-Reactive Protein, and maximal oxygen uptake were assessed using a 3x2 (group x time) mixed ANOVA, with repeated measures on the “time” factor. Measures during the 5 km TT were equated to six time points to correct for different duration of the test between participants: warmup (or rest for lactate) and then quintiles during the run. Lactate, heart rate, and expired gas data from the 5 km TT were assessed using a 3x2x6 (group x time [pre/post intervention] x time point during the run) ANOVA, with repeated measures on the last two factors. Dietary intake was assessed with a 3x5 (group x time) ANOVA with repeated measures on the “time” factor. Tukey’s LSD post-hoc tests were used to compare means if any main effects or interactions were observed. Frequency of symptoms between groups were assessed
using chi-square tests. Effect size for change scores between conditions were calculated using partial $\eta^2$ ($\eta_p^2$) for our primary (ferritin) and secondary (exercise variables; VO$_{2\text{max}}$ and 5 km TT) outcomes. An effect size of 0.01 was considered small, 0.06 as medium, and 0.14 as large. Significance was accepted at $p<0.05$.

**CONSORT 2010 Flow Diagram**

Figure 4.1 Flow diagram of participant recruitment

4.4 Results

Thirty-four women consented to take part in the study (Figure 4.1). Twenty-eight participants were randomized and began the supplement. One dropped out due to an inability to tolerate the
supplement, and another due to reasons unrelated to the study. The final analysis included nine individuals from each of the maltodextrin and lpa pea groups and eight from the regular pea group. Demographic information is presented in Table 4.2. No differences were observed between groups at baseline.

Table 4.2 Participant Demographic Information

<table>
<thead>
<tr>
<th></th>
<th>Maltodextrin</th>
<th>Regular Pea</th>
<th>Low Phytic Acid</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.7±8.6</td>
<td>35.1±13.1</td>
<td>34.9±8.6</td>
<td>0.95</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.3±8.2</td>
<td>62.3±8.6</td>
<td>65.3±6.9</td>
<td>0.29</td>
</tr>
<tr>
<td>VO2max (mL/kg/min)</td>
<td>47.8±8.7</td>
<td>51.6±9.9</td>
<td>53.4±8.4</td>
<td>0.62</td>
</tr>
<tr>
<td>5 km time (min)</td>
<td>30.8±4.1</td>
<td>27.7±2.4</td>
<td>28.5±3.3</td>
<td>0.22</td>
</tr>
<tr>
<td>Running frequency</td>
<td>3.2±1.0</td>
<td>3.9±1.4</td>
<td>3.6±1.3</td>
<td>0.43</td>
</tr>
<tr>
<td>Running volume</td>
<td>134±67</td>
<td>152±58</td>
<td>173±103</td>
<td>0.56</td>
</tr>
</tbody>
</table>

All values are means ±SD; VO2max= maximal oxygen consumption

4.4.1 Compliance and Blinding

All participants randomized to the maltodextrin group correctly guessed the condition they had been assigned to. Three (37.5%) participants in the lpa group and 4 (50%) in the regular pea group correctly guessed their group allocation. Compliance was greater than 80% in all groups (maltodextrin=81.9%; regular pea=85.5%; lpa pea=82.6%). One participant in the maltodextrin
group consumed 1/5 of the prescribed dose and one consumed 2/3 of the prescribed dose. One participant in the lpa pea group consumed 2/3 of the dose, one 3/4 of the dose, and another consumed half the dose. Two participants in the regular pea group consumed 2/3 of the dose. All other participants consumed the supplement as prescribed.

4.4.2 Hematological Measures

*Ferritin.* Changes in ferritin levels between the beginning and end of the trial are depicted in Figure 4.2. Before analysis, two outliers were removed from the maltodextrin group, two from the regular pea group, and one from the lpa group. No group x time interaction was evident (p=0.75; η²=0.014) (Table 4.3). One participant had high C-reactive protein levels at baseline, so a sensitivity analysis was performed with that datum removed, as high inflammation can affect iron status. This did not alter the findings. Further sensitivity analysis was conducted looking at only those participants below the median ferritin value at baseline, with no differences evident in the results. Note that inclusion of outliers in the analysis did not alter the findings. Further, findings were not altered if compliance to the supplement was added as a covariant nor if ferritin data were log transformed or if lpa and the regular groups were compared alone.

*C-Reactive Protein and hemoglobin.* One outlier from the maltodextrin group was removed prior to analysis for C-reactive protein. There was no interaction between group and time for C-reactive protein (p=0.22) or hemoglobin (p=0.31; Table 4.3).
Figure 4.2 Change in ferritin values (ug/L) after the 8-week dietary intervention. Error bars represent standard error of the mean.

4.4.3 Exercise Performance.

$VO_{2max}$. No significant interaction was found between group and time ($p=0.64; \eta^2=0.05$). There was a significant time effect, with baseline $VO_{2max}$ values significantly higher than post-intervention ($p=0.005$). One participant in the lpa group became very ill with COVID-19 and mononucleosis approximately midway through the dietary intervention, impacting her training and fitness. Removal of her data had no impact on the findings (Table 4.3).

5km Time Trial. One outlier from the maltodextrin group was removed prior to analysis. No interaction was found between group and time ($p=0.55; \eta^2=0.06$) (Table 4.3). When the participant who became ill during the intervention was removed, the results were unchanged. During the 5 km run, there were no group x time or group x time x time during the run interactions for oxygen consumption, lactate, respiratory exchange ratio, or heart rate ($p>0.05$) (Table 4.3). As expected, there were main effects of time evident during the run ($p<0.05$), with heart rate being significantly lower during the warmup than during the run, $VO_2$ increasing from
warmup to conclusion of the run, and lactate being lowest at rest and highest at the last collection point (i.e., near the end of the run).

4.4.4 Body Composition

One participant from the maltodextrin group did not undergo baseline body composition assessment due to scheduling issues. No interactions were found between group and time for lean body mass, fat mass, or percent fat (Table 4.3; p>0.05). There was an effect of time for lean mass (p=0.009), with post-intervention assessments being significantly greater than those obtained at baseline (46.5 ±5.0 kg vs. 47.3 ±4.5 kg).

4.4.5 Biweekly Check-Ins

Dietary intakes from both regular dietary intake and the supplement are portrayed in Table 4.4. No differences were observed in energy (p=0.55), carbohydrate (p=0.41), or fat intake (p=0.20) throughout the trial. Both protein (p=0.03) and iron (p=0.01) intake were significantly greater in the regular pea group compared to the maltodextrin group. No other differences in dietary intake were observed. Leisure time exercise questionnaire responses did not differ between groups nor across time points (p=0.69; data not shown).
Table 4.3 Outcome measures at baseline and post-intervention for each group

<table>
<thead>
<tr>
<th>Measure</th>
<th>Time</th>
<th>Maltodextrin (n=9)</th>
<th>Low Phytic Acid Pea (n=9)</th>
<th>Regular Pea (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (ug/L)</td>
<td>Pre</td>
<td>31.3±12.2</td>
<td>33.4±19.2</td>
<td>33.4±22.6</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>30.6±19.4</td>
<td>35.1±25.6</td>
<td>38.2±27.5</td>
</tr>
<tr>
<td>CRP(mg/L)</td>
<td>Pre</td>
<td>0.66±0.66</td>
<td>0.49±0.36</td>
<td>0.46±0.15</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>0.67±0.56</td>
<td>0.51±0.24</td>
<td>0.73±0.46</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>Pre</td>
<td>130.8±9.3</td>
<td>132.3±10.8</td>
<td>129.8±9.1</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>131.4±8.3</td>
<td>130.4±10.3</td>
<td>133.4±9.9</td>
</tr>
<tr>
<td>VO2max (mL/kg/min)</td>
<td>Pre</td>
<td>49.7±7.5</td>
<td>53.4±8.4</td>
<td>53.6±8.8</td>
</tr>
<tr>
<td></td>
<td>Post*</td>
<td>46.8±2.7</td>
<td>48.6±4.8</td>
<td>47.0±3.6</td>
</tr>
<tr>
<td>VO2max (L/min)</td>
<td>Pre</td>
<td>3.4±0.9</td>
<td>3.5±0.6</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td></td>
<td>Post*</td>
<td>3.3±0.6</td>
<td>3.2±0.6</td>
<td>2.9±0.4</td>
</tr>
<tr>
<td>TTE (s)</td>
<td>Pre</td>
<td>501.1±128.8</td>
<td>497.1±110.9</td>
<td>561.1±100.9</td>
</tr>
<tr>
<td></td>
<td>Post*</td>
<td>532.7±139.5</td>
<td>543.9±142.1</td>
<td>592.7±111.4</td>
</tr>
<tr>
<td>5 km TT (s)</td>
<td>Pre</td>
<td>1850.8±246.3</td>
<td>1725.7±210.8</td>
<td>1660.4±142.8</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>1848.6±257.6</td>
<td>1722.7±204.8</td>
<td>1651.2±166.7</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>Pre</td>
<td>17.7±5.8</td>
<td>14.8±4.6</td>
<td>16.5±5.3</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>17.7±5.2</td>
<td>15.3±4.9</td>
<td>16.4±3.9</td>
</tr>
<tr>
<td>Lean mass (g)</td>
<td>Pre</td>
<td>48.5±5.4</td>
<td>47.7±4.7</td>
<td>43.1±3.5</td>
</tr>
<tr>
<td></td>
<td>Post*</td>
<td>49.4±4.5</td>
<td>47.9±4.6</td>
<td>44.5±3.3</td>
</tr>
</tbody>
</table>
Values are mean and SD; CRP=C-Reactive Protein; VO\textsubscript{2max}= maximal oxygen consumption; TTE= time to exhaustion during the VO\textsubscript{2max} test; TT= time trial; *Time main effect (values across groups during post-testing different from baseline; p<0.05)

4.4.6 Dietary Intake

Dietary intakes from both regular dietary intake and the supplement are portrayed in Table 4.4. No differences were observed in energy (p=0.55), carbohydrate (p=0.41), or fat intake (p=0.20) throughout the trial. Both protein (p=0.03) and iron (p=0.01) intake were significantly greater in the regular pea group compared to the maltodextrin group, but no differences were evident for either protein (p=0.14) nor iron (p=0.29) when comparing lpa pea and maltodextrin. No other differences were observed.

Table 4.4 Dietary Intake at Baseline and Every Two Weeks During the Trial

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Week 8</th>
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<tbody>
<tr>
<td><strong>Energy (kcal/day)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Maltodextrin</td>
<td>1849±445</td>
<td>2181±320</td>
<td>2194±494</td>
<td>1916±421</td>
<td>2202±453</td>
</tr>
<tr>
<td>Low Phytic Acid</td>
<td>2078±297</td>
<td>1990±272</td>
<td>1966±316</td>
<td>2053±412</td>
<td>2172±599</td>
</tr>
<tr>
<td>Regular Pea</td>
<td>1762±424</td>
<td>2177±317</td>
<td>1839±529</td>
<td>1816±246</td>
<td>2074±584</td>
</tr>
<tr>
<td><strong>Carbohydrates (g/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>202.4±60.3</td>
<td>287.5±60.6</td>
<td>239.0±27.8</td>
<td>236.0±54.0</td>
<td>266.7±70.4</td>
</tr>
<tr>
<td>Low Phytic Acid</td>
<td>245.2±69.4</td>
<td>205.1±58.5</td>
<td>213.0±38.4</td>
<td>232.3±69.9</td>
<td>230.2±95.9</td>
</tr>
<tr>
<td>Regular Pea</td>
<td>200.1±69.8</td>
<td>249.5±74.0</td>
<td>219.5±42.2</td>
<td>215.0±45.0</td>
<td>227.8±85.5</td>
</tr>
<tr>
<td><strong>Protein (g/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Maltodextrin 97.2±41.3  80.7±28.6  90.7±25.8  88.4±32.6  86.0±32.7  
Low Phytic Acid 86.5±20.8  124.8±36.4  104.8±23.4  119.4±24.9  117.4±38.9  
Regular Pea* 64.9±12.5  111.3±25.9  106.3±21.8  125.8±31.1  114.7±31.4  

Fat (g/day)

Maltodextrin 72.9±19.5  64.6±20.2  61.0±26.9  63.1±19.6  80.4±36.5  
Low Phytic Acid 83.2±13.1  86.0±22.0  68.7±23.4  78.8±16.8  71.6±26.7  
Regular Pea 79.9±33.2  63.2±25.5  55.5±25.4  55.4±14.8  71.5±22.8  

Iron (mg/day)

Maltodextrin 12.7±3.8  12.3±4.4  12.6±7.1  11.3±7.3  11.6±2.4  
Low Phytic Acid 13.7±1.8  16.7±2.6  15.0±3.6  16.4±4.4  14.3±4.7  
Regular Pea* 11.2±3.8  18.2±6.6  17.1±5.8  15.2±2.9  16.3±5.3  

Values are means ±SD; Values indicate the regular diet combined with the pea or placebo supplementation; *Simple main effect of condition was evident for protein and iron intake throughout the intervention, with higher intakes in the regular pea compared to the maltodextrin group (p<0.05). No other statistically significant differences were observed.

4.4.7 Adverse Effects

Approximately 40% of participants reported some variety of symptoms they believed to be related to the supplement during the trial, with more symptoms being reported from the pea groups than the maltodextrin group for the first three check-ins. No statistical differences were observed at week two ($\chi^2=2.841$, p=0.24), week six ($\chi^2=3.646$, p=0.16), or week eight ($\chi^2=0.572$, p=0.75) for prevalence of symptoms. A significant difference in the prevalence of symptoms was observed at week four, with the pea conditions experiencing more symptoms than the
maltodextrin group ($\chi^2=6.766, p=0.03$). Zero to two individuals reported symptoms from the maltodextrin group compared to three to six of participants from the pea groups. At the final check-in, the number of participants that reported symptoms were similar between groups. The most common symptom reported was bloating, followed by gassiness. Other reported symptoms included abdominal pain, constipation, heartburn, decreased appetite, nausea, and diarrhea, though these were less common. The prevalence of symptoms was highest at the first check-in and decreased until the third check-in, at which time they remained stable to the end of the trial.

4.5 Discussion

This research is the first of its kind to investigate the role of a plant-based dietary intervention using peas with increased iron bioavailability on iron levels in female runners. No statistical differences in plasma ferritin levels were observed between the groups, though both pea groups experienced a net increase in serum ferritin, whereas the maltodextrin group did not.

The current findings corroborate other literature investigating food-based approaches to ID in athletes. Alaunyte et al. (2014) failed to observe differences in serum ferritin in deficient females consuming 7 mg/day of iron in the form of an iron-fortified bread for six weeks. Similarly, Burke et al. (2012) reported no significant change in plasma ferritin after both male and female cross-country runners consumed 9 oz red meat per week plus a multivitamin supplement. Burke et al. (2012) did, however, report a non-significant increase in hematocrit. Given that Burke et al. (2012) utilised a pharmaceutical supplement plus animal-sourced iron (i.e., heme iron from red meat), the validity of comparison to the current trial is limited, as the absorption of heme and non-heme iron is substantially different (Piskin et al., 2022; Shubham et al., 2020). Anschuetz et al. (2010) also failed to elicit a significant change in iron status after a four-week intervention in
collegiate runners who received dietary counselling to improve iron absorption. Despite lacking statistical significance, the authors noted that individuals who improved the bioavailability of iron in their diet experienced increases in serum iron parameters.

The results of the current study differ from observations by Ishizaki et al. (2006), who found improved serum ferritin in gymnasts after four weeks of consuming diets designed by a dietitian to provide 15 mg of iron per day through a traditional Japanese diet, though benefits disappeared four weeks after stopping the dietary intervention. Differences observed between studies could be due to a myriad of factors, including the type of iron investigated (heme, non-heme, or a combination), as well as iron levels at baseline. It is well-established that absorption of dietary iron is inversely related to iron status in the body (Kalasuramath et al., 2013), primarily due to the actions of hepcidin (Viatte & Vaulont, 2009). The subjects of the current study were not in a state of iron depletion, with average baseline ferritin values (after removing outliers) of 32.7 ug/L. Despite previous reports failing to detect statistical significance, as mentioned above, net increases in serum ferritin have been recorded. Alaunyte et al. (2014) observed a 5.4% increase in serum ferritin over a six-week trial of consuming an iron fortified bread but did not include a control group for comparison. Burke et al. (2012) observed a 15% increase in serum ferritin in the intervention group compared to a 10% increase in the control group, though this research was not a plant-based approach. Future studies investigating plant-based approaches to improve iron status in athletes with larger sample sizes and/or longer intervention periods are warranted, given the positive findings in both Alaunyte et al. (2014) and the current research, the only two studies known to the authors that explore a plant-based approach to increase ferritin levels.

