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Iron and Vitamin D Status in Female Gee-Gees Varsity Athletes

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A thesis submitted in partial fulfillment of the requirements for the

Master's degree in Interdisciplinary Health Sciences

Interdisciplinary School of Health Sciences

Faculty of Health Science

University of Ottawa

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LIST OF ABBREVIATIONS

AI	Adequate Intake
AMDR	Acceptable Macronutrient Distribution Ranges
ASA24	Automated Self-Administered 24-hour Dietary Assessment Tool
CBC	Complete Blood Count
CFG	Canada Food Guide
DBP	Vitamin D Binding Protein
DMT1	Divalent Cation Transporter 1
DRI	Dietary Reference Intakes
EAR	Estimated Average Requirement
EER	Estimated Energy Requirements
Fe2+	Ferrous Iron
Fe3+	Ferric Iron
Hb	Hemoglobin
Hct	Hematocrit
IDA	Iron Deficiency Anemia
ID	Iron Deficiency
IOC	International Olympic Committee
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume
PTH	Parathyroid Hormone

RBC	Red Blood Cell Count
RDA	Recommended Dietary Allowances
RDW	Red Cell Distribution Width
RNR	Respondent Nutrition Report
SD	Standard Deviation
SF	Serum Ferritin
TS	Transferrin Saturation
Ug	Micrograms
IU	International Units
UL	Tolerable Upper Intake Level
WBC	White Blood Cell Count
25(OH)D	25-hydroxy vitamin D

ABSTRACT

The main objective of this study was to measure the nutritional status of iron and vitamin D in varsity female athletes from the University of Ottawa and the possible relationship with their dietary patterns (mixed diet or plant-based diet). Among 63 athletes interested in participating, 17 completed the study. Nutritional intakes were assessed during sports season using two 24-hour recalls with the self-administered dietary assessment tool (ASA24). A blood sample was taken to measure indicators of iron and vitamin D status. The results revealed that 5% of the athletes suffered from vitamin D deficiency and as much as 47% had suboptimal vitamin D levels. No athletes suffered from iron deficiency anemia (IDA), but 26% had iron deficiency (ID). The vitamin D intake for 94% of athletes was below the estimated average requirements (EAR) and the use of supplements helped athletes attain the EAR. In contrast, 94% of athletes were able to meet the EAR for iron and the use of supplements caused athletes to exceed upper limits (UL). Lastly, due to the small number of plant-based athletes recruited, it was not possible to establish any relationships with this dietary pattern.

Keywords: iron, vitamin D, female, athletes, high performance

RÉSUMÉ

L'objectif principal de cette étude était de mesurer l'état nutritionnel du fer et de la vitamine D chez des athlètes féminines de haut niveau de l’Université d’Ottawa et la relation possible avec l’adoption d’un régime mixte ou régime à base de plantes. Parmi les 63 athlètes intéressées, 17 ont complété l'étude. Les apports nutritionnels ont été évalués pendant la saison sportive en effectuant deux rappels de 24 heures à l'aide de l'outil d'évaluation diététique autoadministré (ASA24). Un échantillon de sang a été prélevé pour mesurer les indicateurs du statut en fer et en vitamine D. Les résultats ont révélé que 5% des athlètes souffraient d’une déficience en vitamine D et que 47% avaient des niveaux sous-optimaux. Aucune athlète ne souffrait d'anémie ferriprive, cependant, 26% avaient de faibles réserves de fer. L'apport en vitamine D de 94% des athlètes était inférieur aux besoins moyens estimés (BME) et l'utilisation de suppléments a aidé les athlètes à atteindre le BME. En revanche, 94% des athlètes ont pu atteindre le BME en fer et l'utilisation de suppléments a entraîné les athlètes à dépasser les apports maximaux tolérables (AMT). Enfin, en raison du faible nombre d'athlètes recrutés suivant un régime à base de plantes, aucune relation n'a pu être établie avec ce régime.

Mots clés : fer, vitamine D, femmes, athlètes, haute performance

ACKNOWLEDGMENT

I would first and for most like to thank all the athletes for taking the time to complete our research project considering they were extremely busy with competitions, training, and exams as well as Danika Smith responsible for varsity operations and student-athletes services for being our liaison with the sports teams throughout the project. She played a major role in our study by facilitating most of our exchanges with the sports teams and coaches.

I am very grateful for my supervisor France Rioux, Ph.D. for all the support and guidance she gave me. She was always available to answer my questions and truly took the time to help me when needed. Aside from a few research classes, this is as far as I've ever dove into research, therefore the extra guidance was much appreciated and valued. She is an amazing teacher and wonderful supervisor and made my master's journey a memorable one.

I would also like to thank my thesis committee: Bénédicte Fontaine-Bisson, Ph.D., Dt.P. and Lara Pilluti, MSc, Ph.D. for their encouragement and helpful feedback.

I want to thank all the sports coaches for allowing us to meet with the athletes before or after practice. Thank you as well to all the physicians associated with each sports team for ensuring that athletes with abnormal blood parameters received the proper follow-up, and specifically to Marie-Josée Klett who accepted to be the physician associated with the research project mainly for the blood test requisition.

Thank you to the Faculty of Health Sciences - funds for scientific research - for funding this research project.

Finally, I would like to thank my parents, sister, and partner for supporting me through it all and encouraging me along the way

CHAPTER 1

INTRODUCTION

The importance of nutrition in high-performance athletes no longer needs to be justified; there is sufficient research evidence demonstrating that the nutritional quality of the diet before, during, and after exercise can have an impact on the performance and recovery of athletes (Rodriguez et al., 2009). Young female athletes represent a group at risk for nutritional iron and vitamin D deficiencies. The iron status of female athletes is often deficient or suboptimal due to low iron intake coupled with higher requirements caused by menstrual loss and repetitive and intensive training (Arne et al., 2009; Pedlar et al., 2018; Sim et al., 2019). Since iron plays an important role in oxygen transport, iron deficiency anemia can affect performance, power, and endurance capacity in athletes (Myhre et al., 2016; Pedlar et al., 2018; Rodenberg & Gustafson, 2007; Sim et al., 2019a). IDA can also affect motivation, concentration, and decision-making (Dziembowska et al., 2019; Samson et al., 2022). As for vitamin D, lower plasma levels are also observed in a significant proportion of Canadians during the winter in which female athletes are no exception (Wilson-Barnes et al., 2020). Vitamin D plays an important role in bone health due to its role in calcium absorption and bone mineralization. A deficiency in this vitamin can have negative consequences on athletic performance, bone health, and overall health (Mahan, L. Kathleen., Raymond, Janice L., 2017).

The vegan diet is gaining more and more popularity in the general population, as well as in the athlete's (Rogerson, 2017; Vitale & Hueglin, 2021). Opting for a completely plant-based diet is a steadily growing trend, primarily in industrialized countries due to its many health benefits (Sisay et al., 2020). Today, almost every sports discipline has a growing number of athletes who are choosing to no longer eat foods derived from animal sources (Rogerson, 2017; Vitale & Hueglin, 2021; K. Wirnitzer et al., 2018). On the other hand, the vegetarian diet, although composed primarily of plant-based foods, includes some types of animal-based foods such as eggs, and dairy products. The adoption of a vegan or vegetarian diet among high-level athletes is an area that has been of interest to scientists for over 150 years (K. C. Wirnitzer, 2018). Since 2017, the publications of review articles on veganism in sports have been higher than ever (K. Wirnitzer, 2020). The literature strongly supports the idea that it is not necessary to consume animal products to excel in high-level sports (H. Lynch et al., 2018). All types of eating patterns can allow optimal performance if they are properly planned and respect the recommended dietary allowances (RDAs). Therefore, a well-planned vegan or vegetarian diet can be suitable for elite athletes (Craddock et al., 2016). Unfortunately, these dietary patterns, if not meticulously designed, may put the athletes at higher risk of having non-optimal iron and vitamin D nutritional status.

CHAPTER 2

LITERATURE REVIEW

2.1 VEGANISM

2.1.1 Definition

A vegan diet is a dietary pattern that includes a variety of plant-based foods such as fruits, vegetables, legumes, grains, nuts, and seeds and excludes all products of animal origin such as meats, seafood, poultry, dairy, and eggs (Dinu et al., 2017).

2.1.2 Background & benefits

There are several benefits to following a vegan diet which includes reducing the risk of cardiovascular disease by lowering cholesterol levels and body weight (Bradbury et al., 2014), lowering the risk of hypertension (Appleby et al., 2002), and the risk of type 2 diabetes (Barnard et al., 2009). In addition, it may also help to reduce the risk of developing some types of cancer such as colorectal cancer (Melina et al., 2016). Aside from the health benefits of adopting this diet, other reasons people may choose to go vegan is for animal welfare (Ghaffari et al., 2022; Janssen et al., 2016), for environmental purposes (Ghaffari et al., 2022; Janssen et al., 2016), for ethical and moral reasons, for taste and enjoyment, for norms and socialization due to their upbringing and/or due to family preferences, for religious and cultural reasons, and lastly, due to low-cost (Ghaffari et al., 2022; North et al., 2021). Moreover, a recent cross-sectional study conducted among 691 self-identified vegans from Australia, the United Kingdom, and the United States has suggested that people may choose to adopt this lifestyle as a form of social identity (Judge et al., 2022).

2.1.3 Key nutrients of concern

Although this diet can meet a person's nutritional needs from infancy to older age, it must be properly planned. Some key nutrients of concern include iron and zinc as the iron contained in plant-based foods is less bioavailable than iron found in animal-based foods (Melina et al., 2016) and plant-based foods contain high amounts of phytates that bind zinc and reduce its bioavailability (Craig, 2009). In addition, vitamin D (Mangels, 2014) and vitamin B_{12} (Majchrzak et al., 2006) are also a concern as the main sources of these nutrients come primarily from animal-based foods.

2.2 VEGETARIANISM

2.2.1 Definition

A vegetarian diet can be defined as a dietary pattern that includes a wide variety of fruits, vegetables, grains, legumes, nuts, and seeds, as well as some animal-based products like dairy and eggs. However, it excludes all meat, seafood, and poultry (Dinu et al., 2017).

2.2.2 Background & benefits

Similarly to the vegan diet, the benefits of following a vegetarian diet may include lowering the risk of developing heart disease by lowering levels of cholesterol and body weight, as well as reducing high blood pressure, reducing the risk of type 2 diabetes (Barnard et al., 2009) and reducing the risk of developing some types of cancer (Appleby & Key, 2016; Melina et al., 2016). People appear to be more lenient toward this type of lifestyle mainly for animal welfare and environmental protection, but also for the health benefits mentioned above, social norms, social image, and religion (Müssig et al., 2022).

2.2.3 Key nutrients of concern

The vegetarian diet can meet all the nutritional requirements at any stage of life if it is carefully planned. Since it includes some foods from animal sources, the risk for nutritional deficiency is lower. However, the nutrients of concern are vitamin D (Mangels, 2014) if not consuming enough dairy products during limited sun exposure months, iron and zinc since those two nutrients are less bioavailable in this diet (Melina et al., 2016), and vitamin B_{12} if not consuming enough eggs or milk (Craig, 2009).

2.3 MIXED DIET

2.3.1 Definition

A mixed diet can be defined as a dietary pattern that includes a wide variety of both plant and animal-based foods including meat, poultry, and seafood.

2.3.2 Background & benefits

According to the literature, people tend to choose this dietary pattern primarily for health, taste, and enjoyment (North et al., 2021). The population's motives to eat a variety of plant and animal-based foods are also linked to traditional eating ways and habits (Müssig et al., 2022). Accordingly, the Canada Food Guide (CFG) recommends including a variety of both plant and lean animal-based foods (Canada, 2020).

2.3.3 Key nutrients of concern

Overall, the nutritional requirements can be easily met when consuming a mixed diet. For this reason, it is associated with the lowest risk for nutritional deficiencies.

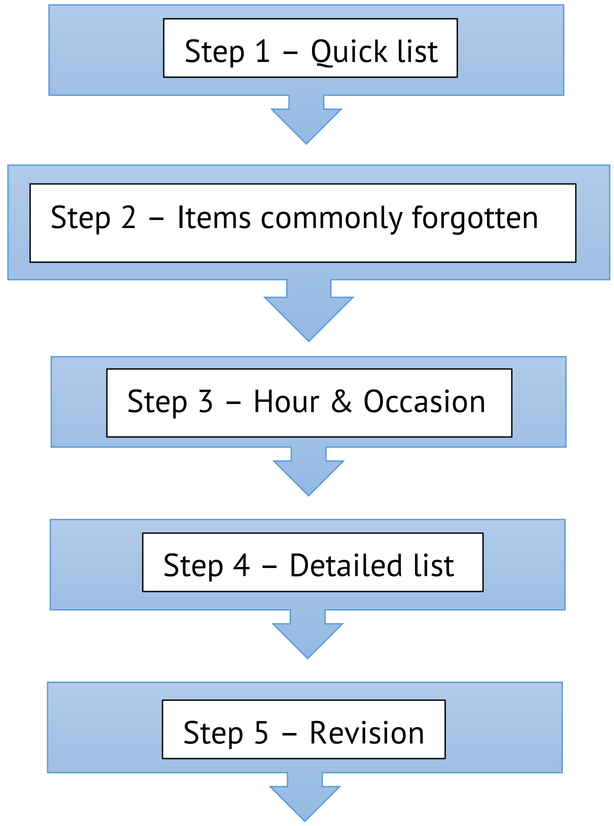
2.4 ASSESSMENT OF NUTRITIONAL INTAKE

2.4.1 Collecting information on dietary intake

The first step to assessing a person's nutrient intake is by obtaining information regarding their typical food patterns. This can include but is not limited to, the quantity and quality of foods and beverages consumed. There are a variety of tools that can help gather this type of data. For instance, there is the 24-hour recall method (Salvador Castell et al., 2015). This method is retrospective and is used in the National Canadian Community Health Survey by Health Canada (Canada, 2018). As outlined in Figure 1, the model consists of five different steps: the first step consists of quickly listing all the foods and beverages consumed in the previous 24 hours. The second step is optional but is a reminder to ensure that no foods or beverages have been forgotten such as alcoholic beverages, sweets, and other foods. Step three consists of including the times that foods and beverages were consumed as well as the type of meal consumed (e.g., breakfast, lunch, dinner, snack). Step four obtains more detailed information on the food or beverages listed in step one (e.g., brand, quantity, size, preparation methods, etc.). At this step, it could be useful to use aids to help estimate portion sizes such as using food models. Finally, step five is an additional step that allows another chance to add any final information (Nieman, 2019). It is also important to take note of whether the data reported represents a typical day of eating. This method is ideal since it is rapid, easy to administer, and is low cost. However, it does rely on memory, contains frequent over/under estimations of actual intake, and requires more than one recall to estimate adequately the usual intake of a person (Huang et al., 2022; Nieman, 2019).

Figure 1

24-hour recall protocol



Another useful method is the food frequency questionnaire. This method asks respondents to report their usual frequency of consumption from a list of foods and beverages for a specific period (Nieman, 2019). It can be quantitative, semi-quantitative, or non-quantitative, it can be completed through an interviewer or individually, and it is the most common tool used in large studies (Thompson & Subar, 2017). This method is advantageous because it is not too demanding, quick to administer, low cost, reflective of the usual consumption of the person and identifies long-term eating patterns in various populations (Cade et al., 2004). It does, however; rely on memory and requires individuals to read and write (Cade et al., 2004; Nieman, 2019).

The food diary is another method used to collect information on dietary intake (Ortega et al., 2015). This method instructs respondents to list all foods and beverages consumed over a certain number of days (Nieman, 2019). Respondents can be asked to weigh foods or even take photos for increased accuracy. This method is ideal since it does not rely on memory, it reflects the usual intake, and it is more accurate than the 24-hour recall and the food frequency methods as foods and beverages are recorded for several days including weekdays and weekends (Crawford et al., 1994). Nonetheless, it can be burdensome for individuals to document their food intake all day long for several days, the respondents must be able to read and write, and it has also been found that some respondents do not add enough detail (Cordeiro et al., 2015; Nieman, 2019).