Supplementing with pea protein increased intakes of iron and protein in those consuming the regular pea protein, but not the lpa pea protein. Differences in protein intake between the two pea
groups can be explained by the regular pea group consuming slightly more supplement than the lpa pea group, and therefore more protein daily (63.7 g/day vs 58.8 g/day). However, the two pea powders were matched for iron such that both conditions consumed ~6 mg/d of extra iron through the pea powder daily. The regular pea group did report slightly better compliance to the supplement than the lpa pea group (85.5% vs. 82.6%), which could explain the differences between these two groups. To our knowledge, no research has investigated the feasibility of a pea protein in improving dietary intakes of iron or protein, though research has reported pea protein to perform similarly to whey in inducing muscle hypertrophy and strength (Banaszek et al., 2019; Babault et al., 2015). Pea protein may therefore be an attractive option to those wishing to supplement protein but wishing to limit consumption of animal-based proteins. A pea protein concentrate may be especially attractive to endurance athletes due to the relatively high carbohydrate content (~39% carbohydrate), which would assist athletes in attaining both their protein and carbohydrate goals (Thomas et al., 2016).

Our study is strengthened by measuring C-reactive protein in addition to serum ferritin, allowing us to eliminate inflammation as a confounding factor on ferritin values (Dignass et al., 2018). However, our study was limited by the wide range of ferritin values of participants. Dietary iron intake was used as a proxy of iron status at baseline, assuming those with low dietary iron intakes would be more likely to have low iron stores. However, some participants presented with very high serum ferritin levels at baseline even after outliers were removed. Future research should investigate the role of pea protein, both regular and lpa, on the iron status of individuals with depleted ferritin at baseline. Further, studies on those iron deficient (measured using ferritin status) as opposed to iron inadequate (determined by intake in our study)
would be prudent, as those with sufficient ferritin are unlikely to respond to an intervention aimed at improving ferritin.

While much of the current literature conducted to improve iron status in women of reproductive age utilize pharmaceutical iron supplements, delivering a similar amount of iron through a pea product is likely infeasible. However, peas may be more cost-effective while providing other nutritional benefits and being culturally appropriate in many areas of the world most affected by iron deficiency. Therefore, future research should investigate whether replacing regular peas with *lpa* peas in the diet of those in areas of the world most impacted by ID, especially those in lower-income countries with a high reliance on plant-based diets, may find similar positive trends in iron status and nutrient intake as observed in the current research (Safiri et al., 2019). Such research should consider longer supplementation periods to counteract the lower amount of iron delivered by the peas.

The timing of our testing may also be a limiting factor, as baseline testing happened mostly in October and November, with post-intervention assessments occurring largely in December and January. This may help to explain the main effect of time displaying a decrease in VO$_{2\text{max}}$ values from baseline to post-interventions across groups. The cold climate at the site of the intervention during the winter months (December and January) may have impacted specific training (i.e., running). While physical activity was measured using the Goodin Leisure Time Exercise Questionnaire, it does not account for specific types of activities engaged in. It is possible that participants decreased their frequency of running and increased it with other varieties of exercise during the colder months, which could explain this change.

In conclusion, supplementation with *lpa* peas did not significantly impact ferritin in female runners, though further investigation with tight inclusionary criteria is warranted. Although the
results of the current study are not statistically significant, the groups consuming the regular and lpa pea powders experienced a small positive effect on plasma ferritin while the maltodextrin group did not. Given the current trend toward reducing consumption of animal products (Iwasa-Madge & Wegener, 2020; Meyer et al., 2020), pea protein is a feasible option to help athletes meet their protein goals. Further research, potentially with more participants and a longer study period, is needed to clarify whether pea-based supplements could be a strategy for improving iron status.

**Competing interests**

The authors declare that they have no competing interests

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**Authors' contributions:** Conceptualization: KAS, GAZ, DLL, TDW, PDC; Methodology: KAS, GAZ, DLL, TDW, PDC; Data collection: KAS, JK; Data analysis; KAS, PDC; Manuscript Preparation: KAS; Manuscript Review and Editing: KAS, GAZ, DLL, TDW, PDC; Supervision: TDW, GAZ, PDC; Funding Acquisition: TDW, GAZ, PDC. All authors have read and agreed to the published version of the manuscript.

4.6 References


4.7 Next Steps

In study 2 we observed beneficial effects of pea protein in general on the iron status and intake, along with protein intake, in female runners. While the findings on iron status did not reach statistical significance, a small effect size was observed. Further, pea protein is a feasible way to increase protein intake, especially in groups that may require greater protein intakes, such as para athletes. Para athletes may also be at risk of insufficient micronutrient intake, especially iron, as discussed in chapter two. Therefore, the aim of study three is to quantify the dietary intake in a diverse group of para athletes (i.e., para cyclists) to better understand potential shortcomings in their diet.
Chapter 5- Study 3- Dietary quality and nutrient intakes of elite paracyclists
5.1 Abstract

Nutrient requirements for para athletes will be influenced by a variety of factors secondary to their impairment and, therefore, recommendations for para athletes cannot be drawn directly from that of able-bodied athletes. Information on the dietary intakes of para athletes is lacking and therefore needs to be examined. In this study, nutrient intakes and diet quality of 31 para cyclists were determined via food frequency questionnaires. Based on the dietary reference intakes, most para cyclists consumed intakes above the recommended dietary allowance (RDA) or adequate intake (AI). Recommendations were not met for iodine in males or females (males=87% RDA; females=62% RDA) or fibre in males (84% AI). A 33% risk of inadequacy was noted for iodine in females and 53% in males, while risk of inadequacy was also noted for vitamins D and E in females (26% risk of inadequacy for both vitamin D and vitamin E). Forty-two percent of females and 75% of males did not meet fibre recommendations (14 g/1000 kcal) and only three athletes (all females) consumed fatty acids in the recommended omega 6 to omega 3 ratio of 4:1 or less. Athletes consumed grains, fruits, and vegetables frequently, though whole grains, pulses, and oily fish were generally consumed less often. Para cyclists appear to be consuming intakes at or above recommendations for most nutrients, though several nutrients were consumed below the recommended amounts; therefore, increasing the variety of foods consumed is suggested.

Keywords: Dietary Reference Intake; Paralympic; Fibre
5.2 Introduction

In the last few decades, para sport has experienced a substantial increase in participation (Flueck, 2021). Despite this, sport science has lagged, limiting the opportunity for evidence-based practice in both health and performance for these athletes. While the evidence informing optimal nutritional practices for able-bodied individuals is abundant (Rodriguez et al., 2009; Thomas et al., 2016; Vitale & Getzin, 2019), the same does not exist for athletes with physical or visual impairments (i.e., para athletes). Alterations in the physiological and metabolic functioning of this population compared to their able-bodied peers make research on able-bodied athletes not directly applicable to those with physical impairments (Gee et al., 2021; Shaw et al., 2021a). Physiological and structural alterations such as decreased muscle mass, spasticity, hypertonia, alterations in sweat response, and differences in energy expenditure will impact the nutritional requirements to optimize health and performance (Scarmella et al., 2018).

Current evidence in the area of sport nutrition for athletes with physical impairments is lacking and often restricted to wheelchair athletes (Madden et al., 2017; Shaw et al. 2021a). Of the research available in other types of impairments, evidence is often limited to clinical settings, not always applying to sports performance (Caplan et al., 2018; Kley et al., 2013; Theis et al., 2021). Many Paralympic sports, such as para cycling, include a variety of impairments, with both ambulatory and non-ambulatory individuals being represented (Flueck, 2021). Due to the lack of para-specific guidelines available, athletes and practitioners often turn to guidelines derived from able-bodied individuals or the dietary reference intakes (DRIs) to inform decision making in these individuals (Scarmella et al., 2018).

Current research in the area has attempted to characterize the habitual intake in para athletes, generally finding consumptions in excess of recommendations for most nutrients, though
inadequate intakes are often reported for vitamin D, folate, fibre, and iron (Goosey-Tolfrey & Crosland, 2010; Krempien & Barr, 2011; Madden et al., 2017; Sasaki et al. 2021). Of these commonly reported inadequacies, folate would be important for those with brain and spinal cord injuries, as folic acid has been demonstrated to improve functional recovery and neurodevelopment (Naim et al., 2011), while vitamin D intakes may be particularly vital for those with spinal cord injuries and amputations due to limitations in weight-bearing activities and implications for bone health (Scarmella et al., 2018). Pertaining to athletes specifically, iron is important for oxygen transport and cell proliferation and insufficient amounts are likely to limit performance. Though some research has attempted to quantify the habitual intake in this population, no study to date has outlined the types of foods consumed by athletes in order to assess diet quality and, therefore, how intakes may be improved. Thus, identifying potential inadequacies in different types of para athletes and the food most commonly consumed is important, not only for sport performance, but also for health. Much of the above-cited research has been conducted on athletes from a variety of different sports within the same sample, potentially causing difficulties in drawing conclusions, as requirements may vary greatly across sports. Para cycling is an interesting population of athletes to study, as a variety of impairment types can be studied while controlling for the demands of the sport.

Our previous research outlined how the COVID-19 pandemic impacted the diet of elite para cyclists (Shaw et al., 2021b). Given the paucity of evidence surrounding the habitual dietary intake of athletes with physical impairments, we opted to analyze the baseline data (i.e., outside of the COVID-19 pandemic period) with additional participants to assess the typical intake of this population, both in terms of types of foods consumed and habitual intake of nutrients.
Therefore, the primary objective of the current research was to assess the quality (type) of foods consumed and the energy, macronutrient, and micronutrient intake provided by those foods in a group of elite para cyclists using the DRIs.

5.3 Methods
Para cyclists from English-speaking countries who had competed at the National or International level in the previous 12 months were invited through email or direct message on social media (i.e., Facebook, Instagram, Twitter) to participate in the research. If athletes indicated their interest, a website link was provided to them, which took them to a food frequency questionnaire (FFQ) administered using the online platform SurveyMonkey (San Mateo, USA). The first page of this questionnaire hosted the consent form for the study. Consent was implied by the participants clicking “continue” to progress to the questions pertaining to their diet. Athletes who were injured or not actively training for any reason were excluded from the research. Ethical approval was obtained from the University of Saskatchewan Research Ethics Board (BIO 3312).

5.3.1 Data Collection
Dietary intake was assessed using the Canadian Diet History Questionnaire II (CDHQII; Csizmadi et al., 2007). This is a 165-item questionnaire that collects dietary history from the past month including food, beverages, and dietary supplements consumed. Participants were presented with food items and asked how often they consumed that particular item in the past month (never, once per month, 2-3 times per month, once per week, 2-3 times per week, 4-5 times per week, once per day, 2+ times per day) as well as how much was consumed each time. This questionnaire was chosen because it has been found to be valid for assessing dietary intake
with no differences being observed between it and a 24-hour food recall (Horne et al., 2020). Our research team has used this FFQ in past studies to capture habitual intake (Bertrand et al., 2021; Shaw et al., 2022). Data from the FFQ were analyzed using the Food Processor Software (ESHA Research, Version 11.1, Salem, Orlando, USA), which provided an estimate of energy, macronutrients, and micronutrients.

The quality of dietary intake was assessed by quantifying the frequency of consumption of key food groups and subcategories within those groups. Specifically, we looked at how many times per day participants consumed the following foods: fruits (fresh, frozen, dried, canned); vegetables; grain products (bagels, bread, rice, pasta); meat alternatives (tofu, soy meat substitutes, pulse-based soups, baked beans, cooked dried beans); nuts and seeds; eggs (white only and whole); meat and fish; and dairy and dairy alternatives (yogurt, cheese, plant-based milk alternatives). We also considered the proportion of these intakes that came from whole grain sources (for grains), processed meat and fish, lean unprocessed meat, oily fish (i.e., fresh tuna, salmon, mackerel), low or non-fat dairy, high protein milk alternatives (soy, pea) and low-protein plant-based milk alternatives (nut milks, rice milk, etc.).

5.3.2 Statistics
Data were assessed using JASP statistical software, version 0.10.2 (2013-2019, University of Amsterdam). Normality was assessed using the Shapiro Wilks test. Due to a significant Shapiro Wilks test, sex differences were assessed using the Mann-Whitney U non-parametric test for alcohol, protein, fibre, iodine, omega-3 fatty acids, omega-6 fatty acids, vitamins B6, B12, C, D, E, and K, folate, selenium, and caffeine. Sex differences for all other nutrients were assessed using an independent samples t-test. Dietary intake was assessed by comparing reported intakes
to the DRIs (Institute of Medicine, 2002). The risk of inadequacy for nutrients with an estimated
average requirement (EAR) was assessed using the EAR cut-point method (Institute of
Medicine, 2001; Krempien & Barr, 2011). For iron, as requirements in women of reproductive
age are not normally distributed, the probability approach was used (Institute of Medicine, 2001).
For nutrients with an adequate intake (AI) rather than a recommended dietary allowance (RDA),
if the median of the group was above the adequate intake, it was assumed that the group’s usual
intake was adequate. Intake of nutrients for males and females as a group were also compared to
the RDA by dividing absolute intake by the RDA for their respective age group and sex and
multiplying by 100 to get a percentage intake relative to the RDA. In situations where a nutrient
does not have an RDA, intakes were compared to the AI. For macronutrients, if intakes fell
within the acceptable macronutrient distribution ranges (carbohydrate: 45%-65%, protein: 10%-35%,
fat: 20%-35% of daily caloric intake), intake was assumed to be sufficient for health.
Individual data for nutrients with a tolerable upper intake level (UL) (vitamin A, vitamin C,
vitamin D, vitamin E, vitamin B₃, vitamin B₆, folate, calcium, iodine, iron, magnesium,
phosphorus, selenium, and zinc) were assessed to gauge the risk of toxicity. Differences in
dietary intake with and without supplementation were assessed using a dependent samples t-test.
Differences in impairment (identified by para cycling class: C=cyclist; H=handcyclist;
T=tricyclist; B=visually impaired cyclist) were characterized, though due to limited sample size
in some classes statistical comparisons were not made. Significance was accepted at p<0.05. All
data are presented as means ± standard deviation.

5.4 Results
Thirty-four individuals consented to the research and began the FFQ. Three individuals (all males) did not complete the FFQ, thus, 31 para cyclists were included in analysis (n=19 females, n=12 males). Twenty-eight of these individuals were classified as “elite” (i.e., race for and supported by their national body) while three were sub-elite (i.e., competed at the National level but not on the national team). Participant sport class is displayed in Table 5.1.

Table 5.1 Classification of Participants (n=31)

<table>
<thead>
<tr>
<th>Bike Class</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C2</td>
<td>1</td>
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</tr>
<tr>
<td>C3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>C4</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>C5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>T1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>T2</td>
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<td>4</td>
</tr>
<tr>
<td>H1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>H2</td>
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<td>0</td>
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<td>H3</td>
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<td>0</td>
</tr>
<tr>
<td>H4</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*H= handbike; T= tricycle; C= bicycle; B= tandem. Lower numbers (ex., C1) indicate greater degree of impairment*
5.4.1 Diet Quality

Daily frequency of consumption for food items of interest is presented in Table 5.2. Generally, grains, fruits, and vegetables were consumed frequently (3.5, 2.9, and 4.2 times per day, respectively), though the proportion of grains that came from whole sources was low (18%). Meat alternatives (pulses) and nuts and seeds were consumed less frequently (0.31 and 0.69 times per day, respectively). Dairy and dairy alternatives were consumed 1.75 times per day, with approximately half of all dairy coming from low or non-fat dairy (53%) and 11% coming from low-protein milk alternatives (i.e., almond milk, rice milk).