2.4.2 Quantifying nutritional intake

Once the information on food and beverage intake is recorded, the energy and nutrient intake is quantified using different tools that have been created for this purpose. Additionally, there is an abundance of software available online (e.g., Food Tracker, ProfilAN, Eatracker.ca, ASA24, etc.) or through apps (e.g., My Fitness Pal, Cronometer, etc.). Dietary assessment tools based on new technologies, such as apps and online software, offer opportunities to facilitate food intake measures and are shown to be adequate for both participants and researchers (i.e., more cost-effective and time-saving) (Khazen et al., 2020). No official standards yet exist to evaluate health-related mobile apps making it more challenging to choose the right app; however, from a researcher's point of view, academic apps offer more advantages than consumer-grade apps, as they are developed with scientific input and are compared with a standard method for validation purposes (Khazen et al., 2020).

2.4.3 Evaluation of the nutritional adequacy of a diet

The evaluation of nutritional adequacy can be done by comparing the person's food pattern with the recommendations made by Health Canada through the CFG and general guidelines for healthy eating (Canada, 2021). The latest CFG from 2019 encourages the public to eat a variety of foods each day, make water their beverage of choice and provides recommendations for food portions using a plate as the template (i.e., half the plate should be filled with fruits and vegetables, a quarter of the plate with protein foods and the last quarter with whole grain foods) (Canada, 2021). In comparison, the 2007 CFG included four food groups (Vegetables and Fruit, Grain Products, Milk and Alternatives, Meat and Alternatives) presented in a rainbow with specific guidance on numbers of servings for children over 2 years, adults over 50 and women (pregnant, breastfeeding). The 2007 CFG is still used when needing to compare the consumption of the different food groups and number of portions namely for pregnant women as well as in the ASA24 tool. Both food guides, although portrayed differently, emphasized the importance of fruits and vegetables by making this group more prominent (e.g., half the plate filled with fruits and vegetables versus fruits and vegetables being the most visible arc in the rainbow).

The evaluation of nutritional adequacy of a person's diet is also commonly done by comparing the consumption of the different nutrients with the DRIs. The DRIs include the acceptable macronutrient distribution range (AMDR) associated with a reduced risk of chronic disease that is defined as "a range of intake for [either protein, fat, or carbohydrate] expressed as a percentage of total energy (kcal)" (Canada, 2005). The DRIs also include the RDA defined as "the average daily dietary intake level that is sufficient to meet the nutrient requirements of [97-98% of] healthy individuals [at any stage of life] and sex group" (Canada, 2005). The adequate intake (AI) can be used as a reference when there is insufficient data to set the RDA for a given nutrient. And finally, the tolerable upper intake level (UL) is considered to be the highest average daily intake that will not generate negative health effects and should not be exceeded (Canada, 2005).

2.5 IRON

2.5.1 Bioavailability and dietary sources

Iron is a mineral that is naturally present in many foods such as heme and nonheme iron (Hurrell & Egli, 2010). Heme iron is bound into a heme molecule which is part of hemoglobin, whereas nonheme iron is an iron that is not attached to a heme molecule. The iron found in meat, fish and poultry is mainly heme iron (World Health Organization, 2004) and accounts for about 10% of the average daily iron intake, with an absorption rate of about 15-35% (Hurrell & Egli, 2010). On the other hand, iron found in foods derived from plants such as vegetables, fruits, nuts, legumes, and grains, as well as the iron found in milk and eggs is nonheme iron (World Health Organization, 2004). It accounts for the remaining 90% of the average daily iron intake, but it is less well absorbed with an absorption rate of about 2-20% (Hurrell & Egli, 2010). Some factors are known to increase nonheme iron absorption, such as ascorbic acid (vitamin C) by reducing ferric iron to ferrous iron, a more soluble and absorbable state, and by acting as a weak chelator (Li et al., 2020; S. R. Lynch & Cook, 1980). Therefore, it is recommended to consume a source of vitamin C when consuming a source of nonheme iron from plant-based foods. It is important to note that cooking, manufacturing, and storage all destroy the ascorbic acid and therefore, take away its ability to increase nonheme iron absorption (Teucher et al., 2004). In addition, poultry, fish, and meat are also known to increase nonheme iron absorption; however the exact mechanism of how it does so remains unclear (Bach Kristensen et al., 2005; Björn-Rasmussen & Hallberg, 1979; Reddy et al., 2006). In contrast, some factors are shown to decrease and/or inhibit iron absorption; these include phytates found in whole grains, seeds, legumes and some nuts as they bind themselves to iron and form insoluble complex that cannot be absorbed (Hallberg et al., 1989), polyphenols found in vegetables, fruits, some cereals, tea, coffee, and wine for the same reason mentioned above (Abbaspour et al., 2014; Siegenberg et al., 1991), calcium from dairy products as it competes for the same receptor as iron (Lönnerdal, 2010), and protein found in milk (whey and casein) and eggs (albumin) as it binds itself to iron slowing down iron absorption (Cook & Monsen, 1976). Moreover, due to competition for similar pathways for absorption, excessive intake of zinc can reduce iron absorption (Whittaker, 1998) and copper may interfere with iron absorption by binding mucosal transferrin (Collins et al., 2010). Overall, individuals who chose to adopt a plant-based diet are at higher risk of developing IDA since plant foods consist primarily of less well-absorbed iron and contain higher amounts of compounds such as phytates and polyphenols that reduce iron absorption, thus reducing its bioavailability.

2.5.2 Metabolism and functions

Iron is an abundant element on earth and an essential element for almost all living species, including the human body, as it has no means of creating its supply (J. Beard & Han, 2009). Iron absorption mainly happens in the duodenum and upper jejunum (Muir & Hopfer, 1985). Once digested and having made its way to the duodenum, the heme-containing iron from meat, fish, or poultry will be transported directly through the heme transporter into the enterocyte (J. Wang & Pantopoulos, 2011). Once in the enterocyte, the heme molecule will be hydrolyzed to release ferrous iron (Fe^{2+}) (Muir & Hopfer, 1985). As for the nonheme iron mainly contained in plant foods, iron tends to be mostly in its ferric form (Fe^{3+}) -an unstable form- which will need to be converted to its Fe^{2+} form – a more stable form- by duodenal cytochrome b (DCYTB) for absorption. Then, iron can be transported into the enterocyte through the divalent cation transporter 1 (DMT1) (Abbaspour et al., 2014). The Fe^{2+} found in the enterocyte will either be stored as ferritin and used for energy metabolism or transported across the basolateral membrane by the transporter ferroportin, which is inhibited by hepcidin (main regulator of iron homeostasis secreted by the liver) (Abbaspour et al., 2014). To enter and circulate in the bloodstream, the Fe^{2+} will first be oxidized back to Fe^{3+} by hephaestin and then bind itself to transferrin (iron transport protein) (Abbaspour et al., 2014).

In humans, 65% of iron is found in hemoglobin (Hb) in the bone marrow (Trumbo et al., 2001). Here, transferrin will bind itself to transferrin receptors on the erythroblast to release iron for hemoglobin synthesis and red blood cell production. When the red blood cells die, macrophages remove them, the Hb gets degraded, and the iron is released. Once again, the iron is either stored as ferritin or released back into circulation by ferroportin and used for the synthesis of myoglobin part of muscle tissue or the synthesis of enzymes (Theil et al., 2012). Some iron is also stored in the liver as ferritin (Abbaspour et al., 2014).

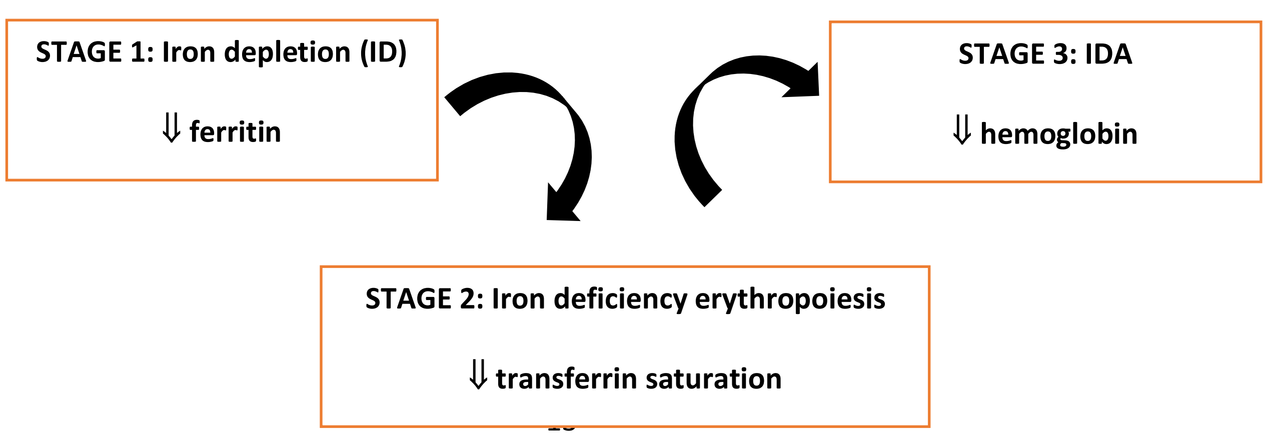
Iron is an oxidative substance and will therefore always be attached to protein to prevent the liberation of free radicals. As there are no known mechanisms to secrete excess iron, which could have negative effects due to its oxidative properties, iron storage and use are well regulated by the peptide hormone, hepcidin (Finberg, 2011). Plasma levels of hepcidin are influenced by different stimuli such as anemia and plasma iron. If iron levels begin to rise, hepcidin will bind ferroportin (iron transporter found on the cells of the duodenum, macrophages, and placenta cells) and cause the destruction of ferroportin and thus, entry of iron into the plasma. In contrast, if iron levels began to decline, there will be a decreased expression of hepcidin, an increased cell surface of ferroportin and an increased entry of iron into the plasma (Nemeth & Ganz, 2006).

2.5.3 Stages of IDA & laboratory diagnosis of ID & IDA

Iron deficiency anemia occurs in three different stages (Mahan, L. Kathleen., Raymond, Janice L., 2017) as illustrated in Figure 4. The first stage is iron depletion in which iron stores will begin to decrease and there will be a reduction in ferritin levels. The second stage is iron-deficiency erythropoiesis, where iron transport will be decreased, and transferrin will be reduced. The last stage is IDA where there is a decreased production of normal red blood cells, a reduced production of heme and an inadequate amount of Hb to transport oxygen (Sim et al., 2019). At this last stage, evident signs and symptoms of iron deficiency anemia will be apparent including pale skin, fatigue, reduced work performance, and impaired immune and cognitive functions (S.-R. S. Pasricha et al., 2010; Sim et al., 2019a). Initially, research suggested that symptoms only began to show in the later stages of IDA; however some studies are suggesting that symptoms such as reduced performance can begin as early as stage one with a reduction in ferritin levels (DellaValle & Haas, 2011; Fernández-Lázaro et al., 2020; Rogerson, 2017; Sim et al., 2019).

Figure 2

The three stages of IDA



Different diagnostic criteria may be used to define both ID and IDA. For instance, in a recent study among athletes, ID was defined as serum ferritin (SF) levels <30 ug/L (Sims et al., 2022), while ferritin levels <35 ug/L were considered low and <12 ug/L as critically low in an earlier study (Koehler et al., 2012). The diagnostic criteria for IDA and ID among recreationally active males and females were defined as SF levels<16 ug/L and Hb <120 g/L and SF levels equal or <16 ug/L and Hb >120 g/L, respectively (Sinclair & Hinton, 2005). Lastly, a narrative review considering iron status for athletes defined ID as ferritin <35 ug/L, Hb >115 g/L, transferrin saturation (TS) >16% and IDA as ferritin <12 ug/L, Hb <115 g/L TS <16% (Sim et al., 2019). Therefore, in this present study, the diagnostic criteria to define ID and IDA were a SF of <35 ug/L, a Hb>115 g/L and a TS >16% and a Hb of <115 g/L, a SF of <12 ug/L and a TS of <16%, respectively.

When assessing athletes' iron status, it is crucial to measure serum ferritin levels as an indicator of iron stores, which allows for the identification of individuals who are at higher risk of developing IDA. Subsequently, this can guide the appropriate care and treatments to replenish their stores and prevent IDA and further complications (Bouri & Martin, 2018). Although ferritin is an acute phase reactant and levels could be misleading in the presence of inflammation (Henwood et al., 2015), it remains an adequate screening method and should be looked at in combination with other blood indicators such as Hb and TS (Sim et al., 2019).

2.5.4 Impact of iron on sports performance

Due to iron's important role in energy metabolism and body oxygen transportation (J. L. Beard, 2001), the athlete's iron dietary intake can have a major effect on physical performance, training, and resistance, particularly in endurance sports (Alaunyte et al., 2015; Hinton, 2014). Therefore, iron depletion with or without anemia can impact physical performance by decreasing maximal oxygen update during physical activity (Fernández-Lázaro et al., 2020). The relationship between physical activity, physical performance, and iron status was examined in 109 women aged 18-45 years old. The results found that after controlling for fat-free mass and intense physical activity, females with ID had a significantly lower maximum rate of oxygen used by the body (VO_2 max) during physical activity than those with normal iron levels (P=0.05) (Crouter et al., 2012). Moreover, another study determining the impact of iron depletion without anemia on performance in 165 female rowers from five colleges and universities had 30% of the nonanemic iron depleted (SF <20 ug/L) rowers reporting being 21 seconds slower in their two kilometer times than rowers with normal iron status (DellaValle & Haas, 2011). Lastly, a reduction in ferritin levels is also shown to increase fatigue, delay skeletal muscle recovery, and decrease strength (Fernández-Lázaro et al., 2020). IDA can strongly limit sports performance by strongly reducing oxygen transport to the exercising muscle and increasing anaerobic metabolism, thus lowering blood pH and depleting the muscle of glycogen (Sim et al., 2019). A slight decrease in Hb levels can decrease exercise power by 20% (Deakin & Peeling, 2015). Overall, athletes must maintain adequate iron status, including their iron stores, to maintain adequate sports performance.

2.5.5 Nutritional status of iron in female athletes according to dietary patterns

According to the literature, female athletes, especially those participating in endurance or aesthetic sports, are routinely diagnosed with ID (Bass & McClung, 2011; Beck et al., 2021; Deakin & Peeling, 2015; McClung et al., 2014). A recent study examining SF levels in 39 female university student runners aged 18-25 found that all but two athletes had SF levels below 35 ug/L (DiSilvestro et al., 2020). Female athletes are particularly at higher risk of iron deficiency than male athletes due to menstrual bleeding (Badenhorst et al., 2021; McClung et al., 2014; Sim et al., 2019). In addition, many female athletes do not meet the recommended daily iron intake even though they consume higher energy diets (Alaunyte et al., 2014; Koehler et al., 2012). Whereas men tend to consume more iron and have lower requirements as they hold greater storing capacity than women and do not menstruate. Therefore, they are more likely to meet and even exceed their recommendations (Hentze et al., 2010). The prevalence of iron deficiency in athletes is around 15-35% for women compared to only 3-11% for men (Badenhorst et al., 2021; Nabhan et al., 2020). A retrospective cohort study looking at 1085 elite adult athletes' electronic medical records found that 52% of female athletes had suboptimal iron status (<35 ng/ml) compared to 15% of males (Nabhan et al., 2020). Consequently, the International Olympic Committee Consensus Statement on Period Health Evaluation of Elite Athletes rationalized the inclusion of a routine hematological assessment due to the higher prevalence of decreased iron stores among athletes with an emphasis on female athletes (Arne et al., 2009). Overall, regardless of gender, the athletes' iron requirements are higher due to several factors influencing iron metabolism such as erythropoiesis, hemolysis, hematuria, gastrointestinal bleeding, urinary losses, inflammatory processes, and anti-inflammatory drugs (McClung et al., 2014; Pedlar et al., 2018; Shaw et al., 2022). The estimated average iron requirement of omnivore athletes increases by 1.3-1.7 times compared to the requirement of non-athletes and can be up to 70% higher during intense training (Whiting & Barabash, 2006). Moreover, the inflammatory response to exercise is associated with the transient increase in the iron regulatory hormone hepcidin, which would decrease its absorption following exercise, therefore its bioavailability (Pedlar et al., 2018; Sim et al., 2019). Thus, all these factors may provide evidence of the ID commonly measured in athletes (Sim et al., 2019). Research has also identified athletes with IDA but with lower prevalence compared to ID. A recent study aiming to determine the frequency of ID in competitive athletes by investigating the data of 629 female and male athletes who presented for their basic sports annual exam found that 35.9% of female athletes had ID (normal Hb, low ferritin) and only 2.1% had IDA (low Hb, low ferritin) (Roy et al., 2022). A 2020 study aiming to quantify and compare iron status in 152 professional female athletes from various senior teams to nonathletes found that 39 athletes (45.9%) had ID compared to three athletes (3.5%) with IDA (Ponorac et al., 2020). Lastly, a cross-sectional study assessing the prevalence of ID and IDA in adolescent athletes engaging in ball games among both sexes (350 males and 126 females) identified 53.2% of females with ID (SF< 30 ug/L) and 4% of females with IDA (SF< 20 ug/L and Hb <12 g/dL) (Nicotra et al., 2023).