Table 5.2 Daily intake of food groups and types

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Frequency (per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains</td>
<td>3.5</td>
</tr>
<tr>
<td>Of which is whole grains</td>
<td>0.63</td>
</tr>
<tr>
<td>Meat Alternatives</td>
<td>0.31</td>
</tr>
<tr>
<td>Vegetables</td>
<td>4.20</td>
</tr>
<tr>
<td>Fruit</td>
<td>2.85</td>
</tr>
<tr>
<td>Nuts and Seeds</td>
<td>0.69</td>
</tr>
<tr>
<td>Meat and fish</td>
<td>2.12</td>
</tr>
<tr>
<td>Of which is lean unprocessed*</td>
<td>0.98</td>
</tr>
<tr>
<td>Of which is processed</td>
<td>0.31</td>
</tr>
<tr>
<td>Of which is oily fish</td>
<td>0.16</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.54</td>
</tr>
<tr>
<td>Dairy and Dairy Alternatives</td>
<td>1.75</td>
</tr>
<tr>
<td>Of which is low or non fat dairy</td>
<td>0.92</td>
</tr>
<tr>
<td>Of which is soy milk</td>
<td>0.02</td>
</tr>
</tbody>
</table>
5.4.2 Macronutrient and Energy Intake

Consumption of all macronutrients was aligned with acceptable macronutrient distribution ranges (Table 5.3). Males consumed significantly more total fat (p=0.048), and saturated fat (p=0.01) compared to females, with a trend for increased energy (p=0.06). Both males and females consumed less than 10% of total daily energy from saturated fat, as recommended by the (World Health Organization, 2023). Macronutrient by sport class is displayed in Table 5.4. Tandem athletes consumed more energy and greater amounts of all macronutrients compared to the other classifications. As a percentage of total intake, consumption of the different macronutrients was comparable across all classifications. Consumption of macronutrients and energy were comparable between cyclists, handcyclists, and tricyclists, though cyclists appear to have consumed more fibre (33.4±16.1 g) compared to handcyclists and tricyclists (28.0±6.6 g and 28.7±12.2 g, respectively).

When considering the AI for fibre (38 g/day for males, 25 g/day for females), adequate fibre was consumed by females (33 g/day) but not males (29 g/day) (Figure 5.1). When fibre intake was assessed based on relative recommendations (i.e., relative to caloric intake), 42% of females (n=8) and 75% of males (n=9) did not meet recommendations of 14 g/1000 kcal (Institute of Medicine, 2002).
Males consumed an average of 19.3±7.8 g and 2.1±0.9 g of omega-6 and omega-3 fatty acids, respectively, while females consumed an average of 15.7±8.3 g and 2.1±1.2 g of omega-6 and omega-3 fatty acids, respectively. These intakes exceed the AI values of 17 g/day and 1.6 g/day for intakes of omega-6 and omega-3 fatty acids in males and 12 g/day and 1.1 g/day for intakes of omega-6 and omega-3 fatty acids in females (Institute of Medicine, 2002). Notably, only 3 athletes (all females) consumed omega 6 to omega 3 fatty acids in a ratio of 4:1 or less, as recommended (Mariamenatu & Abdu, 2021). The ratio of omega-6 to omega-3 was not different between sexes (p=0.51).

5.4.3 Micronutrient Intake
Dietary intake by sex, including supplements, is displayed in Table 5.3. Males consumed significantly more selenium compared to females (p=0.04). No other differences were evident between sexes. A high risk of inadequacy was observed in both males and females for iodine (males 33% risk; females 53% risk). Females exhibited a high risk of inadequacy for vitamin D (26%) and vitamin E (26%), while males did not (Figure 5.2). Micronutrient intake appears to be relatively similar between sport classes, with tandem athletes consuming greater amounts of most micronutrients compared to other sport classes, except for vitamins E, C, B1, B2, B6, and B12 (Table 5.4).
Table 5.3 Daily Dietary Intake of Para Cyclists by Sex

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Males (n=12)</th>
<th>Females (n=19)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2563 ± 946</td>
<td>2015 ± 631</td>
<td>0.06</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>308±116</td>
<td>248±87</td>
<td>0.10</td>
</tr>
<tr>
<td>Carbohydrate (% kcal)</td>
<td>48.0±2</td>
<td>50.0±6.5</td>
<td>0.50</td>
</tr>
<tr>
<td>Total fibre (g)</td>
<td>29.5±9.8</td>
<td>33.1±16.1</td>
<td>0.92</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>132±59</td>
<td>109±51</td>
<td>0.15</td>
</tr>
<tr>
<td>Protein (% kcal)</td>
<td>20.1±4.3</td>
<td>22.1±5.6</td>
<td>0.32</td>
</tr>
<tr>
<td>Total Fat intake (g)</td>
<td>90±36</td>
<td>68±25</td>
<td>0.048</td>
</tr>
<tr>
<td>Total Fat intake (% kcal)</td>
<td>32.4±9.0</td>
<td>28.9±3.7</td>
<td>0.15</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>26±12</td>
<td>17±6</td>
<td>0.01</td>
</tr>
<tr>
<td>Saturated fat (% kcal)</td>
<td>8.4±2.1</td>
<td>7.9±1.8</td>
<td>0.52</td>
</tr>
<tr>
<td>Monounsaturated fat (g)</td>
<td>32±13</td>
<td>24±12</td>
<td>0.10</td>
</tr>
<tr>
<td>Polyunsaturated fat (g)</td>
<td>23±9</td>
<td>19±9</td>
<td>0.27</td>
</tr>
<tr>
<td>Omega-3 Fatty Acid (g)</td>
<td>2.1±0.9</td>
<td>2.1±1.2</td>
<td>0.64</td>
</tr>
<tr>
<td>Omega-6 Fatty Acid (g)</td>
<td>19.3±7.8</td>
<td>15.7±8.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Omega 6/omega 3 ratio</td>
<td>8.7±3.0</td>
<td>8.0±2.8</td>
<td>0.51</td>
</tr>
<tr>
<td>Vitamin A (µg RAE)</td>
<td>2132±519</td>
<td>2818±2262</td>
<td>0.31</td>
</tr>
<tr>
<td>Vitamin B1 (mg)</td>
<td>7.7±14.0</td>
<td>13.4±21.7</td>
<td>0.42</td>
</tr>
<tr>
<td>Vitamin B2 (mg)</td>
<td>6.1±3.8</td>
<td>7.1±7.2</td>
<td>0.71</td>
</tr>
<tr>
<td>Vitamin B3 (mg)</td>
<td>51.5±22.0</td>
<td>40.8±17.8</td>
<td>0.15</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>18.4±43.4</td>
<td>51.0±79.7</td>
<td>0.80</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>726±209</td>
<td>727±405</td>
<td>0.56</td>
</tr>
<tr>
<td>Vitamin B12 (µg)</td>
<td>96.0±286.6</td>
<td>272.8±469.4</td>
<td>0.86</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>333±430</td>
<td>383±430</td>
<td>0.98</td>
</tr>
<tr>
<td>Vitamin D (µg)</td>
<td>28.3±14.9</td>
<td>19.6±12.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>46±74</td>
<td>24±16</td>
<td>0.27</td>
</tr>
<tr>
<td>Vitamin K (µg)</td>
<td>513±279</td>
<td>811±814</td>
<td>0.89</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1558±474</td>
<td>1590±608</td>
<td>0.88</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>589±138</td>
<td>583±209</td>
<td>0.93</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>2070±798</td>
<td>1830±732</td>
<td>0.40</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>4033±1632</td>
<td>3980±1480</td>
<td>0.93</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>4410±1511</td>
<td>3635±1348</td>
<td>0.15</td>
</tr>
<tr>
<td>Iodine (µg)</td>
<td>130±60</td>
<td>93±71</td>
<td>0.10</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>26.8±8.0</td>
<td>29.1±11.9</td>
<td>0.57</td>
</tr>
<tr>
<td>Selenium (µg)</td>
<td>213±70</td>
<td>161±67</td>
<td>0.041</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>25.6±17.7</td>
<td>29.6±24.9</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Intakes include consumption from both food and supplements. RAE=retinol activity equivalent
Table 5.4 Daily Dietary Intake of Para Cyclists by Sport Class

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Handbike (n=4)</th>
<th>Bicycle (n=17)</th>
<th>Tandem (n=3)</th>
<th>Tricycle (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2143±694</td>
<td>2185±809</td>
<td>3003±384</td>
<td>2063±922</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>242±68</td>
<td>273±116</td>
<td>333±34</td>
<td>260±103</td>
</tr>
<tr>
<td>Carbohydrate (% kcal)</td>
<td>45.6±5.5</td>
<td>49.4±7.9</td>
<td>44.5±2.7</td>
<td>52.8±8.3</td>
</tr>
<tr>
<td>Total Fibre (g)</td>
<td>28.0±6.6</td>
<td>33.4±16.1</td>
<td>43.1±15.9</td>
<td>28.7±12.2</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>114±50</td>
<td>117±50</td>
<td>191±81</td>
<td>103±51</td>
</tr>
<tr>
<td>Protein (% kcal)</td>
<td>20.8±3.2</td>
<td>21.8±5.7</td>
<td>24.9±7.7</td>
<td>19.1±3.2</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>81±27</td>
<td>73±31</td>
<td>106±11</td>
<td>68±36</td>
</tr>
<tr>
<td>Total Fat (% kcal)</td>
<td>34.1±3.4</td>
<td>30.1±7.5</td>
<td>32.4±6.9</td>
<td>28.3±5.6</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>22±8</td>
<td>19±9</td>
<td>27±4</td>
<td>20±13</td>
</tr>
<tr>
<td>Saturated fat (% kcal)</td>
<td>9.1±1.3</td>
<td>7.9±2.0</td>
<td>8.1±1.7</td>
<td>8.2±2.2</td>
</tr>
<tr>
<td>Monounsaturated fat (g)</td>
<td>29±12</td>
<td>26±13</td>
<td>39±7</td>
<td>23±12</td>
</tr>
<tr>
<td>Polyunsaturated fat (g)</td>
<td>22±6</td>
<td>20±10</td>
<td>29±5</td>
<td>17±8</td>
</tr>
<tr>
<td>Omega-3 Fatty Acid (g)</td>
<td>2.5±0.9</td>
<td>2.1±1.2</td>
<td>3.5±1.5</td>
<td>1.8±0.9</td>
</tr>
<tr>
<td>Omega-6 Fatty Acid (g)</td>
<td>17.5±6.4</td>
<td>16.8±9.2</td>
<td>23.5±5.8</td>
<td>14.6±6.7</td>
</tr>
<tr>
<td>Omega 6/omega 3 ratio</td>
<td>7.7±3.7</td>
<td>8.5±2.4</td>
<td>8.3±5.9</td>
<td>7.9±1.7</td>
</tr>
<tr>
<td>Vitamin A (µg RAE)</td>
<td>1669±606</td>
<td>2840±2263</td>
<td>3676±1606</td>
<td>2293±1315</td>
</tr>
<tr>
<td>Vitamin B1 (mg)</td>
<td>15.0±24.6</td>
<td>12.1±20.0</td>
<td>4.2±1.0</td>
<td>9.9±19.8</td>
</tr>
<tr>
<td>Vitamin B2 (mg)</td>
<td>7.4±6.8</td>
<td>7.1±6.2</td>
<td>5.9±1.4</td>
<td>5.9±7.3</td>
</tr>
<tr>
<td>Vitamin B3 (mg)</td>
<td>39.1±19.3</td>
<td>44.0±18.0</td>
<td>71.2±22.0</td>
<td>39.3±19.0</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>41.3±75.5</td>
<td>38.7±74.2</td>
<td>9.8±4.1</td>
<td>48.2±75.8</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>654±145</td>
<td>709±383</td>
<td>984±298</td>
<td>771±399</td>
</tr>
<tr>
<td>Vitamin B12 (µg)</td>
<td>322.0±621.9</td>
<td>230.9±423.7</td>
<td>16.6±3.6</td>
<td>153.2±382.1</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>155±66</td>
<td>309±386</td>
<td>517±455</td>
<td>675±539</td>
</tr>
<tr>
<td>Vitamin D (µg)</td>
<td>31.2±7.0</td>
<td>18.5±11.7</td>
<td>34.6±15.1</td>
<td>24.0±17.6</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>23±9</td>
<td>38±64</td>
<td>34±13</td>
<td>26±16</td>
</tr>
<tr>
<td>Vitamin K (µg)</td>
<td>460±175</td>
<td>751±745</td>
<td>1263±898</td>
<td>636±594</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1633±342</td>
<td>1594±509</td>
<td>1924±586</td>
<td>1430±757</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>511±150</td>
<td>581±180</td>
<td>829±117</td>
<td>564±220</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>1662±535</td>
<td>1974±694</td>
<td>2996±916</td>
<td>1637±797</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>3571±1251</td>
<td>4068±1430</td>
<td>5827±2184</td>
<td>3746±1654</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>3778±1199</td>
<td>3863±1483</td>
<td>5309±1531</td>
<td>3695±1441</td>
</tr>
<tr>
<td>Iodine (µg)</td>
<td>79±61</td>
<td>108±66</td>
<td>143±91</td>
<td>109±80</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>22.4±2.0</td>
<td>31.4±11.6</td>
<td>32.5±4.9</td>
<td>23.2±10.7</td>
</tr>
<tr>
<td>Selenium (µg)</td>
<td>161±52</td>
<td>183±71</td>
<td>265±68</td>
<td>157±74</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>28.9±20.7</td>
<td>26.5±20.7</td>
<td>41.8±32.9</td>
<td>25.4±25.0</td>
</tr>
</tbody>
</table>

Intakes include consumption from both food and supplements; RAE = retinol activity equivalent
When group averages were compared to the recommended daily intake (RDA or AI), most micronutrients were consumed in excess with the exception of iodine in both males and females (Figure 5.1). When considered as a group average, no nutrients were consumed in excess of the UL. However, when assessed individually, intakes greater than the UL were consumed by five individuals for vitamin B₃ (1 male, 4 females), six individuals for vitamin B₆ (1 male, 5 females), three individuals for calcium (all females), two individuals for iron (both females), and six individuals for zinc (1 male, 5 females). The average consumption over the UL was minimal for calcium (1%), moderate for iron (12%) but rather substantial for vitamin B₃ (237% above UL), B₆ (72% above UL), and zinc (75% above UL).

![Figure 5.1](image-url)

**Figure 5.1.** Intakes from food and supplements of male and female para cyclists expressed as a percent of daily recommended intake. Dashed line represents recommended intake. Nutrients with * indicate reference values are adequate intake. Reference values for all other nutrients are
recommended dietary allowance. Female intakes of vitamin B1 and male and female intakes of vitamin B6 and B12 exceed 1000% of recommended intake. RAE= retinol activity equivalents.

**Figure 5.2** Estimated prevalence of inadequacy (%) based on EAR cut-point method for nutrients from food only (FO) and food plus supplement intake (F+S) in male and female para
cyclists. RAE= retinol activity equivalents. Where no bars are present, risk of inadequacy was below determinable.

5.4.4 Supplement Use

The use of supplements was reported by 27 (87%) athletes. The most commonly used supplements were vitamin D (n=16), vitamin C (n=14), Multivitamin and mineral (n=12), probiotics (n=10), and omega-3/fish oil (n=10). Other reported supplements included iron (n=8), magnesium (n=7), sport supplements (n=6) (our questionnaire did not specify which sport supplements), calcium (n=6), zinc (n=6), vitamin B complex (n=5), glucosamine (n=5), vitamin B12 (n=4), coenzyme Q10 (n=2), echinacea (n=2), garlic (n=2), ginger (n=2), vitamin E (n=1), and folic acid (n=1). Supplement consumption significantly increased micronutrient intakes for vitamin B1 (p=0.01), vitamin B2 (p=0.002), vitamin B12 (p=0.01), vitamin C (p=0.001), vitamin D (p<0.001), folate (p=0.005), iodine (p<0.001), iron (p=0.002), and zinc (p=0.001). The risk of inadequacy was slightly increased (<10% increase in risk) when considering food only compared to when considering food and supplements in males for vitamin B1, vitamin B2, vitamin B6, vitamin E, iron, and selenium and in females for folate and vitamin C. Both sexes had a slight increase in risk of inadequacy (<10% increase in risk) for vitamin B12, magnesium, and zinc when supplements were not considered. A notable increase in the risk of inadequacy (>10% increase in risk) was observed for vitamin C and folate in males (25% and 17% increase in risk, respectively) and vitamin E for females (16% increase in risk). Both sexes had a notable increase in risk (>10%) for vitamin D (males 83% increase in risk, females 57% increase in risk) and iodine (50% increase in risk for males, 37% increase in risk for females) when considering food only (Figure 5.2). Of those who consumed over the UL for the above-noted nutrients, all
supplemented with the nutrients they consumed in excess except one female for each vitamin B6 and calcium, for which intakes came from diet only.

5.4.5 Caffeine Intake
No differences were evident between males and females for caffeine intake (p=0.25). Males consumed an average of 179±245 mg/day, while females consumed 174±99 mg/day.

5.4.6 Alcohol Intake
Thirteen individuals reported consuming no alcohol (n= 8 females, 5 males). There were no differences between males and females, with males consuming an average of 1.6±1.9g of alcohol per day and females consuming 2.1±3.3g per day (p=1.0). When only considering those who consume alcohol (i.e., removing the zero data points), males consumed 2.8±1.7g per day and females consumed 3.6±3.7g per day (p=0.55).