Whether or not the dietary pattern of a female athlete has an impact on her iron status is certainly of interest. Specifically in terms of if female athletes following a plant-based diet are at greater risk of developing ID or IDA. The total iron intake of plant-based individuals is similar to or even higher than individuals who adopt a mixed diet (Neufingerl & Eilander, 2021). However, as previously mentioned, the nonheme iron from plants is less bioavailable. It has been estimated that 20-40% of women consuming a diet with moderate to low iron bioavailability, i.e., a plant-based diet, do not meet their iron requirements (Deakin & Peeling, 2015). Thus, SF levels are found to be lower in vegans than in omnivores non-athletes (Shaw et al., 2022). The estimated average iron requirements of vegetarians, among the general population, increases 1.8 times compared to the requirements of non-vegetarians (Canada, 2005). Since high-level physical activity can further increase iron needs, it is reasonable to believe that athletes on a plant-based diet would be at greater risk of developing IDA. Surprisingly, there are not many studies in the literature that have focused on this aspect. Gibson-Smith and colleagues (2020) recently assessed the dietary intake using three-day food diaries reported within a 7-day period and iron status in

40 (20 men and 20 women) elite climbers aged between 18 and 46 years old according to their dietary patterns. As a result, they found that 45% of females had sub-optimal iron status (ferritin <35ug/L and/or transferrin saturation <20%) and that SF was significantly lower in vegan and vegetarian athletes compared to omnivore athletes in females only ($p=0.05$). In addition, Nebl and colleagues (2019) aimed to assess the micronutrient status and related biomarkers for 26 vegetarian and 28 vegan recreational runners compared to 27 omnivores recreational runners of both genders aged between 18 and 35 years old. Supplement intake and type of diet followed was obtained through questionnaires and overnight fasted blood samples were taken to assess micronutrient and related biomarker status. In result, they found that regardless of dietary pattern, mean ferritin levels were in reference ranges for all dietary groups, that iron deficiency (SF <15 ug/L) were only seen in women without significant differences between dietary groups and that biomarkers for iron were not linked to iron supplement intake.

2.6 VITAMIN D

2.6.1 Metabolism, sources, and functions

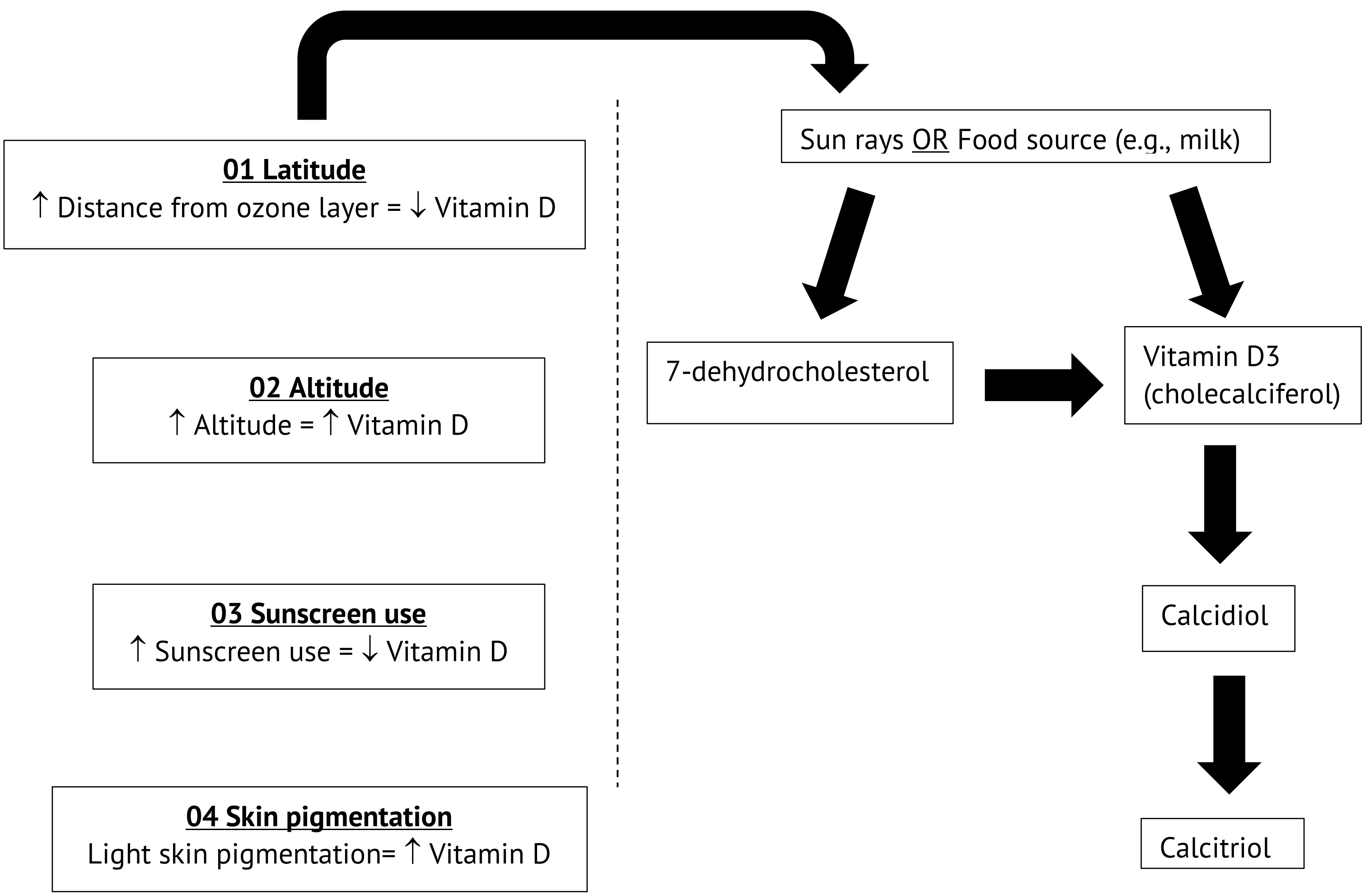
Vitamin D can be obtained naturally through sun exposure, a process by which the sun's energy converts 7-dehydrocholesterol (an intermediate in the synthesis of cholesterol found in the sebaceous glands) into pre-vitamin D_3 (pre-calciferol), then vitamin D_3 (cholecalciferol) (Bikle, 2000). Once cholecalciferol is produced by the skin, it binds itself to vitamin D binding protein (DBP) to make its way to the liver. In the liver, cholecalciferol is converted to calcidiol for which most will be secreted back into the blood bound to DBP. Calcidiol in circulation is the greatest reserve of vitamin D. To be activated, the kidneys need to convert calcidiol into calcitriol (Bikle, 2000).

Sun-induced vitamin D is greatly influenced by several factors including the season, time of day, latitude, altitude, skin pigmentation, and sunscreen use (Wacker & Holick, 2013). People who live further North from the ozone layer, including people living in Canada, often cannot make any vitamin D_3 through their skin for up to six months of the year since the sun's light is too weak to convert 7-dehydrocholesterol into cholecalciferol. In addition, those living in higher altitudes have a higher D_3 production due to the shorter distance for the sun rays to travel; those who apply sunscreen with an SPF of 30 will have a reduced capacity of skin production of vitamin D_3 as opposed to their counterparts who don't use sunscreen, and those with lighter pigmentation will have better absorption of sun rays than those with a darker pigmentation (Wacker & Holick, 2013).

Vitamin D can also be obtained through foods. The best sources of vitamin D come primarily from animal-based foods such as fish (e.g., salmon, tuna, sardines), eggs, and fortified cow's milk and margarine (Wilson-Barnes et al., 2020). The only plant-based source of vitamin D_2 (ergocalciferol) is mushrooms which appears to be less bioavailable than D_3 (Palacios & Gonzalez, 2014; Trang et al., 1998). For this reason, vitamin D intake is often reduced in vegans (Hansen et al., 2018; Rogerson, 2017; Ströhle & Hahn, 2018). Individuals who adopt a vegan diet and have limited sun exposure may be at nutritional risk for vitamin D deficiency given the limited intake of natural and fortified vitamin D foods (Crowe et al., 2011; Wilson-Barnes et al., 2020). However, inadequate vitamin D status is not only associated with individuals following a plant-based diet but is a common problem in the general population (Palacios & Gonzalez, 2014). Dietary intake data from the 2004 Canadian Community Health Survey (CCHS) identified approximately 90% of Canadians not meeting the nutritional recommendations (Canada, 2008). From 2004 to 2015, the CCHS identified that the usual intake of vitamin D from food has significantly reduced by 1 ug/d in vitamin D supplement nonusers, that the prevalence of vitamin D supplementation has increased by 5 to 15% and that the prevalence of vitamin D inadequacy has decreased from 20% to 14% among vitamin D supplement users compared to nonusers (Vatanparast et al., 2020). The supplementation of vitamin D is often the only way to ensure adequacy (Nebl et al., 2019; Vermeulen et al., 2021).

Figure 3

Sources of vitamin D and factors influencing its absorption



Vitamin D is a fat-soluble vitamin primarily stored in the body's adipose tissue. Therefore, the human body can mobilize its reserve. Some of vitamin D's functions include primarily calcium and phosphorus homeostasis playing an important role in bone health (Bikle, 2014; Wilson-Barnes et al., 2020). When blood calcium levels are low, the parathyroid hormone (PTH) will be secreted by the parathyroid gland into the blood which will stimulate the kidneys to increase calcium reabsorption to increase blood calcium and stimulate the conversion of calcidiol into its active form calcitriol. Calcitriol will stimulate the absorption of calcium in the intestine and once again increase blood levels of calcium. Lastly, an increase in PTH levels will cause an increase of calcitriol to the bones which in turn will increase the resorption of calcium and phosphorus from the bones (Calcium et al., 2011; Silver et al., 1986). Additional roles of vitamin D consist of growth and regulation, immunomodulatory effects (Nieman & Wentz, 2019), cardiovascular effects by decreasing the risk of hypertension, type 2 diabetes, and heart failure (de la Guía-Galipienso et al., 2021), and neuromuscular effects (Gunton & Girgis, 2018).

2.6.2 Vitamin D deficiency & laboratory diagnosis of vitamin D insufficiency & deficiency

The best indicator of vitamin D status is serum concentrations of 25-hydroxy-vitamin-D (25(OH)D) (Holick, 2009). A recent review article published in 2020 indicated levels over 125 nmol/L as toxic, levels between 50-125 nmol/L as adequate, levels between 30-50 nmol/L as insufficient, and levels below 30 nmol/L as deficient (de la Puente Yagüe et al., 2020). Other recent studies looking at vitamin D status in athletes have defined vitamin D deficiency as levels below 25nmol/L and insufficient levels between 25-75nmol/L (Malczewska-Lenczowska et al., 2018; Nebl et al., 2019; Sekel et al., 2020) .

2.6.3 Impact of vitamin D on sports performance

Vitamin D is known to affect muscular function (Gunton & Girgis, 2018), but its effect on strength and power remains inconclusive (Wilson-Barnes et al., 2020). In its active form, vitamin D is believed to regulate the expression of approximately 900 genes (T.-T. Wang et al., 2005) shown to have an impact on some performance-related factors such as glucose metabolism, cardiovascular health, exercise-induced inflammation, neurological function, and bone health (Dahlquist et al., 2015). More specifically, vitamin D's effect on muscles may be linked with satellite cell activity. Indeed, the activation of vitamin D receptors, that mediate biological effects of vitamin D, are present in satellite cells and satellite cells control the future of the cell. As a result, this is said to cause an increase in type II muscle fibres leading to an increase in speed and strength (Hamilton, 2010; Olsson et al., 2016). One study aiming to examine the effects of 5000 UI a day for eight weeks on musculoskeletal performance in a placebo-controlled trial where athletes were randomly allocated using a computer-based random number generator to vitamin D (n=5) or placebo (n=5) treatment either receiving a daily supplement of 5000 UI of vitamin D3 or a cellulose placebo, respectively. In result, they found that there was a significant increase in the 10-meter sprint times (P=0.008) and vertical jump (P=0.008) in the group taking vitamin D compared to the placebo group (Close et al., 2013). Another possible influence of vitamin D on performance could be linked to testosterone concentrations, as vitamin D receptors are transcription factors that also belong to the same nuclear family as testosterone, a hormone well proven to enhance performance by reducing body fat and increasing muscle growth (Dahlquist et al., 2015). Lastly, vitamin D is crucial for the proper absorption of calcium, which plays a crucial role in muscle contraction and relaxation, directly affecting sports performance and injury preventions (Fuhrman & Ferreri, 2010).

Therefore, vitamin D deficiency can increase the risk of stress fractures and bone disorders in athletes due to its vital role in bone health (Davey et al., 2016; Grieshober et al., 2018).

2.6.4 Nutritional status of vitamin D in athletes according to training environment (latitude/sun exposure) and dietary patterns

According to a study among high-level college athletes, men (62.1%) are more likely to achieve their vitamin D RDAs than women, likely due to their higher caloric intake, higher consumption, and/or increased intake of vitamin D supplements (Leitch et al., 2021). However, daily vitamin D intake in athletes (∼300 IU/day) appears to be well below the RDA of 600 IU (Leitch et al., 2021). Consequently, approximately 56% of global athletes have been found to have inadequate vitamin D status (<32 ng/mL), a proportion obtained in a meta-analysis regrouping 23 studies with 2,313 athletes from both sexes (Farrokhyar et al., 2015). As previously discussed, the vitamin D status of an athlete is also affected by the latitude of the training environment. For instance, a cross-sectional study published in 2020 demonstrated that elite athletes who reside at high latitudes (≥40°N), or who train indoors are at high risk for vitamin D insufficiency (25(OH)D <50 nmol/L), or even deficiency (25(OH)D <25-30 nmol/L) (Wilson-Barnes et al., 2020). In the United States (44.9°N), 37.7% of college athletes had suboptimal vitamin D levels (25(OH)D <80 nmol/L) in the spring (Fitzgerald et al., 2015). Another cross-sectional study at a lower latitude (30.5°N) also identified 50% of a cohort of university athletes with inadequate 25(OH)D values [<87 nmol/l] (Forney et al., 2014). Training outdoors during the summer time will also have a major impact on the athlete's vitamin D status as the vitamin D_3 synthesis during this time is optimal compared to the winter months or if training indoors (Wilson-Barnes et al., 2020). Athletes with darker skin will also be at higher risk of vitamin D deficiency since they do not absorb the sun rays as well as their lighter skin tone colleagues (Beck et al., 2021).

To this date, very few studies have examined vitamin D status, particularly in vegetarian or vegan athletes. One cross-sectional study published in 2019, compared the micronutrient status including the serum vitamin D among 27 omnivores, 26 vegetarians, and 28 vegan recreational runners (men and women) aged between 18-35 years old. A questionnaire was used to assess lifestyle factors and supplement intake and blood tests were conducted after a 10-hour fasting period. More specifically, levels of 25(OH)D were measured in serum and levels <25 nmol/L were considered deficient, levels between 25-50 nmol/L were considered insufficient and levels >75 nmol/L were optimal. The results showed that among the three dietary groups including three omnivore athletes, five vegetarian athletes and five vegan athletes, less than 20% of athletes had levels of 25(OH)D <50 nmol/L. However, all participants who took supplements had 25(OH)D values over 50 nmol/L and the blood vitamin D levels were higher in individuals during summer, without any supplement intake, than during the wintertime (P=0.021). Furthermore, only two athletes (7%) were found to be deficient and followed a vegan dietary pattern. Thus, this study suggests, based on the results, that a well-planned vegetarian or vegan diet including supplements can meet the athlete’s vitamin D requirements (Nebl et al., 2019). In contrast, other studies among nonathletes have found that adopting a vegan diet might predispose individuals to macronutrient and micronutrient (vitamin D) deficiencies (Appleby & Key, 2016; Hansen et al., 2018; Marsh et al., 2012).