5.5 Discussion
This study characterized the dietary intakes of elite para cyclists and assessed intakes against the DRIs. We found that males and females largely met or exceeded the RDA for most nutrients with few exceptions. Athletes consumed fruits, vegetables, and grains frequently, with consumption of whole grains and meat alternatives being quite low. The intakes of fruit and vegetables consumed by athletes in this study are generally in line with recommendations of two servings of fruit and three servings of vegetables daily, which has been found to be associated with lower mortality. However, intakes of whole grain products appear to be well below recommendations from around the world (van der Kamp et al. 2021). Intakes of legumes appear to be similar in the current sample compared to the general population, as Miller and colleagues
(2017) found legume consumption to be 0.4 times per day. The intake reported in the current research (0.31 times per day) is lower than most recommendations globally, where the consumption of legumes is recommended daily (Hughes et al., 2022). However, dairy intake appears to be generally in line with global recommendations in which most countries recommend daily intake (Comerford et al., 2021). Of those who chose plant-based milk alternatives, very little was from high protein milk alternatives (i.e., soy, pea milk). Soy and pea milks are generally considered to be nutritionally similar to cow’s milk due to the high protein content, (cow’s milk= 8 g/240 mL; soy milk= 7 g/240 mL), while other plant-based milk alternatives (i.e., almond and other nut milks, rice milk, coconut milk) have 0-1 g/ 240mL (Schuster et al., 2018). This may be an important consideration for athletes who are aiming to replace dairy milk with a plant-based alternative while getting the same nutritional benefits.

5.5.1 Energy and Macronutrient Intake

No statistical difference was observed between sexes in relation to energy intake, although males had an increased intake of energy compared to their female counterparts approaching significance (p=0.06). Similarly, the intakes of carbohydrates and proteins were greater in males than females, though not significantly so. Males did, however, consume significantly more total fat and saturated fat, though when expressed relative to total caloric intake, a significant difference was no longer evident. Differences in energy intake between sexes are expected, as well as the large range of intake in athletes with impairments (Goosey-Tolfrey & Crosland, 2010; Grams et al., 2016; Madden et al., 2017). The variability in intakes is similar to that seen in able-bodied cyclists (Coo & Dobbin, 2022) and supports the need for individualized recommendations in this population. Intakes of each macronutrient as a percentage of total
caloric intake fall within the acceptable macronutrient distribution ranges, suggesting that the proportion of energy from each macronutrient is likely sufficient for the maintenance of health. However, carbohydrate intakes for both males and females were at the low end of the acceptable macronutrient distribution range (45-65% daily caloric intake). As athletes, in particular endurance athletes, require higher carbohydrate intakes to support training and recovery, greater carbohydrate intakes are likely required to fuel optimal performance (Thomas et al., 2016). Athletes and support staff should monitor measures such as body weight and training tolerance to assess the suitability of total energy intake and encourage adequate carbohydrate intake to optimize health and performance.

The current research reported no differences in carbohydrate or protein intake between males and females, although males consumed more total and saturated fat than females. Our results are similar to those observed by Gerrish et al (2017), who found no statistical differences between males and females in terms of macronutrient intake in athletes with spinal cord injury, as well as no differences in energy intake. Our results differ from those observed by Goosey-Tolfrey & Crosland (2010) and Madden et al. (2017) who reported females consumed lower intakes of protein and carbohydrates, respectively. Comparing the current research to those mentioned above should be done with caution, as Gerrish et al. (2017) and Goosey-Tolfrey and Crosland (2010) studied wheelchair athletes, while our research included only four wheelchair athletes (i.e., handcyclists). Participants studied by Madden et al. (2017) were not adequately described to determine the level of impairments. The majority of respondents in the current research were ambulatory (i.e., cyclists), making direct comparisons with previous research difficult.
The results of this research are particularly unique, as we have characterised the omega 3 and 6 intakes of the athletes. While much research has assessed the health impacts of omega-3 fatty acids (Djuricic et al., 2021; Shahidi et al., 2018), looking at both of these fatty acids together may provide more insight into potential health implications rather than looking at either omega-3 or omega-6 fatty acids independently. Reducing the omega-6 to omega-3 ratio in the diet reduces cardiovascular disease, inflammation, and overall mortality, with ratios of 4:1 (omega-6:omega-3) or less being considered optimal (Mariamenatu & Abdu, 2021; Simopoulos). Ratios of 15:1 or 20:1 are frequently described in a typical Western diet (Mariamenatu & Abdu, 2021). Males and females in our sample had ratios of 8.7:1 and 8.0:1, respectively. While this is a marked improvement compared to findings in the general population, further improvements in health may be gained by reducing this ratio. Our research found intakes of fatty fish to be 0.16 times per day, a frequency which aligns with approximately one time per week. Most countries recommend eating fatty fish at least twice a week (de Roos et al., 2017). A para athlete population in particular may benefit from including more sources of omega-3 fatty acids in their diet such as fatty fish, walnuts, flaxseeds, and soybeans to improve this ratio, which may reduce inflammation and oxidative stress, states which are common in those with chronic conditions (Shaw et al., 2021a).

The insufficient intake of fibre observed in the current research (42% of females and 75% of males consuming less than 14 g/1000 kcal) is in line with the findings of Eskici and Ersoy (2021) as well as Krempien and Barr (2011). While athletes may opt for foods lower in fibre to decrease feelings of satiety and meet their energy needs (Thomas et al., 2016), sufficient intakes of fibre are still important to regulate fecal bulk (Institute of Medicine, 2001) and to limit the risk of hypercholesterolemia and metabolic conditions (Kazemi et al., 2018; Zello, 2006). Decreasing
the risk for hypercholesteremia and metabolic conditions may be important for this population given the increased age of many para athletes, and potentially decreased muscle activation during regular activities in non-ambulatory athletes (Chilibeck & Guertin, 2017). This may also be important for athletes with spinal cord injuries to reduce instances of constipation (Khalil et al., 2013; Sezer et al. 2015), although excessive intake of fibre may exacerbate symptoms and thus intakes should be adjusted as necessary to achieve desired bowel function (Khalil et al., 2013). Intakes between 18-31g per day of fibre have been recommended for those with spinal cord injury (Perret & Stoffel-Kurt, 2011), which is lower than the current DRIs for males (38 g/day) but in line with DRIs for females (25 g/day). Our research found consumption of whole grain products to be quite low (approximately four times per week). Increasing the proportion of grain products from whole grain sources may help to increase the fibre intake in this population. Our research also found consumption of pulses (e.g., lentils, chickpeas, beans, yellow peas) and nuts and seeds to be quite low (~2 times per week and 4 times per week, respectively). Increasing consumption of this type of food will also assist in meeting fibre recommendations.

5.5.2 Micronutrients

Given the lack of specific recommendations for an athletic population or for those with physical impairments, the intakes of the current sample have been assessed compared to the DRIs (Institute of Medicine, 2001). Our results are similar to much of the literature, observing intakes meeting or exceeding recommendations in this population for most micronutrients (Goosey-Tolfrey & Crosland, 2010; Grams et al., 2016; Madden et al., 2017; Potvin et al., 1996; Sasaki et al. 2021). Though recommendations for specific micronutrient intakes in para athletes are not known, exceeding the DRIs values is probably beneficial as the stressing of metabolic pathways
induced by exercise may increase the requirements for some micronutrients (Thomas et al., 2016). No nutrient was consumed in excess of the UL at the group level, though some individuals consumed more than the UL for vitamin B3, vitamin B6, calcium, iron, and zinc. Of those who consumed in excess of the UL, most excess intakes were achieved through supplementation. Consuming nutrient intakes above the UL has no known benefit and increases the risk for adverse effects (Barr, 2006). Athletes should be aware of their general nutrient intake to not exceed the UL for specific nutrients, and only supplement when necessary as advised by nutrition professionals.

The results of the current study differ from much of the published literature, which has found low intakes of vitamin D in a para athlete population (Goosey-Tolfrey & Crosland, 2010; Gordon et al., 2022; Krempien and Barr, 2011; Madden et al., 2017; Sasaki et al., 2021). In our current sample of elite para cyclists, the risk of inadequacy for vitamin D intake was quite high when considering levels from food only but was largely corrected through supplementation. Some of this research (Gordon et al., 2022; Krempien & Barr, 2011; Sasaki et al., 2021) specified that supplements were considered in their dietary analysis, though it is unclear whether supplements were considered in the reporting of dietary intake by Madden et al. (2017) and Goosey-Tolfrey and Crosland (2010). Our results also differ from much of the literature concerning intakes of calcium and magnesium, as Krempien and Barr (2011 and Sasaki et al., 2021) have reported inadequate intakes relative to guidelines. Calcium and magnesium may be especially important for athletes with physical disabilities, as deficiencies may lead to a decline in neuromuscular function and impair sport performance (Doubelt et al, 2015). Differences across studies could be related to the method of collection (food records vs. FFQ) the sports studied, or the population studied (ambulatory vs non-ambulatory athletes; type of impairment).
Although the majority of nutrients were consumed in excess of the DRIs, intakes of iodine were below recommendations in both males and females. Iodine is an essential component of thyroid hormones, which are particularly important for myelination in the central nervous system (Institute of Medicine 2001). Optimizing iodine may therefore be of particular interest in para athletes with conditions in which myelination is compromised such as multiple sclerosis and Charcot-Marie-Tooth disease. For any athlete, able-bodied or para, iodine is important to optimize thyroid function and metabolism (Rogerson et al., 2017), and thus is important for maintaining the energy levels required for training and recovery. Given that iodine is consumed through iodized table salt, which was not directly measured in the FFQ used in the current research, it is possible that intakes of iodine were underestimated. Other sources of iodine (besides iodized table salt) include seaweed, shellfish, eggs, and dairy.

5.5.3 Alcohol and Caffeine

Evidence suggests alcohol to be deleterious pre- and during training due to impacts on metabolism, thermoregulation, and skills/concentration. As such, the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine recommend athletes consider public health guidelines around alcohol consumption and consider minimizing or avoiding alcohol consumption (Thomas et al., 2016). While public health policies vary from country to country, the World Health Organization reports that alcohol is responsible for three million deaths annually, with no safe consumption level (World Health Organization, 2020). In Canada, public health now recommends reducing alcohol intake, regardless of your current habits, and limiting intake to two standard drinks per week to minimize health risks (Paradis et al., 2023). The athletes in the current sample reported consuming ~2 g of alcohol per day (3 g per
day if not including those who abstain), which is equivalent to approximately one to 1.5 drinks per week.

Caffeine intake has been studied widely in an athletic context for its ergogenic effect on a variety of types of exercise performance (Barreto et al., 2022; Guest et al., 2021; Naulleau et al., 2022). Health Canada has suggested 400 mg daily to be an amount well tolerated by most individuals (Health Canada, 2022). Caffeine intake in the current sample was reported at 176 mg per day, well below the recommended upper limit. The current questionnaire, however, did not take into account race day habits, during which caffeine intake may increase substantially.

5.5.4 Strengths and Limitations

Our study is strengthened by studying a single subgroup of para athletes (i.e., para cyclists), therefore controlling for the demands of the sport. Further, our study assessed the intake of specific polyunsaturated fatty acids, which is a novel finding unique to our research. However, our study is limited by the distribution of athletes from the different sport classes (C, H, T, and B). Of the 31 participants included, over half were cyclists (n=17), with significantly fewer tandem riders (n=3), handcyclists (n=4), and tricyclists (n=7). Therefore, making comparisons between classes (and different severities/types of impairment) is difficult. The FFQ used relies on recalling the consumption of various foods over a period of time which could result in recall error. However, Labonté et al. (2012) have found this questionnaire to be both valid and reproducible at both the individual and group levels. We did not collect body weight data of the participants in this study, so we cannot characterize macronutrient intake relative to body mass, which is how current sport nutrition guidelines are reported. Even with these limitations, any dietary information in this population is novel.
5.5.5 Conclusion

The current research has provided information on the dietary intake and diet quality of elite para cyclists, which will be useful to coaches and healthcare providers of the athletes to help them optimize their nutrition to maximize performance. Future studies should investigate how intakes differ between different types and degrees of impairment and work toward generating dietary recommendations for this population.

Author Contributions: The study was designed by KAS, PDC, and GAZ; the data were collected by KAS; the data were analyzed by KAS; the data interpretation and manuscript preparation were under-taken by KAS, GAZ and PDC. All authors approved the final version of the paper.

5.6 References


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https://doi.org/10.1016/j.nut.2020.110992


https://doi.org/10.3390/nu11061289


http://www.ncbi.nlm.nih.gov/books/NBK593397/
5.7 Next Steps

In study three, we observed intakes meeting or exceeding recommendations for most athletes; however, fibre intake was highlighted as an area in which intakes may not meet requirements for all athletes. Further, while adequacy of macronutrient intake cannot be determined from study three due to the lack of participant body weight measures, the gross carbohydrate intake appears to be rather low for elite athletes participating in an aerobic sport such as road cycling.

Research suggests para athletes, particularly those with SCI, may require similar or higher protein intakes compared to able-bodied individuals in order to stimulate muscle protein synthesis and manage concerns such as pressure ulcers, but likely require less total energy intake due to less active tissue (Flueck & Parnell, 2021). Taken together, our pea protein concentrate may be beneficial for those with spinal cord injuries, as it provides both carbohydrates and protein to help meet dietary needs while providing a small amount of fibre. Other research in para athletes has also documented low iron intake in wheelchair athletes, as noted in chapter two and five. Thus, the final study of this dissertation aims to assess the role of our lpa pea protein concentrate on the iron status, exercise performance, body composition, and dietary intake in active individuals with SCI.

Chapter 6- Study 4- Low phytic acid peas have no effect on iron status in physically active individuals with spinal cord injury but improve nutrient intake
6.1 Abstract

Iron plays a critical role in physiological systems, yet iron deficiency is the most prevalent micronutrient worldwide. Individuals with spinal cord injuries may be at increased risk for iron deficiency due to increased requirements, reduced absorption of dietary iron, and poor intake. The purpose of this research is to examine the efficacy of a pea protein with high iron bioavailability on iron status, exercise performance, and dietary intake in active individuals with spinal cord injury. Four individuals underwent measures of cardiovascular fitness (VO\textsubscript{2peak}), exercise performance (10 km time trial), body composition, iron status (ferritin and hemoglobin), and bowel function before and after supplementing with a pea protein bred to have high iron bioavailability for eight weeks. Ferritin (p=0.21) and hemoglobin (p=0.89) values were unchanged following supplementation. Supplementation increased iron intake (p=0.047) and a trend for increased protein intake (p=0.07) was evident. Increases were particularly notable in those with low intakes at baseline. In one participant, this increase in protein corresponded with an increase in lean body mass. One participant experienced improved indicators of bowel function and no participants experienced symptoms related to the supplement that hindered their consumption. Pea protein may be beneficial for improving dietary intake and, in some individuals, may improve bowel function.

Keywords: protein; plant-based; body composition; bowel function
6.2 Introduction

Iron is an essential micronutrient, playing important roles in energy metabolism, oxygen transport and storage, synthesis of hormones and neurotransmitters, and immune function (Abbaspour et al., 2014; Ganz & Nemeth, 2015). However, iron deficiency (ID) is the most prevalent nutrient deficiency globally, affecting nearly two billion individuals (Gedfie et al., 2022). Deficiencies in iron lead to decrements in work output, fatigue, reduced metabolic efficiency, headache, and irritability (Soppi, 2018). Active individuals tend to be at a greater risk for ID relative to their sedentary peers due to increased requirements to support erythropoiesis and to offset reductions in absorption secondary to exercise (Peeling & McKay, 2023). Given the reduction in training capacity and recovery observed in iron deficient individuals, athletes are often encouraged to undergo regular iron screenings to ensure adequate stores across the training cycle (Sim et al., 2019).

Athletes with spinal cord injuries (SCI) may be at an increased risk of ID due to high incidences of chronic inflammation following SCI (Alison & Ditor, 2015). Increased inflammation upregulates the iron regulating protein, hepcidin, which degrades the iron transport protein ferroportin (Peeling & Mckay, 2023; Zimmermann & Hurrell, 2007). As ferroportin is the only known pathway for iron to be transported from the enterocyte to the blood, this ultimately leads to a reduction in iron absorption. Further, those living with SCI often must deal with pressure ulcers as a result of their paralysis and lack of sensory perception (Groah et al., 2015). Iron plays an important role in the healing of such wounds, potentially increasing the requirement in this population (Taylor, 2017). When considering intake of dietary iron, research in para athletes from a variety of different sports suggests intakes of iron in this population are typically low relative to government recommendations (Eskici & Ersoy, 2016; Jeoung & Kim,
2021; Madden et al., 2017). Due to decreased absorption, increased requirements, and reduced intake, these individuals may be at increased risk of ID.