Considering the current state of knowledge and the importance of optimal nutritional status in athletes, the current thesis aims to examine the iron and vitamin D status in varsity female Gee-Gee athletes and the possible relationship with their dietary patterns.

CHAPTER 3

RESEARCH HYPOTHESES & OBJECTIVES

3.1 Main objective:

This research project aims to examine the nutritional status of iron and vitamin D in varsity female athletes from the University of Ottawa and to explore its possible relationship with their dietary patterns: mixed diet and plant-based diet (vegetarian or vegan).

3.2 Research hypotheses:

1. A quarter of the athletes will have adopted a plant-based diet.
2. More than half the female athletes will not meet the EAR for vitamin D and iron.
3. The prevalence of IDA will be negligible, but the prevalence of ID will be around 30% and more prevalent in plant-based participants.
4. The prevalence of vitamin D deficiency will be negligible, but the prevalence of vitamin D insufficiency will be around 50%, more prevalent in the plant-based participants and those playing indoor sports.

3.3 Specific objectives:

1. To determine the proportion of female athletes adopting a mixed diet compared to a plant-based diet.
2. To determine the prevalence of ID and IDA among participants.
3. To determine the prevalence of vitamin D insufficiency and deficiency among participants.
4. To determine the proportion of female athletes who do not meet the EAR for iron and vitamin D.

CHAPTER 4

METHODOLOGY

At the University of Ottawa, sports such as basketball, hockey, rugby, soccer, swimming, volleyball, track, and cross country are varsity or high-performance sports for women.

4.1 Current medical and nutritional services

High performance athletes from the University of Ottawa have access to a sports team dietitian. Their main role is to support the athletes by providing educational nutrition workshops on a variety of relevant topics such as how to prepare meals and how to properly hydrate and fuel before, during, and after exercising, competitions, or while on the road. They also offer cooking classes, one-on-one counselling sessions, prepare menus for athletes, and support the coaches with their expertise when needed.

Currently, the sports physicians responsible for the University's female athletes, follow the International Olympic Committee (IOC) guidelines for pre-participation physicals. According to the IOC guidelines, the physical exam should include an assessment of pulmonary, hematological, dermatological, urological, gastrointestinal, neurological, and endocrine systems, as well as assess for any allergies, infections, and ears, nose, and throat status. It should also include an assessment of the athlete's medical history to identify the presence of any non-cardiac conditions and blood tests consisting of a Complete Blood Count (CBC) and ferritin levels to assess for ID and IDA (Arne et al., 2009). With regards to treatments, athletes from the University of Ottawa who suffer from IDA (<35ug/L) are given oral iron supplementation and those who suffer from ID (35-50 ug/L) are given nutritional counselling with the goal of re-establishing iron levels through iron-rich and bioavailable foods. It is important to note that only first-year athletes are targeted for these pre-participation physical exams. Therefore, athletes in the 2nd, 3rd, and 4th years are not being systematically targeted for follow-up. In addition, vitamin D levels are not included in the IOC guidelines.

4.2 Study design, sample characteristics, and methods of recruitment

This study used a cross-sectional quantitative descriptive research method design. All female athletes participating in a high-intensity varsity sport such as volleyball, basketball, rugby, swimming, hockey, soccer, and track and cross country were invited to participate in the study. To be eligible to participate, athletes were required to speak French and/or English. It was also required for them to be available to complete two 24-hour recalls, the online questionnaire and consent to have a blood sample taken. Overall, 63 athletes demonstrated interest in participating, however only 17 completed all the necessary steps of the study (volleyball: n=4, rugby: n=4, swimming: n= 5, hockey: n=2, soccer n=2). Six participants withdrew from the study due to scheduling conflicts, academic priorities, and physical illness. Forty athletes did not provide any reasons for withdrawal.

An initial email was sent out to all the coaches of the different sports teams to describe the research project and to arrange a brief, in-person meeting with the athletes either prior to or after one of their practices. During each of the meetings, the current study's researchers (i.e., Professor France Rioux and myself, Thalie Soulière, M.A. student) provided a brief PowerPoint presentation describing the purpose of the study and the nature of the athlete's participation in the project. Afterwards, the athletes received a participation form requesting their name, email address, telephone number and to indicate whether they were interested in participating. It was also requested that they specify their dietary preferences (i.e., a vegan diet defined as consuming no foods from animal origin, a vegetarian diet defined as consuming eggs and dairy products but no meat, fish, or poultry, or a mixed diet defined as consuming a wide variety of animal and plant-based foods). Athletes were provided with the option to leave the form following the meeting or to drop it off, within 24 hours, at Professor France Rioux's office located on campus as indicated on the form.

The athletes that expressed interest in participating received an email outlining the instructions on the tasks they were required to complete, which included: 1) to complete, sign, and return the consent form attached, 2) to complete a short Survey Monkey questionnaire as provided in the email, 3) to complete the two 24-hour recalls using ASA24 website for which a demonstration video, a username, and password were provided, and 4) to attend a Dynacare clinic for a blood test with the requisition form attached to the email. Athletes were given a time frame of two weeks to complete the above tasks and were then advised to inform the researchers upon completion. This study was approved by the University of Ottawa ethics committee.

4.3 Blood collection and biochemical analysis

The blood samples were collected at one of the three Dynacare locations (e.i.,100 Marie Curie Private, 381 Kent St, and 150 Montreal Rd in Ottawa, Ontario) from October 3rd of 2022, up until December 30th of 2022. The requisition form required for the athlete's blood tests was provided by Dynacare.

The blood analysis included a Complete Blood Count (CBC) consisting of Hb, hematocrit (Hct), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell count (WBC), WBC Differential (absolute), platelet count and mean platelet volume (MPV). It also included SF, TS, and 25(OH)D.

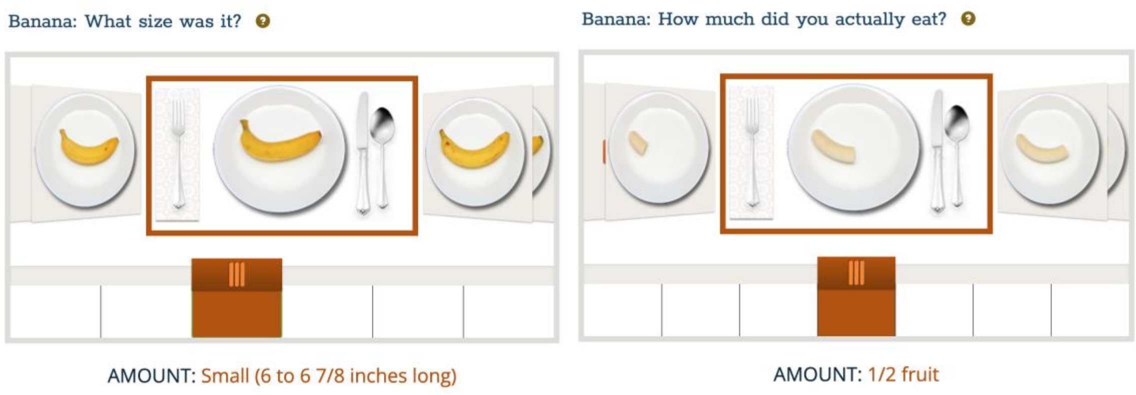
IDA was defined as having all three of the following criteria: a Hb of <115 g/L, a SF of <12 ug/L and a TS of <16%. ID was defined as having all three of the following criteria: a SF of <35 ug/L, a Hb>115 g/L and a TS >16%. The diagnostic criteria for the nutritional status of iron were based on both literature findings and the Dynacare clinic laboratory reference values. The Dynacare clinics defined an insufficient level of vitamin D between 25-75 nmol/L, a deficient level below <25 nmol/L and an adequate level between 76-250 nmol/L. Accordingly, vitamin D deficiency was characterized in our study by a serum vitamin D level of <25 nmol/L and insufficiency by a serum level between 25-75 nmol/L.