While pharmaceutical iron interventions tend to be efficacious in improving iron status (Pasricha et al., 2014), such interventions have been reported to cause gastrointestinal disturbances (Sim et al., 2019), limiting compliance. Negative gastrointestinal effects may be a greater barrier in those with SCI compared to those without, as these individuals often experience neurogenic bowel dysfunction (NBD) secondary to their injury, leading to symptoms such as abdominal pain, discomfort, and constipation. Given the overlap in symptoms of neurogenic bowel and pharmaceutical iron supplementation, those with SCI may opt to not use such supplementation to avoid worsening of such symptoms even if they have suboptimal iron stores.

Food-based approaches to improve iron status may therefore be of particular interest to those with SCI as a strategy to improve their iron without the side effects of pharmaceutical supplementation. Researchers at the Crop Development Centre at the University of Saskatchewan have developed lines of field peas that may be an attractive approach to improving iron status (Warkentin et al., 2012). These peas have been bred to have reduced concentrations of phytic acid, a storage form of phosphorus that chelates divalent minerals such as iron, reducing bioavailability. These low phytic acid (lpa) peas have been shown to have approximately 60% less phytic acid than the parent lines, resulting in 1.4 to 1.9 times higher iron bioavailability (Liu et al., 2015). Research from our lab has found that female runners supplementing with these peas saw a modest improvement in iron status (Shaw et al., 2023).

Field peas may be an ideal vehicle for delivering bioavailable iron to this population, as peas are rich in a variety of other nutrients such as complex carbohydrates, protein, and various
vitamins and minerals. Field peas are high in oligosaccharides, a form of carbohydrate that may improve bowel function and reduce the degree of NBD experienced (Singh et al., 2017). The energy requirements of those with SCI may be lower due to decreased active tissue, though relative protein requirements may be higher compared to able-bodied individuals to support wound recovery and training adaptations (Flueck & Parnell, 2021). As our previous research (study three) observed lower carbohydrate intakes than is likely required in athletes, a pea protein concentrate may be especially useful, as it provides carbohydrates as well as protein and other micronutrients. Therefore, field peas and other pulses may be beneficial for this population due to their rich micronutrient profile and high protein level. This unique composition may assist these individuals in not only meeting their macronutrient goals but also their micronutrient needs, even if their overall energy requirements are lower.

The objective of the current research is to assess the role of a high protein powder made of *lpa* peas on iron stores and nutrient intake in physically active individuals with SCI. Based on our previous research with this product (Shaw et al., 2023; chapter 4 of this thesis), we hypothesize that supplementation will provide a modest improvement in iron stores and improve intakes of dietary protein.

6.3 Methods

6.3.1 Subjects and Study Design

Six individuals with traumatic SCI (5 males, 1 female) from Regina and Saskatoon, Canada (4 from Saskatoon, 2 from Regina) consented to take part in this research. Participants supplemented for eight weeks with a high-protein pea concentrate bred to have *lpa*. Inclusionary criteria included being 18 years of age or older, meeting the physical activity recommendations
for individuals with SCI (i.e., at least 20 minutes of physical activity on two or more days per week; Ginis et al., 2011), having sustained their injury at least 12 months prior, and were not currently supplementing with iron. If participants were deemed to have met inclusionary criteria, they were invited to participate in baseline testing, involving dietary intake, measures of NBD, exercise performance, iron status, and body composition. Following completion of baseline testing, participants supplemented with 80 g of lpa pea protein concentrate (Table 6.1) and 250 mg of vitamin C for eight weeks. After the supplementation period, participants repeated measures assessed at baseline. During the eight-week supplementation period, diet, physical activity, and NBD scores were monitored biweekly by questionnaire. This research was approved by the University of Saskatchewan Biomedical Research Ethics Board (BIO 1207). All participants provided written informed consent prior to any measurements. All post-intervention visits occurred at the same time of day (± 90 minutes) as the corresponding baseline visit to avoid any effects of circadian rhythm. Participants were instructed to maintain their regular physical activity habits and were informed of the nutritional information of the supplement so they could consume the supplement without significantly increasing their typical intake.

6.3.2 Dietary Intervention

Participants consumed 40 g of pea protein concentrate and 125 mg of vitamin C twice per day for eight weeks. Vitamin C was consumed with the pea protein to maximize iron absorption, as vitamin C acts as a reducing agent in the intestines, converting ferrous iron (Fe$^{3+}$) to the absorbable ferric (Fe$^{2+}$) state. Nutritional information of the supplement is displayed in Table 6.1. Participants were instructed to consume the supplement in the manner that best supported compliance but were instructed to avoid dairy and dairy alternatives for two hours before and
after consuming the supplement to maximize iron absorption. Participants reported compliance with the supplement in a tracking log and unused powder was returned and weighed to confirm compliance.

**Table 6.1 Nutritional Information of Supplement**

<table>
<thead>
<tr>
<th></th>
<th>Per serving (40 g)</th>
<th>Per day (80 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (kcal)</strong></td>
<td>158</td>
<td>317</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td>15.7</td>
<td>31.4</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>19.6</td>
<td>39.2</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>1.9</td>
<td>3.8</td>
</tr>
<tr>
<td><strong>Iron (mg)</strong></td>
<td>2.1</td>
<td>4.1</td>
</tr>
</tbody>
</table>

*Participants consumed two 40 g doses daily*

6.3.3 Exercise Performance

*Peak Oxygen Uptake (VO$_{2peak}$).* Participants were instructed to follow their typical pre-exercise food, hydration, and caffeine intakes and avoid moderate to strenuous exercise in the 24 hours before coming in for testing. Upon arrival, participants sat quietly for five minutes before having their heart rate and blood pressure assessed. After resting measures were recorded, a five-minute warm-up was completed, during which participants chose a comfortable cadence. After the five-minute warm-up, participants engaged in a five-minute passive rest period, during which a heart rate monitor (Polar, Kempele, Finland) and gas collection mask were fitted. Peak oxygen uptake (VO$_{2peak}$) was assessed using an arm or leg ergometer (Monark, Vansbro, Sweden) based on the individual’s abilities. Exercise intensity started at 50W and was increased by five or ten watts every two minutes, depending on the participant’s ability and fitness. The test was terminated when the individual could not maintain a cadence within two revolutions per minute of their
chosen cadence despite encouragement from the researcher. Expired gases (Vmax Series 29 Calorimeter, SensorMedics, USA) and heart rate were collected and averaged over 20 seconds for analysis.

**Time Trial (TT).** During baseline testing, a familiarization of the 10 km TT occurred 30 minutes after the VO$_2$peak test, during which the protocol for the time trial was followed but no data were collected. At least 36 hours after the assessment of VO$_2$peak, participants returned for another 10 km TT, during which expired gases and heart rate were collected throughout. The TT was completed at 75% of the resistance achieved during the VO$_2$peak test. Prior to beginning the test, participants warmed-up for 1 km at the resistance used in the test and the cadence chosen for the VO$_2$peak test. Once 1 km had passed, they began the TT. Participants were instructed to complete the 10 km as quickly as they could and no encouragement from researchers was provided.

6.3.4 Hematology

Hemoglobin was assessed via a capillary sample (HemoCue, Ängelholm, Sweden). Ferritin was assessed via a venous blood sample collected by a researcher trained in phlebotomy. Samples were then centrifuged at 3000g for 10 minutes and 10°C before separation of plasma. Plasma was stored at -80°C until analysis. Determination of plasma ferritin was completed using fluorescence Immunoassay (AFIAS 6; Boditech, Gang-won-do, Korea). The working range for this assay is 10-1000 µg/L and the intra-assay CVs ranged from 1.2% to 6.2%. Two trials of all measures were completed and averaged for analysis. If measures were not congruent, a third measure was taken, and the two closest measures averaged.
6.3.5 Body composition

Body composition measurements were obtained by DEXA (Hologic© Discovery Wi; Bedford, MA) using QDR software for Windows XP (QDR Discovery, Hologic, Inc.). All measurements were completed by a certified radiology technologist. Coefficients of variation of lean and fat mass using this protocol have been demonstrated to be 1% and 3%, respectively (Chilibeck et al., 2023). Participants arrived at the lab in a normally fed and hydrated state and were instructed to avoid vigorous physical activity in the 24 hours prior.

6.3.6 Biweekly Check-Ins

Throughout the trial, participants were instructed to maintain their habitual physical activity and dietary behaviours. To assess this, participants were sent a questionnaire every two weeks to assess indicators of NBD, physical activity, and dietary intake. Dietary intake was assessed using 24-hour recall, physical activity was assessed using the Godin Leisure Time Exercise Questionnaire (Godin & Shephard, 1985), and indicators of NBD were assessed using a questionnaire developed and validated by Krogh et al. (2006) for assessing NBD. At this time, participants were also provided an opportunity to report any symptoms they were experiencing that they thought might be related to the supplement. Dietary intakes were assessed using Cronometer (https://cronometer.com).

6.3.7 Statistical Analysis

Statistical analyses were completed using JASP statistical software (version 0.16.4). Dependant t-tests (pre-post) were used to assess changes in VO$_2$max, ferritin, and hemoglobin. A repeated
measures ANOVA was used to assess NBD and dietary intake at biweekly check-ins. If a significant finding was observed for the ANOVA, a least significant difference post-hoc test was used. No statistical tests were run on body composition data, as data is only available for two participants. For exercise performance, no statistical tests were run due to differences in protocol for one of the three participants with available data. For data presentation of exercise performance, performance relative to baseline was calculated using the following formula to get performance as a percentage of baseline:

\[
\frac{Baseline - Post\ Intervention}{Baseline} \times 100
\]

For the participants who did a TT, the result was subtracted from 100 (where a negative value [indicating a lesser time to complete the TT and therefore improved performance] ended up being added). For the participant that did a time to exhaustion trial, the resultant value was added to 100 (where a negative value [indicating a lesser time to exhaustion and therefore worse performance] ended up being subtracted from 100). Individual data is presented for each variable. Significance was accepted at p<0.05.

6.4 Results

Two participants (both males from Saskatoon) were lost to follow-up after providing consent but before engaging in any baseline measures for reasons unrelated to the study. Four individuals (three males, one female) completed the intervention and are described in Table 6.2. One participant (Participant 2) did not complete exercise measures (VO\textsubscript{2}peak or performance trial), as the equipment was not accessible to him based on hypertonia in his upper body. Participants 1 and 3 completed exercise measures using an arm ergometer while Participant 6 used a leg ergometer. Physical activity remained unchanged throughout the trial for all participants.
Compliance with the supplement was very high, for all participants (compliance= Participant 1-98%; Participant 2- 92%; Participant 3- 95%; Participant 4- 100%).

Table 6.2 Participant characteristics

<table>
<thead>
<tr>
<th>Participant</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Level of Injury</th>
<th>Complete/Incomplete</th>
<th>Time since injury (years, y, months, m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>21</td>
<td>T5</td>
<td>Complete</td>
<td>1y, 10m</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>31</td>
<td>C4-5</td>
<td>Incomplete</td>
<td>18y, 3m</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>27</td>
<td>C5</td>
<td>Incomplete</td>
<td>7y, 4m</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>56</td>
<td>C6</td>
<td>Incomplete</td>
<td>5y, 9m</td>
</tr>
</tbody>
</table>

*Level of injury indicates vertebra at which injury occurred; complete injury refers to incidences where there is a complete loss of function below the level of the injury; incomplete refers to incidences where some movement or feeling is evident below the level of injury*

6.4.1 Hematological Values

A venous blood sample was not possible to obtain for Participant 2 due to atrophy impacting the ability to access the vein. Therefore, ferritin measures are only available for three participants (1, 3, and 4). Ferritin values decreased in Participants 1 and 4 and remained stable in Participant 3 (Figure 6.1). No significant difference was observed from baseline to post-intervention (p=0.21). Hemoglobin was assessed in all participants. Values decreased in Participants 1 and 2, stayed relatively stable with a modest decrease in Participant 4, and increased substantially in Participant 3 (Figure 6.2). No significant difference was observed from baseline to post-intervention (p=0.89).
6.4.2 Body Composition

Body composition was only available for two participants (3 and 4) due to scheduling conflicts that could not be overcome. Lean body mass remained largely unchanged in Participant 3 (Baseline lean body mass= 49.9 kg; post-intervention lean body mass=48.9 kg) though fat mass increased slightly (baseline fat mass= 13.4 kg; post-intervention fat mass= 14.4 kg), while a notable increase in lean body mass was observed in Participant 4 (baseline lean body mass= 44.8 kg; post-intervention lean body mass= 48.9 kg).
kg; post-intervention lean body mass = 46.2 kg) alongside a decrease in fat mass (baseline fat mass = 27.2 kg; post-intervention fat mass = 25.9 kg).

6.4.3 Neurogenic Bowel Dysfunction Score

NBD score is indicated in Table 6.3. Scores remained relatively unchanged in Participants 2 and 4, decreased substantially in Participant 3, and increased modestly in Participant 1. Though Participant 1 experienced only a slight increase in score, the change was significant enough to alter the severity of dysfunction based on the scoring system outlined by Krogh et al. (2006) (Table 6.3). The decrease in score of Participant 3 was substantial enough to move from “moderate” to “very minor” dysfunction. However, no statistical difference was observed (p=0.82).

**Table 6.3** Neurogenic bowel dysfunction score

<table>
<thead>
<tr>
<th>Participant</th>
<th>Baseline</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Week 8</th>
</tr>
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<tr>
<td>1</td>
<td>13</td>
<td>13</td>
<td>15</td>
<td>15</td>
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<td>2</td>
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<td>13</td>
<td>11</td>
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<td>3</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
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</table>

*Scoring system based on Krogh et al. (2006); Legend: 0-6=very minor; 7-9=minor; 10-13= moderate; 14+= severe*

6.4.4 VO₂peak

VO₂peak remained unchanged for all three participants who completed the measure (Participant 1: 29.9 mL/kg/min pre-intervention vs 30.1 mL/kg/min post-intervention; Participant 3: 11.0 mL/kg/min pre-intervention vs 10.9 mL/kg/min post-intervention; Participant 4: 25.6 mL/kg/min pre-intervention vs 23.7 mL/kg/min post-intervention). No significant differences in oxygen consumption were observed for relative (mL/kg/min) or absolute (L/min) values.
6.4.5 Exercise Performance

Due to limitations in equipment, one participant (3) completed a time to exhaustion trial at 75% of the resistance that was achieved during the VO$_2$peak test rather than the 10 km time trial completed by participants 1 and 4. Similar to VO$_2$peak values, exercise performance remained relatively unchanged from baseline to post-intervention. Post-intervention results expressed as a percentage of baseline performance are depicted in Figure 6.3. Physiological data during the performance trial is displayed in Table 6.4. Due to a measurement error, heart rate data was not available for participant 3.

![Figure 6.3](image)

**Figure 6.3** Exercise performance relative to baseline following an 8-week intervention period consuming a low phytic acid pea protein; *due to equipment limitations, participant 3 completed a time to exhaustion trial at 75% resistance reached in VO$_2$peak test rather than 10 km time trial completed by participants 1 and 4. Dotted line represents no change from baseline performance.
Table 6.4 Expired gas and heart rate data during 10 km time trial

<table>
<thead>
<tr>
<th>Participant</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart Rate (bpm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>Max</td>
</tr>
<tr>
<td>1</td>
<td>Average 164</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>Max 185</td>
<td>168</td>
</tr>
<tr>
<td>3*</td>
<td>Average -</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Max -</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Average 145</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>Max 175</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>VO₂ (mL/kg/min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>Max</td>
</tr>
<tr>
<td>1</td>
<td>Average 22.4</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>Max 29.2</td>
<td>30.2</td>
</tr>
<tr>
<td>3*</td>
<td>Average 7.9</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>Max 12</td>
<td>13.0</td>
</tr>
<tr>
<td>4</td>
<td>Average 22.4</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>Max 24.8</td>
<td>29.4</td>
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<tr>
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<tr>
<td></td>
<td>Max</td>
<td>Max</td>
</tr>
<tr>
<td>1</td>
<td>Average 1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Max 1.09</td>
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</tr>
<tr>
<td>3*</td>
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<td></td>
<td>Max 1.23</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Due to limitations in equipment, participant 3 completed a time to exhaustion trial rather than a 10 km time trial; VO₂= oxygen consumption; RER= respiratory exchange ratio

6.4.6 Dietary Intake

Dietary intake at baseline and during the intervention for each participant is displayed in Table 6.5. Intake during the intervention includes consumption of the supplement, as reported in the 24-hour food recall. No differences were observed for energy (p=0.63), fat (p=0.50), carbohydrate (p=0.70), protein (p=0.30), or fibre (p=0.71). A significant increase was observed in iron intake (p=0.047). Post-hoc testing revealed intakes at six weeks were greater than at baseline.
(p=0.03) and at four weeks (p=0.03). When comparing baseline values to eight-week values, there was a trend for increased protein (p=0.07).

Table 6.5 Individual Dietary Intake at Baseline and Biweekly During Intervention

<table>
<thead>
<tr>
<th>Participant</th>
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<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
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<td></td>
<td></td>
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<tr>
<td>4</td>
<td>1434</td>
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<td>1938</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>59.2</td>
<td>38.7</td>
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</table>

PRO (% kcal)
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<td>2.0</td>
<td>1.9</td>
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</tr>
<tr>
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<td>2.0</td>
<td>2.1</td>
<td>1.7</td>
<td>2.7</td>
</tr>
<tr>
<td>3</td>
<td>1.7</td>
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<td>2.7</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Fibre (g/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19.5</td>
<td>21.8</td>
<td>13</td>
<td>25.8</td>
<td>11.9</td>
</tr>
<tr>
<td>2</td>
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<td>20.6</td>
<td>33</td>
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</tr>
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<td>4</td>
<td>26.6</td>
<td>19.8</td>
<td>25.3</td>
<td>24.6</td>
<td>20.8</td>
</tr>
</tbody>
</table>

Includes consumption of supplement, as recorded in 24-hour food recall

6.4.7 Adverse Effects

All but one participant (Participant 1) reported some variety of symptom they thought to be related to the supplement at some point during the intervention. After two weeks of consuming the supplement, participant 2 reported constipation but noted that it resolved after a few days. Participant 3 reported bloating, and Participant 4 reported increased flatulence. For the following six weeks of consuming the supplement, only participant 4 reported symptoms (increased gas at each biweekly check-in), but no other participants reported any symptoms.

6.5 Discussion

From this research, we obtained preliminary data to suggest that individuals with SCI tolerate a pea-based protein concentrate with little gastrointestinal effects and potential benefits on bowel function and nutrient intake. To our knowledge, this is the first research study to investigate the role a plant-based protein supplement might have on the health and performance of individuals with SCI. We found that supplementation with a lpa pea protein concentrate had very individual
effects on measures of NBD, with most participants experiencing no change but one participant experiencing a marked improvement. We also found supplementation with the *lpa* pea protein significantly increased iron intake from baseline to week six, and protein intakes trended towards significantly increased from baseline to week eight (*p*=0.07). No differences in other aspects of dietary intake were observed. Plasma ferritin as well as hemoglobin did not significantly differ from baseline to post-intervention, though values decreased slightly or remained unchanged over the course of an 8-week intervention consuming a pea protein concentrate with *lpa*. Further, there were no effects on peak oxygen uptake, exercise performance, or physiological response during exercise.

Most individuals with SCI experience some degree of bowel dysfunction secondary to the injury to the central nervous system due to autonomic dysfunction, sensory deficits, paralysis, and immobility (Johns et al., 2021). NBD may lead to discomforts such as constipation and abdominal pain or symptoms such as fecal incontinence (Johns et al., 2021) and is a leading cause of re-hospitalization in those with SCI (Tate et al., 2020). Despite the deleterious effects that NBD can have on the quality of life in those with SCI, progress in developing treatments and optimal management for bowel dysfunction has been relatively slow (Tate et al., 2020; Qi et al., 2018). Our research found that our pea protein concentrate had little to no effect in two of our four participants, increased scores of NBD in one participant, and improved indicators of bowel function markedly in one participant.

Improvements in bowel function in apparently healthy populations are typically observed with the consumption of more pulses in the diet (Murty et al., 2010; Pittaway et al., 2007; Venkidasamy et al., 2019), though many of such benefits are due to high amounts of fibre found in pulses (Venkidasamy et al., 2019). Our specific product was air-classified to produce a fine
fraction, reducing much of the fibre content (1.45% crude fibre) and producing a high-protein, high-iron concentrate. However, our product still contained approximately 40% carbohydrates (Table 6.1). Much of this carbohydrate was likely resistant starch, a polysaccharide that has been found to positively modulate gut health. In addition to starch, oligosaccharides such as raffinose and stachyose are also likely prevalent, which are found more densely in the cotyledons of seeds rather than the seed coat (which was removed in the processing of our product) (Singh et al., 2016). These oligosaccharides have been found to have beneficial effects on the gut microbiota and bowel function in human (Li et al., 2017) and animal (Guo et al., 2023; He et al., 2020; Liang et al., 2022) models.

While many food-based approaches to improve iron status often fail to observe any statistically significant differences, a general upward trend in iron status is often observed (Alaunyte et al., 2014; Anschuetz et al., 2010; Shaw et al., 2023). In the current research, we found no difference in ferritin or hemoglobin after the 8-week dietary intervention, which is contrary to our hypothesis that such a dietary intervention would increase these measures, but in line with other research utilizing this supplement in female runners (Shaw et al., 2023). This lack of change may be related to the baseline status of the individuals in the current research, who all had very high ferritin levels. Increased ferritin levels up-regulate hepcidin, decreasing iron absorption (Kalasuramath et al., 2013; Peeling & McKay, 2023). However, it’s possible that low-grade inflammation secondary to their injury increased expression of ferritin, as ferritin is an acute phase protein that increases in response to inflammation in order to decrease available iron and protect against free radical damage, rather than as an indicator of iron storage, as it is typically interpreted (Mahroum et al., 2022). Pulses are rich in polyphenols such as flavonoids and phytosterols, compounds which are suggested to have anti-inflammatory effects (Maleki et
al., 2019; Singh et al., 2016) and have been suggested as a therapeutic compound for SCI (Zhang et al., 2017). Decreased inflammation could lead to a reduction of ferritin that is expressed due to high levels of inflammation, which may limit our ability to observe improvements in iron status following consumption of a pulse-based supplement. Our small sample size likely limited the statistical interpretation of results, but provide promising preliminary data, nonetheless.

Our research is in line with previous research in our lab using a lpa pea concentrate (Shaw et al., 2023), which observed no differences in exercise performance following supplementation. Little differences also appear to be evident in the metabolic response to exercise (measured through expired gases). Previous research has found increased reliance on fat metabolism and a subsequent decrease in carbohydrate metabolism (Bennett et al., 2012; Kaviani et al., 2020) and improved performance (Kaviani et al., 2020) when consuming lentils compared to a potato and egg white meal matched for energy and macronutrients, thought to be related to the low glycemic index of the lentils. However, much of this research has utilized lentils as a pre-exercise meal, which is different than the chronic supplementation protocol utilized in the current research. Future research should assess the impact of a plant-based meal or supplement in the pre-, peri-, and/or post-exercise timeframe in a population with SCI.

Consumption of our supplement resulted in increased dietary iron intakes and a trend for increased protein intake while having minimal impacts on other aspects of dietary intake assessed, which is in line with our hypothesis and what we have observed previously in our lab (Shaw et al., 2023). Protein intakes trend towards increased when expressed as total intake (g/day) and appear to be increased relative to body weight (g/kg/day) in all participants. One participant (3) had an increase in energy intake throughout the study, so protein relative to energy intake (% kcal) did not change. This increase in energy intake likely explains the slight
increase in fat mass observed in this participant. Protein intake relative to caloric intake appears to have increased in the other three participants who maintained a relatively similar caloric intake throughout the study but increased protein. Interestingly, Participant 4, who saw a rather large increase in protein intake (0.9 g/kg at baseline; 1.7 g/kg at week eight) also experienced a rather notable increase in lean body mass (+1.4 kg). Pea protein may therefore be beneficial for improving body composition when it increases dietary protein to levels sufficient to support muscle protein synthesis. Increasing the amount of protein consumed relative to total intake and body weight is likely a benefit for those with SCI, as delayed gastrointestinal transit time, impaired nutrient absorption, and high incidence of pressure sores all increase protein requirements in this population (Figel et al., 2018; Flueck & Parnell, 2021). Similar to the results observed in study three, participants tended to have reduced carbohydrate intake as a percentage of total intake, which was unaltered by the supplement. Increasing intake of complex carbohydrates such as pulses or whole grain cereals may assist individuals in meeting both carbohydrate and fibre recommendations, potentially benefitting both health and performance. Iron intake was significantly increased from baseline to week six, which is in line with our previous research with this supplement in female runners (Shaw et al., 2023). In the current research, iron intake increased in those who had low baseline intakes of iron (2 and 4), but did not appear remarkably different in those who had higher baseline intakes of iron. This increase in dietary iron intake did not correspond to an increase in ferritin levels, likely due to the high ferritin values observed at baseline.

Our study is not without limitations. Our small sample size and lack of a control group limit the conclusions that can be drawn from the current research, a concern that is further impacted by the lack of accessible equipment for one of the participants. Further, the participants in our
study all had quite high ferritin values at baseline, likely limiting the effectiveness of an intervention designed to improve iron status. Given the high likelihood of chronic inflammation in his population, future research investigating iron status in this population should assess markers of inflammation to consider as a confounding factor on indicators of iron storage. We also acknowledge that delivering an amount of iron through a food-based approach similar to what would be delivered by a pharmaceutical is not feasible. However, food-based interventions may be more cost-effective in the long term while providing other nutritional benefits. Future research using food-based approaches, particularly utilizing plant-based food, should consider a longer intervention period to counteract the lower amount of iron delivered by the product.

6.5.1 Conclusion
Preliminary data suggest that lpa pea supplement did not influence iron status, or exercise performance in a small population of active individuals with SCI, but tended to increase iron and protein intakes and improved symptoms of NBD in one participant. Further, increases in protein intake due to a pea protein supplement may improve body composition in individuals with SCI. Pea protein concentrates may be a beneficial product for those with SCI to increase their protein and iron intake while delivering complex carbohydrates that may have benefits on bowel function.

6.6 References


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https://doi.org/10.3389/fnut.2022.1039403


7.1 Discussion

The results of this thesis inform on PB solutions to improve nutrient intake and health outcomes in female runners and athletes with physical impairments through supplementation with a biofortified field pea to the regular diet. Improving diet quality in such a manner is likely to have a lower environmental impact than using animal-based products. As global food production increases to feed the growing population, maximizing planetary health has been a prominent goal globally as policymakers have realized that feeding the world today and in the future requires paying attention not only to diet quality and nutrition but also to the environmental impact of food production and distribution along with the socioeconomic impacts of the global food system (Béné et al., 2019). In 2015, the United Nations published a list of seventeen development goals aimed at finding win-win solutions for environmental health along with human well-being, equity, and prosperity (https://sdgs.un.org/goals). Specific to this thesis is sustainable development goal 2, to “End hunger, achieve food security and improved nutrition and promote sustainable agriculture” and goal 3, “Ensure healthy lives and promote well-being for all at all ages”. Central to both of these goals is the food consumed and the systems in which such food is produced, as food systems have been identified as having the potential to nurture human health and support a thriving ecosystem, or to be a major driver of poor health and environmental degradation (Willett et al., 2019). Indeed, it has been suggested that a healthy diet is a sustainable diet (Béné et al., 2019).

The amount of food required to feed the growing population is at its highest, while the need to re-imagine our food systems is at its most dire if we want to meet the goals of the Paris Agreement of limiting atmospheric warming to less than a 2°C of pre-industrial levels (Vicedo-Cabrera et al., 2018). In 2019, a Commission of global experts called for a “Great Food
Transformation” (Willett et al., 2019). This transformation aims to provide nutritious diets for an estimated population of 10 billion by the year 2050 while promoting sustainable agricultural practices such that we stay “within a safe operating space” (Willett et al., 2019). This vision of transformation is that the reference diet will consist largely of fruits, vegetables, whole grains, legumes and pulses, nuts, and unsaturated oils while shifting away from animal products, added sugar, refined grains, and starchy vegetables. Such shifts will require a substantial change in the general consumption and production patterns, as the global supply of cereals and red meat vastly exceeds what would be required for a nutritious diet, yet investments continue to be spent on such products (Béné et al., 2019).

While it may be tempting to simply suggest all individuals consume a PB diet, even those avoiding animal products completely tend to rely heavily on cereal grains. Such dietary patterns could potentially limit the nutritional value of the diet (Marinangeli et al., 2021). That is to say, simply removing animal products from the diet is not sufficient for a nutritious and sustainable diet – purposeful effort must be given on both the part of the individual and the food system itself to optimize nutritional intake while minimizing environmental harm. Further, simply increasing the proportion of nutrition received from PB food products such as field peas and other pulses and a subsequent decrease in animal products is likely to improve the health and sustainability of the diet (Shaw et al., 2022; Willett et al., 2019). It is this goal that drives the primary ambition of the current thesis – to assess the role that biofortified pulses might have in improving the dietary intake of vulnerable athletic populations and to evaluate how such products might improve the health, well-being, and performance of these athletes.

While the evidence suggests that PB diets can support health and longevity when planned appropriately (Shaw et al., 2022), there remain areas in which we can maximize the benefits of
such a dietary pattern, particularly in the most vulnerable populations. This thesis focused on two vulnerable populations of interest—female runners and athletes with SCI. Research in these two populations is particularly unique and needed because 1) both of these populations are thought to be at disproportionate risk for dietary insufficiencies (Madden et al., 2017; Sasaki et al., 2021; Wilson et al., 2023), and 2) both of these populations are vastly understudied in the exercise science and nutrition literature (Cowley et al., 2021; Shaw et al., 2021).

As this thesis outlined, field peas are a rich source of many nutrients that are key to optimizing health, but also contain antinutrients that limit bioavailability. Studies one, two, and four of this thesis investigated how a biofortified pea might influence the health and well-being of two vulnerable athlete populations. These studies aimed to assess, for the first time in humans, how a biofortified pea might influence the dietary intake and health parameters in our populations of interest. While no significant effect was found on clinical measures of iron, our primary outcome, a positive trend in iron stores was observed in female runners, suggesting a potential benefit with long-term supplementation. In study four, though our sample size was limited by recruitment, our data appear to suggest that, in some individuals with SCI, a pea-based protein supplement may improve bowel function. Studies one, two, and four all displayed increases in protein and iron intakes, particularly in those with low intakes at baseline. As much of the literature including more PB food sources in the diet of athletes warns of increased satiety from such a dietary pattern (Cialdella-Kam et al., 2016; Melin et al., 2016; Strasser et al. 2021), our research is novel in identifying a nutrient-packed pea-based supplement that is beneficial in improving dietary intake without influencing overall caloric intake. These results highlight the role of PB food and supplements in sustainably optimizing nutrient intake.
In study three, we highlighted potential nutritional shortcomings in a sample of elite para cyclists, including fibre, which could be remedied by following the recommendations made by the “Great Food Transformation” (Willett et al., 2019). While dietary intake appears to exceed the recommended intakes of most nutrients, a risk of inadequacy for many nutrients was evident and only corrected through supplementation. Given that a food-first approach is advocated by many experts to maximize the health and performance of the athlete (Close et al., 2022), a biofortified pea powder, such as the one utilized in our research, may benefit these sporting populations. Though our developed product was not necessarily a whole-food product, the consumption of this concentrated form of pea may be an efficacious way to increase nutrient intake without significantly altering energy intake. This product may be of particular interest to athletes as it is a food-based product that is an easily transportable and accessible way to meet their nutritional needs, particularly when travelling for training or competition.

While we assessed the role of a lpa pea on human health, the application of lpa crops has potential environmental benefits as well. As monogastric animals cannot break down phytate, 90% of the phosphorus in the plant (stored as phytate) is excreted, leading to phosphorus pollution in the environment (Colombo et al., 2020). Excess phosphorus runoff into bodies of water leads to excessive algae growth which impacts water quality and leads to oxygen depletion, ultimately decreasing aquatic biodiversity in areas affected (Gilbert, 2017; Mallin & Cahoon, 2020; Raboy, 2020). Further, since monogastric livestock cannot access most of the phosphorus bound in seed crops due to the lack of phytase, farmers must provide supplementary feed with phosphorus and cations that are inaccessible in the presence of phytic acid (Colombo et al., 2020). Therefore, the utilization of lpa seed crops in the agricultural sector has the potential
to decrease agricultural spending and improve environmental well-being (Raboy, 2020), aspects that align directly with the sustainable development goals outlined above.

7.2 Strengths and Limitations

The studies completed for this thesis are novel in the use of biofortified peas to improve dietary intake and clinical markers of nutritional insufficiency in two athletic populations—female runners and active individuals SCI. These studies are strengthened through the use of the gold-standards of measuring body composition, DEXA, and the research-standard indirect calorimetry to assess maximal and peak oxygen uptake as well as fuel contributions to exercise. However, the methodology employed is only one of the many strengths of these studies. Given the novelty of our population (particularly in study four), the sample size is a serious concern and a major limitation in this research, though there are certainly other aspects of our interventions that could and should be improved upon for future research in the area.

7.2.1 Strengths

Our trial in female runners (study two) is strengthened by first completing a feasibility trial (study one) to ensure the supplement was tolerable and the methods feasible. Another major strength of study two is the utilization of a double-blind, randomized controlled trial to accurately assess the influence of our intervention supplement (lpa peas). Further, by using not one, but two comparator supplements (maltodextrin and regular phytic acid pea), we are better able to discern if any potential benefits are due to the lpa nature of our intervention supplement or characteristics inherent to the peas themselves. This methodology sets the precedent for future research assessing biofortified food products in human health. Such research should use an
unrelated product as the comparator as well as the parent line of the biofortified product to ensure any effect is related to the biofortification and not other aspects of the product. The high compliance to the supplement across studies one, two, and four is also a strength of this research, indicating that long-term usage of this product is likely a feasible strategy to increase intakes of bioavailable iron in athletic populations. Study three is strengthened in the population assessed, as most studies assessing dietary intake in a para athlete population do so in athletes from various sports, which have different dietary requirements. By assessing a single sport (cycling), we are able to control for the demands of the sport. Cycling is also an interesting sport to assess since a variety of physical impairments are represented and the type of impairment can be grossly categorized based on cycling class (C, H, T, or B). Cycling classification allows differences in intake relative to impairment to be assessed. Study four is strengthened through the assessment of bowel function, a measure that is typically overlooked in dietary interventions in para athlete populations.

7.2.2 Limitations

As noted above, the primary limitation in studies two and four is sample size. Particularly in study four, our population of interest is very small and the area in which we are drawing from (Regina and Saskatoon) is also relatively small. Therefore, recruiting a well-powered sample of the population would require recruiting a proportion of the overall population across multiple provinces, which is not feasible. Another limitation is the length of the intervention. While the 8-week intervention is in line with other studies in the field, the lower amount of iron delivered by a food-based product compared to pharmaceutical supplements may require a longer intervention period to decipher possible benefits. Finally, most of the participants in studies one, two, and
four had adequate ferritin at baseline, likely limiting the effectiveness of an intervention designed
to improve iron status.

7.3 Future Directions

The strengths and limitations of the studies completed for this thesis provide important insight
for future research to consider. The use of the parent line of pea as a comparator for the \textit{lpa} pea
allows us to disseminate benefits due to the biofortified nature of the pea compared to
characteristics inherent to peas in general. Future research should also allow for longer
intervention periods (16+ weeks) in order to counteract the lower amount of iron delivered
through a food-based approach compared to pharmaceuticals. Further, exclusionary criteria
based on baseline ferritin measures would also allow for a sample with inadequate iron stores at
baseline, likely increasing the efficacy of the intervention. On a related note, the implementation
of \textit{lpa} peas in areas of the world most impacted by iron deficiency, such as developing countries,
should be completed, and the ability to grow such products locally in these developing countries
assessed. Findings from such research have the potential to not only improve the health of the
local population but also the economic wellbeing through food production. Future research
should continue to assess the efficacy of pea protein and other PB interventions in those with
SCI, utilizing a larger sample size and a comparator (e.g., dairy protein, isoenergetic control) to
better understand how such dietary practices might improve the health and performance of these
individuals. Finally, little is known about how dietary requirements might change in individuals
with physical impairments. Thus, future research should aim to quantify micro- and
macronutrient requirements in different types of impairments such that guidelines can be put in
place to maximize the health and wellbeing of these individuals.
7.4 Conclusions

The findings of this thesis are that supplementation with a *lpa* pea protein does not significantly improve iron status in female runners or individuals with SCI. However, perhaps the most important takeaway from this thesis overall is that a PB protein supplement, particularly a protein concentrate consisting of both carbohydrates and protein, successfully increases the intakes of protein and iron (at least in those consuming inadequate amounts at baseline) and that compliance with such a product is high. No detrimental effects on iron status nor performance were observed. These findings suggest that increasing the proportion of the diet from PB sources is not only feasible in these populations but may improve the nutrient intake of the athlete. Specific to *lpa* peas, they did not appear to perform differently than the parent line used as a comparator in studies one and two, so the implementation of *lpa* food products has important environmental and economic impacts that have the ability to support the “Great Food Transformation” outlined by Willett et al. (2019).

7.5 References


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Appendix A - Participant Consent Forms
TITLE: Effect of low-phytate pea powder with high iron bioavailability on iron status of female runners

PRINCIPAL INVESTIGATOR: Dr. Philip D. Chilibeck, Ph.D., Professor, College of Kinesiology, University of Saskatchewan, 306-966-1072

Co-INVESTIGATORS: Dr. Gordon Zello, Ph.D., Professor, College of Pharmacy and Nutrition (306-966-5825), Dr. Tom Warkentin, Ph.D. Professor, Plant Sciences (306-966-2371).

Student researcher: Keely Shaw, M.Sc., (Ph.D. student, supervised by Phil Chilibeck), 306-966-1082), College of Kinesiology, University of Saskatchewan

Sponsor: Agriculture Research Branch, Government of Saskatchewan

24 hour emergency cell phone: 306-230-3849 (Phil Chilibeck)

INTRODUCTION
You are invited to take part in this research study because you are a female runner who has low dietary iron intake. The goal of the study is to determine whether 8 weeks of daily consumption of a powder made with peas with high iron bioavailability can improve iron status and endurance performance.

Your participation is entirely voluntary, so it is up to you to decide whether or not you wish to take part. If you decide not to take part, you do not have to provide a reason and it will not affect your relationship with the researchers and will not affect your academic standing at the university. If you decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

This consent form may contain words that you do not understand. Please ask the researchers to explain any words or information that you do not clearly understand. You may ask as many questions as you need to understand what the study involves. Please feel free to discuss this with your family, friends or family physician.

There will be a maximum of 45 people participating in this study.

WHO IS CONDUCTING THE STUDY?
The sponsor of this study is the Agriculture Research Branch, Government of Saskatchewan who have given the researchers a grant through the Agriculture Development Fund. They will reimburse Dr. Chilibeck and the University of Saskatchewan for the costs of undertaking this study. However, the investigators will not receive any direct financial benefit from conducting this study.
WHO CAN PARTICIPATE?
You can participate if you are a female runner and are determined to have low dietary iron intake. Please note that visitors to the University of Saskatchewan campus must confirm that they are either fully vaccinated or have received a negative COVID test recently.

STUDY PROCEDURES
If you choose to participate in the study you initially be given a 24-hour food recall questionnaire to determine whether you you have low dietary iron intake. If you are ineligible for the study based on this food recall questionnaire, your data will be discarded.

If you have a low intake, you will be invited to participate in the remainder of the study. This involves a number of laboratory visits for baseline testing:

1) 10 mL blood collection from the forearm to assess iron status. This will be collected in room 108 of the Williams building by a trained phlebotomist (i.e. someone who is trained specifically in taking blood samples).
2) Body composition assessment by dual energy X-ray absorptiometry in room 108 of the Williams Building (221 Cumberland Ave. N.). This involves lying on a table while an X-ray machine assesses your body composition. This test will last about 15 minutes.
3) On a separate day, you will perform an exercise test on a treadmill to determine your peak aerobic capacity (as an assessment of your aerobic fitness). This involves running on a treadmill with workload increasing every two minutes until you reach exhaustion and cannot keep up with the speed of the treadmill. This test will last about 10-15 minutes depending on your aerobic fitness. During this test you will breathe through a mouthpiece so that we can collect respiratory gases to determine the amount of oxygen you consume. You will be given a 30 minute break and then will perform a practice trial of a 5 km time trial on the same treadmill (without a mouthpiece). This requires you to run as fast as possible to cover 5 km on the treadmill (you will be able to see the distance covered on the treadmill display).
4) Approximately a week later, you will perform the same 5 km time trial on the treadmill; this will count as the test of exercise performance for the study. During this test you will be required to wear a mouthpiece so that we can collect respiratory gases to determine your energy expenditure, and we will collect a finger-prick blood sample at baseline and every 5 minutes throughout the test to determine blood lactate levels as an indicator of anaerobic metabolism (this involves collection of one drop of blood approximately five to six times throughout the test).

After the baseline testing you will be randomized (i.e. assigned by chance by a computer) to one of three groups:

1) Consumption of 140g (~47g 3 times per day) for 8 weeks of a powder containing peas with high iron bioavailability
2) Consumption of 140g (~47g 3 times per day) for 8 weeks of a powder containing regular peas
3) Consumption of 140g (~47g 3 times per day) for 8 weeks of maltodextrin

You will be given 125 mg vitamin C tablets to consume three times per day throughout the 8 weeks
because vitamin C helps with iron absorption.

You will not know which powder you are receiving (i.e. the study is “blinded”) until the end of the study.

During the 8 weeks we will ask you to complete a 24-hour food recall and a physical activity questionnaire every 2 weeks. The 24-hour recall will ask you to recall all the foods and drinks you have consumed in the past 24 hours. The physical activity questionnaire will ask you which physical activities you did during the past week, along with the frequency (i.e. times per week) and the duration of each exercise session.

You will not be allowed to take any vitamin or mineral supplements during the 8 week intervention.

At the end of the 8-week intervention you will do the same testing as done at baseline, except that you will only do the 5 km time trial once (i.e. a week after the aerobic capacity test). These tests include: 10 mL blood collection from the forearm to assess iron status, the aerobic capacity test, the 5 km time trial, and body composition assessment.

Some of the measurements in the study (i.e. iron status, exercise performance) may be affected by different phases of the menstrual cycle. We will record your menstrual cycle stage during baseline testing and will perform final study measures during the same menstrual cycle phase. This means that the final testing may vary slightly from the 8-week time point.

Your overall time commitment to the study will be eight hours for lab testing and completion of questionnaires.

COVID-RELATED RISKS AND MITAGATION

• Our research site is located at the University of Saskatchewan, under the jurisdiction of Saskatoon public health. We are taking all safety precautions to reduce the risk of spread of COVID-19 and expect you to follow public health directives as well.
  • If you feel that you are from a vulnerable group with respect to COVID-19 effects (e.g., immuno-compromised), please discuss your participation with the research team before consenting. You are under no obligation to participate and nothing bad will happen if you change your mind about participating in the research.
  • We will be collecting personal contact information that we must retain in order to follow up with you and/or conduct contact tracing if you may have been exposed to COVID-19 in coming to the research site.
  • Contact information will be kept separate from data collected through the research study to allow for de-identification of the research data (if applicable, as detailed in the protocol).
  • You maintain your right to withdraw from the study at any time, including research data (if applicable). If you do withdraw, we will continue to maintain your contact information and will only give it to the Saskatchewan Health Authority if required for contact tracing.
  • We cannot guarantee anonymity as the personal contact information identifies you as a participant.
BENEFITS
If you choose to participate in this study, you may or may not see an improvement in your iron status and your exercise performance. These benefits are not guaranteed.

RISKS AND DISCOMFORTS
Risks associated with blood draws include bruising and infection at the site of the blood draw. You may also feel light headed and/or dizzy. These risks will be minimized by following proper sterilization procedures and blood draws will be done by a qualified individual.

Risks with exercise testing include muscle pulls and strains. This will be minimized by having you perform a proper warm up before exercise testing.

There is a small amount of radiation exposure from the dual energy x-ray absorptiometry scans. This is the equivalent of ~10 µSv. For reference, a cross-country flight could expose a person to about 30 µSv of radiation and the average dental X-ray exposes a person to 4 µSv of radiation.

The powder may cause gastrointestinal side effects such as bloating or upset stomach, but from our past research with similar products, this is rare.

COST AND REIMBURSEMENTS
You will not be charged for any research-related procedures. An honorarium of $300 will be provided to cover your time and out-of-pocket expenses such as travel and parking. If you decide to withdraw early from this study, your compensation will be proportional to your time in the study. This remuneration is subject to declaration to Revenue Canada and for that purpose you will be required to supply your social insurance number.

WHAT HAPPENS IF SOMETHING GOES WRONG?
In the unlikely event of an adverse event arising related to the study procedures, trained staff will be available throughout the conduct of the study who can respond immediately. Necessary medical treatment will be made available at no additional cost to you. As soon as possible, notify the research team. By signing this document, you do not waive any of your legal rights.

CONFIDENTIALITY
In Saskatchewan, the Health Information Protection Act (HIPA) defines how the privacy of your personal health information must be maintained so that your privacy will be respected. The study data will be stored securely (in a locked cabinet contained within a locked office under the supervision of the principle investigator) by the study team for a minimum of 5 years after the final results are published. Research records and medical records identifying you may be inspected by the University of Saskatchewan Biomedical Research Ethics Board, or representatives of the sponsor of the study for quality assurance and monitoring purposes.

It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be disclosed.

VOLUNTARY WITHDRAWAL FROM THE STUDY
If you do decide to take part in this study, you are still free to withdraw at any time and without giving reasons for your decision. There will be no penalty or loss of benefits to which you are otherwise entitled, and your relationship with the researchers and academic standing will not be affected.
If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during enrolment in the study will be retained for analysis up to the point of your withdrawal.

AFTER COMPLETION OF THE STUDY
Participants will be sent a letter at the end of the study with their individual results and with a summary of the results of the study.

CONTACT INFORMATION
If you have any questions about this study, or desire further information about this study before or during participation, you can contact Phil Chilibeck at 306-966-1072, 306-230-3849, or phil.chilibeck@usask.ca.

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, contact the Chair of the University of Saskatchewan Research Ethics Board, at 306-966-2975 (out of town calls 1-888-966-2975). The Research Ethics Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Research Ethics Board.
CONSENT TO PARTICIPATE

Study Title:
Effect of low-phytate pea powder with high iron bioavailability on iron status of female runners

- I have read the information in this consent form.
- I understand the purpose and procedures and the possible risks and benefits of the study.
- I was given sufficient time to think about it.
- I had the opportunity to ask questions and have received satisfactory answers.
- I am free to withdraw from this study at any time for any reason and the decision to stop taking part will not affect my future relationships at the university.
- I agree to follow the principal investigator's instructions and will tell the principal investigator at once if I feel I have had any unexpected or unusual symptoms.
- I have been informed there is no guarantee that this study will provide any benefits to me.
- I understand that by signing this document I do not waive any of my legal rights.
- I will be given a signed and dated copy of this consent form.

- I agree to participate in this study:

Printed name of participant: ______________________
Signature _______________________ Date___________________________

Printed name of person obtaining consent: _____________________
Signature _________________________ Date___________________________
Title: Evaluating the dietary intakes of elite para-cyclists

Principal Investigator/Supervisor:
Dr. Phil Chilibeck, Ph.D., College of Kinesiology, University of Saskatchewan, 1-306-966-1072, phil.chilibeck@usask.ca;

Student Researcher(s):
Keely Shaw, MSc, PhD. Candidate (supervised by Phil Chilibeck and Gord Zello), College of Kinesiology, University of Saskatchewan, keely.shaw@usask.ca

Co-Investigator:
Dr. Gord Zello, Ph.D., College of Pharmacy and Nutrition, University of Saskatchewan, gordon.zello@usask.ca

24-hour contact number (Phil Chilibeck): 1-306-230-3849

Introduction:
You are invited to take part in this research study because you are a Paralcyclist.

Your participation is entirely voluntary, so it is up to you to decide whether or not you wish to take part. If you decide not to take part, you do not have to provide a reason and it will not affect your relationship with the researchers. If you decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

This consent form may contain words that you do not understand. Please ask the researchers to explain any words or information that you do not clearly understand. You may ask as many questions as you need to understand what the study involves. Please feel free to discuss this with your family, friends, coaches, or family physician.

Who is conducting the study?
This study is being conducted by researchers from the Colleges of Kinesiology and Pharmacy and Nutrition at the University of Saskatchewan. The researchers are not being paid to conduct this study.

Purpose and Objective of the Research:
The purpose of this research is to determine the dietary intakes of Para-cyclists. By quantifying the intakes of the different sport classes (C, H, T, B), we hope to better understand the requirements of the different classes, which will inform researchers to develop para-specific nutritional recommendations and assist practitioners in supporting athletes in their nutrition in order to optimize training and recovery to ultimately maximize performance.

Procedures:
If you choose to participate in the study you will be asked to self-report your dietary intake using a validated food frequency questionnaire administered via the online portal Survey Monkey. This questionnaire will ask you how often you consume different types of foods and drinks as well as the amount you consume each time. The questionnaire will take about 45 minutes.

We plan to enrol a maximum of 40 participants

Potential Risks:
As we are using the online portal, Survey Monkey, there is a risk for the inadvertent release of personal information. This risk will be mitigated by the questionnaire not collecting identifying information from you.

Potential Benefits:
There are no direct benefits of participating in this study. However, the results of the study may help inform you of your dietary habits and assist coaches and dietitians in counselling you in regards to your dietary intake.

**Costs and Reimbursements:**
Participation in this study will not involve any additional costs to you. You will not be compensated for completion of the questionnaire.

**Confidentiality:**
Research records identifying you may be inspected in the presence of the Investigator or his designate by representatives of the University of Saskatchewan Biomedical Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent.

Your identity will remain anonymous throughout the study, as you will not be asked to report any identifying information. If you are interested in the results of this research, there an opportunity for you to provide your email prior to submission of the questionnaire. This is not a requirement for taking part in the study.

Study data (which does not contain information that may identify you), will be kept for at least 5 years and will be stored in a secure data server, and will be password protected.

If the results of this study are published, your identity will remain private. It is expected that the information collected during this study will be published/presented to the scientific community at meetings and in journals.

**Right to Withdraw:**
If you do decide to take part in this study, you are still free to withdraw at any time prior to submission of the questionnaire and without giving reasons for your decision. There will be no penalty or loss of benefits to which you are otherwise entitled, and your relationship with the researchers will not be affected. Once the questionnaire has been submitted, you will not be able to withdraw your data, as we will not know which data set is yours.

**Contact Information:**
If you have any questions about this study, or desire further information about this study before or during participation, you can contact Phil Chilibeck at 1-306-966-1072, 1-306-230-3849, or phil.chilibeck@usask.ca

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, contact the Chair of the University of Saskatchewan Research Ethics Board, at 1-888-966-2975. The Research Ethics Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Research Ethics Board.

By completing and submitting the Food Frequency Questionnaire through Survey Monkey, your free and informed consent is implied and indicates that you understand the above conditions of participation in this study.
**TITLE:** Effect of low-phytate pea powder with high iron bioavailability on iron status of individuals with spinal cord injury

**PRINCIPAL INVESTIGATOR:** Dr. Philip D. Chilibeck, Ph.D., Professor, College of Kinesiology, University of Saskatchewan, 306-966-1072

Co-**INVESTIGATORS:**
Dr. Gordon Zello, Ph.D., Professor, College of Pharmacy and Nutrition (306-966-5825), Dr. Tom Warkentin, Ph.D. Professor, Plant Sciences (306-966-2371), Bruce Craven, MSc., BSc(PT), BSPE, Dip Sport (PT), CSCS, Owner and Physiotherapist, Craven SPORT services (306-934-2011),

**Student researcher:** Keely Shaw, M.Sc., (Ph.D. student, supervised by Phil Chilibeck), 306-966-1082), College of Kinesiology, University of Saskatchewan

**Sponsor:** Agriculture Research Branch, Government of Saskatchewan

24 hour emergency cell phone: 306-230-3849 (Phil Chilibeck)

**INTRODUCTION**
You are invited to take part in this research study because you are an individual with a spinal cord injury. The goal of the study is to determine whether 8 weeks of daily consumption of high-protein powder containing peas with high iron bioavailability can improve iron status, endurance performance, and the gut microbiome.

Your participation is entirely voluntary, so it is up to you to decide whether or not you wish to take part. If you decide not to take part, you do not have to provide a reason and it will not affect your relationship with the researchers and will not affect your academic standing at the university. If you decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

This consent form may contain words that you do not understand. Please ask the researchers to explain any words or information that you do not clearly understand. You may ask as many questions as you need to understand what the study involves. Please feel free to discuss this with your family, friends or family physician.

There will be a maximum of 30 people participating in this study.

**WHO IS CONDUCTING THE STUDY?**
The sponsor of this study is the Agriculture Research Branch, Government of Saskatchewan who have given the researchers a grant through the Agriculture Development Fund. They will reimburse Dr. Chilibeck and the University of Saskatchewan for the costs of undertaking this study. However, the investigators will not receive any direct financial benefit from conducting this study.

**WHO CAN PARTICIPATE?**
You can participate if you are an individual between the ages of 18-50 with a spinal cord injury. You will not be included in the study if you are deemed to be anemic at baseline (hemoglobin levels <115 g/L) or if you have taken supplemental iron in the past six weeks.

**STUDY PROCEDURES**

If you choose to participate in the study you will initially complete a 24-hour food recall to assess baseline dietary intake. This involves you writing down everything you eat and drink in a 24-hour period. Your intake of energy, carbohydrates, fats, protein, and iron will be analysed by a researcher using an online food database. You will then be invited to participate in the remainder of the study. This involves a number of laboratory visits for baseline testing:

5) Hemoglobin status will be assessed via fingertip sample. This involves collection of one drop of blood.
6) Body composition assessment by dual energy X-ray absorptiometry in room 108 of the Williams Building (221 Cumberland Ave. N.). This involves lying on a table while an X-ray machine assesses your body composition. This test will last about 15 minutes.
7) 10 mL blood collection from the forearm to assess iron status. This will be collected in room 108 of the Williams building by a trained phlebotomist (i.e. someone who is trained specifically in taking blood samples).
8) To assess your gut microbiome, you will be asked to provide a faecal sample. You will receive instructions and a sample collection kit that will allow you to collect the sample in your own home that we will then ask you to bring to the testing site.
9) On a separate day, you will perform an exercise test to determine your peak aerobic capacity (as an assessment of your aerobic fitness). This involves performing exercise on an arm ergometer. The intensity of the test will increase every 2 minutes until you can no longer maintain the desired output. This test will last about 10-15 minutes depending on your aerobic fitness. During this test you will breathe through a mouthpiece so that we can collect respiratory gases to determine the amount of oxygen you consume. You will be given a 30 minute break and then will perform a practice trial of a 10 km time trial on the arm ergometer. This involves covering 10 km as fast as possible on the arm ergometer.
10) Approximately a week later, you will perform the 10 km time-time on the arm ergometer again; this will count as the test of exercise performance for the study. During this test you will be required to wear a mouthpiece so that we can collect respiratory gases to determine your energy expenditure, and we will collect an earlobe-prick blood sample at baseline and every 2 minutes throughout the test to determine blood lactate levels as an indicator of and how your body is creating energy (this involves collection of one drop of blood approximately four to five times throughout the test).

After the baseline testing you will receive your supplement, a high-protein pea powder made from peas with high iron bioavailability, of which you will consume 80g (40gx2) daily for eight weeks.

During the 8 weeks we will be ask you to complete a 24-hour food recall, a physical activity questionnaire, and a questionnaire about your bowel function every 2 weeks. The 24-hour recall will ask you to recall all the foods and drinks you have consumed in the past 24 hours. The physical activity questionnaire will ask you which physical activities you did during the past week, along with the frequency (i.e. times per week) and the duration of each exercise session. The bowel questionnaire will ask about symptoms you may or may not be experiencing about your bowels (bloating, constipation, etc.).
You will not be allowed to take any vitamin or mineral supplements during the 8-week intervention. You will be given 125 mg vitamin C tablets to consume two times per day throughout the 8 weeks because vitamin C helps with iron absorption.

At the end of the 8-week intervention you will do the same testing as done at baseline, except that you will only do the 10 km time trial once, using the mouthpiece. These tests include: a fingertip sample to assess hemoglobin, a DEXA scan for analysis of body composition, 10 mL blood collection from the forearm to assess ferritin, a faecal sample to assess gut microbiome, the aerobic capacity test, and the 10 km time-trial test.

Some of the measurements in the study (i.e. iron status, exercise performance) may be affected by different phases of the menstrual cycle in female participants. For females choosing to participate in the study, we will record your menstrual cycle stage during baseline testing and will perform final study measures during the same menstrual cycle phase. This means that the final testing may vary slightly from the 8-week time point.

Biological samples (i.e., blood and stool) will be stored in a container labelled with your unique participant ID number (no identifying information) and stored in a -80°C freezer until data collection for all participants is complete, after which the samples will be analysed in a lab by experienced professionals. After we have analysed the data, samples will be disposed of by individuals trained in handling biological samples.

Your overall time commitment to the study will be eight weeks of dietary intervention plus approximately 8 hours of in-lab testing and completion of questionnaires.

COVID-RELATED RISKS AND MITAGATION

• Our research site is located at the University of Saskatchewan, under the jurisdiction of Saskatoon public health. We are taking all safety precautions to reduce the risk of spread of COVID-19 and expect you to follow public health directives as well.

• If you feel that you are from a vulnerable group with respect to COVID-19 effects (e.g., immunocompromised), please discuss your participation with the research team before consenting. You are under no obligation to participate and nothing bad will happen if you change your mind about participating in the research.

BENEFITS
If you choose to participate in this study, you may or may not see an improvement in your iron status, your body composition, and your exercise performance. These benefits are not guaranteed.

RISKS AND DISCOMFORTS
Risks associated with blood draws include bruising and infection at the site of the blood draw. You may also feel light headed and/or dizzy. These risks will be minimized by following proper sterilization procedures and blood draws will be done by a qualified individual.

Risks with exercise testing include muscle pulls and strains. This will be minimized by having you perform a proper warm up before exercise testing.

There is a small amount of radiation exposure from the dual energy x-ray absorptiometry scans. This is the equivalent of ~10 μSv. For reference, a cross-country flight could expose a person to about 30 μSv of radiation and the average dental X-ray exposes a person to 4 μSv of radiation.
The powder may cause gastrointestinal side effects such as bloating or upset stomach, but from our past research with similar products, this is rare.

COST AND REIMBURSEMENTS
You will not be charged for any research-related procedures. An honorarium of $300 will be provided to cover your time and out-of-pocket expenses such as travel and parking. If you decide to withdraw early from this study, your compensation will be proportional to your time in the study. This remuneration is subject to declaration to Revenue Canada and for that purpose you will be required to supply your social insurance number. Any personal information collected as a record of honorarium payment will be stored separately from the data for 7 years for auditing purposes.

WHAT HAPPENS IF SOMETHING GOES WRONG?
In the unlikely event of an adverse event arising related to the study procedures, trained staff will be available throughout the conduct of the study who can respond immediately. Necessary medical treatment will be made available at no additional cost to you. As soon as possible, notify the research team. By signing this document, you do not waive any of your legal rights.

CONFIDENTIALITY
In Saskatchewan, the Health Information Protection Act (HIPA) defines how the privacy of your personal health information must be maintained so that your privacy will be respected. The study data will be stored securely (in a locked cabinet contained within a locked office under the supervision of the principle investigator) by the study team for a minimum of 5 years after the final results are published. Research records and medical records identifying you may be inspected by the University of Saskatchewan Biomedical Research Ethics Board, or representatives of the sponsor of the study for quality assurance and monitoring purposes.

It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be disclosed. Any samples will be stored with labels associated with your unique participant identification code without any identifying information in a -80°C freezer until analysis. The master list of names associated with each code will be stored in a password-protected excel sheet on a password protected computer. Stool samples will be transferred from the site of collection (Saskatoon or Toronto) to a third-party lab in Vancouver (Microbiome Insights) for analysis.

VOLUNTARY WITHDRAWAL FROM THE STUDY
If you do decide to take part in this study, you are still free to withdraw at any time and without giving reasons for your decision. There will be no penalty or loss of benefits to which you are otherwise entitled, and your relationship with the researchers and academic standing will not be affected.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during enrolment in the study will be retained for analysis up to the point of your withdrawal.

AFTER COMPLETION OF THE STUDY
Participants will be sent an email at the end of the study with a summary of the results of the study.

CONTACT INFORMATION
If you have any questions about this study, or desire further information about this study before or during participation, you can contact Phil Chilibeck at 306-966-1072, 306-230-3849, or phil.chilibeck@usask.ca.
If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, contact the Chair of the University of Saskatchewan Research Ethics Board, at 306-966-2975 (out of town calls 1-888-966-2975). The Research Ethics Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Research Ethics Board.
CONSENT TO PARTICIPATE

Study Title: Effect of low-phytate pea powder with high iron bioavailability on iron status of individuals with spinal cord injury

- I have read the information in this consent form.
- I understand the purpose and procedures and the possible risks and benefits of the study.
- I was given sufficient time to think about it.
- I had the opportunity to ask questions and have received satisfactory answers.
- I am free to withdraw from this study at any time for any reason and the decision to stop taking part will not affect my future relationships at the university.
- I agree to follow the principal investigator's instructions and will tell the principal investigator at once if I feel I have had any unexpected or unusual symptoms.
- I have been informed there is no guarantee that this study will provide any benefits to me.
- I understand that by signing this document I do not waive any of my legal rights.
- I will be given a signed and dated copy of this consent form.

- I agree to participate in this study:

Printed name of participant: ______________________
Signature _______________________ Date___________________________

Printed name of person obtaining consent: ______________________
Signature _______________________ Date___________________________
Appendix B- Pea Supplement Preparation Protocol

Step 1- Milling and Air Classification

Client: University of Saskatchewan, Crop Development Centre
Primary Contact: Tom Warkentin, PhD
Project Title: Pilot Scale Milling and Air Classification of Dehulled Peas
Contract Services Agreement Date: April 27, 2020
Completed by: Richardson Centre for Functional Foods and Nutraceuticals

I. Background
Prof. Warkentin (U of S) contracted RCFFN to mill and air classify two (2) lots of dehulled peas labelled CDC Bronco (RCFFN code RC053-20) and 4802-8-874-L (RCFFN code RC054-20) for RCFFN to mill and air classify generating elevated protein concentrates and to test the starting materials and fine fractions for crude protein, moisture, dry matter, and crude fibre contents.

II. Methodology
The Client provided two (2) lots of dehulled yellow peas labelled CDC Bronco (RC053-20) and 4802-8-874-L (RC054-20) weighing 852.2 kg and 845.0 kg, respectively.

Each lot of dehulled yellow peas was milled using the Prater-Sterling M-21 pilot impact mill set to 40 Hz equipped with 1.0 mm screens generating a pre-break coarse flour collected in food grade polypropylene woven bags with plastic lining.

Each lot of pre-break coarse flour was then milled using the Prater-Sterling M-21 pilot impact mill equipped with 0.3 mm screens set to 40 Hz followed by in-line air classification at primary and secondary air flow settings of 75 and 20, respectively, and a power setting of 25 Hz.

Fine and coarse fractions were collected separately in food grade polypropylene woven bags with plastic lining then shrink-wrapped and palleted for shipping.

A total of four (4) samples were collected according to Table 1 for crude protein, moisture, dry matter and crude fibre content testing.
Step 2- Product Cooking, Drying, and Milling

**Equipment Used:**
- Electric kettle – Savage Kettle 2410 – Savage Bros Co. – Chicago Illinols (fig. 1)
- Tray oven – Changzhou Yixin Drying Equipment Co. Ltd (fig. 2)
- Hammer mill – Fitzpatrick DA-06 – Elmhurst, Illinois – the Fitzpatrick Company (fig. 3)

**Process:**
1. Combine water (80%) and flour (20%) in Savage kettle and heat to 90°C. Hold at 90°C for 30 minutes, stirring continuously.
2. Transfer to sheet pans and dry in tray oven (70°C, 16 - 20 hours)
3. Hammer mill in three steps (17.1 mm, 30 mesh, 100 mesh).

Note: Three steps were necessary for milling, rather than the usual two, due to the dried product being unusually hard. Typically, it is first coarsely milled thru 30 mesh followed by a 100 mesh screen.

**Images:**

![Fig. 1 Electric kettle – Savage Kettle 2410](image1)

![Fig. 2 Tray oven – Changzhou Yixin Drying Equipment](image2)

![Fig. 3 Hammer mill – Fitzpatrick DA-06](image3)

*Step 2 was completed Saskatchewan Food Industry Development Centre Inc. (Saskatoon, SK) for study 1. Above images and protocol were provided by Saskatchewan Food Industry Development Centre Inc. Step 2 for study 2 and 4 was completed by ARD-Food Development Centre (Portage la Prairie, MB). Protocol and materials may differ slightly for product used in...*
studies 2 and 4 due to a different company processing the peas, but the nature of the process and the final product was similar.
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