4.4 Food intake estimation tool

The current research project has used the Automated Self-Administered 24-hour Dietary Assessment Tool to quantify athletes' nutritional intakes for the following reasons. Firstly, ASA24 is a free web-based tool provided by the National Cancer Institute, that generates multiple, automatically coded self-administered 24-hour recalls and/or multi-day food diaries (ASA24&Reg; Dietary Assessment Tool | EGRP/DCCPS/NCI/NIH, 2022). It consists of two websites, one for the researchers to manage study logistics and access all data on respondent's intake and the other for the participants to enter food intake data. This tool allows researchers to select one or several recalls per participant and allows respondents to search for any foods, beverages, or supplements. Additionally, it uses images to help respondents estimate portion sizes (see Figure 2), has several reminders to indicate if any food was forgotten and instructions on how to proceed. Lastly, it is available in both French and English.

Figure 4

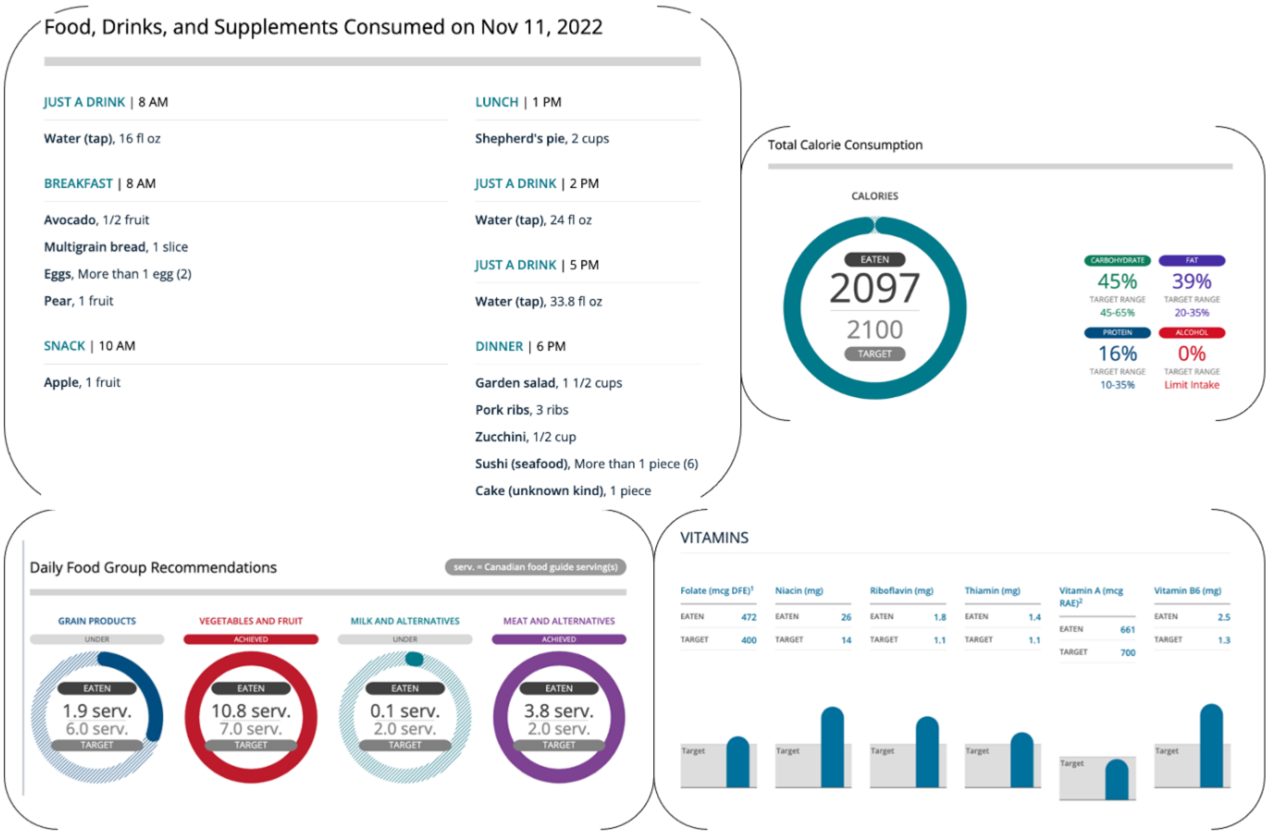
ASA24 portion and size guide reference



Through ASA24, a Respondent Nutrition Report (RNR) is generated for each participant to compare the person's daily nutritional intake to Canadian-specific dietary norms such as the Dietary Reference Intakes (DRI) (ASA24&Reg; Dietary Assessment Tool | EGRP/DCCPS/NCI/NIH, 2022)**.** An example report can be found in Figure 3. Not all the elements from the full report are included in this figure.

Figure 5

Example RNR from a participant



The most recent version of the software (i.e., ASA24-Canada-2018) was selected. This version of the tool allowed respondents to report, save, and modify recipes and includes in the RNR a comparison of daily intakes with the 2007 Canadian Dietary Guidelines and the RDAs. Moreover, the user interface from this version includes more user-friendly features such as increased accessibility on all web browsers and enables data on supplements at the same time as food and beverages rather than in a separate module.

The nutrient database uses the Canadian Nutrient File and a Canadian recipe database used for surveillance by Health Canada. Portion sizes are adapted to reflect the Canadian food supply and dietary supplements database is based on the Licensed Natural Health Product Database and supplemented by the NHANES Dietary Supplement Database 2007-2008.

An unscheduled recall study was selected, which allows participants to log in at any time to report all food eaten within the previous 24 hours until the maximum number of recalls was reached. The two-recalls needed to include one weekday and one weekend during training or competition and all supplements/vitamins taken, if any. We allowed the study to run from September 19th, 2022, to December 23rd, 2022. Lastly, through ASA24, an option was given to the researchers to either allow or deny access to the athletes to obtain their nutritional analysis, also known as the Respondent Nutrition Report (RNR), following the completion of their recall. To control for any potential biases regarding the completion of the second recall, it was decided that athletes be denied access to their RNR at the end. Those wishing to obtain their RNRs, received them once the study was completed.

4.5 Structured online questionnaire

A brief, 11-question, bilingual questionnaire was created using Survey Monkey and was administered to obtain general information on the athletes, such as age, current weight and height, current sport enrolled in, current club year enrolled in, number of children (if any), description of menstrual cycle, use of contraceptives, presence of medical conditions, skin pigmentation, and previous history of IDA (Appendix A).

4.6 Statistical analysis

Since the current study's sample size was small (n=17), the only descriptive statistical analyses that were performed included means, standard deviations (SD), and proportions expressed as percentages (Microsoft Excel version 16.69.1).

CHAPTER 5

RESULTS

SUBJECTS

Objective 1: To determine the proportion of female athletes adopting a mixed diet compared to a plant-based diet.

Sixty-three athletes in total demonstrated interest in participating in the study; however, only 17 completed the entire study. Out of these 63 athletes, 58 (92%) followed a mixed diet and five (8%) followed a mostly plant-based diet. No athlete from the basketball, track, or cross-country teams were able to complete the study in its entirety.

Table 1 outlines some characteristics of our female elite athletes obtained through our Survey Monkey questionnaire.

Table 1

Characteristics of the athlete participants

Characteristics	*N* = 17*
	Mean±SD
Age (years)	19.47±1.23
Diet	*n*
Mixed diet	15
Mostly plant-based diet	2
Sports year	
1st year	5
2nd year	5
3rd year	3
4th year	4
Contraceptive use	
Yes	7
No	9
Children	
Yes	0

No	17
<u>Skin tone</u>	
Extremely fair skin tone	1
Fair skin tone	11
Medium skin tone	2
Olive skin tone	2
Dark skin tone	1
Extremely dark skin tone	0
<u>Menstrual cycle</u>	
No period	2
Light	2
Medium – regular	11
Heavy	2
<u>Presence of medical condition</u>	
Yes	0
No	17
<u>Previous diagnosis of IDA</u>	
Yes	1
No	16

*Volleyball: n=4, rugby: n=4, swimming: n= 5, hockey: n=2, soccer n=2.

Out of our sample of 17 athletes including athletes from all four sports years, 41% reported using a contraceptive method, 100% reported having no children, 65% reported having fair skin, and 65% reported having a medium regular menstruation cycle. Finally, all athletes reported having no medical condition aside from two athletes reporting having Raynaud's phenomenon (i.e., "spasms in small blood vessels in [the] fingers and toes [that] limits blood flow and leads to symptoms like skin colour changes, cold skin and a pins and needles sensation") (*Raynaud's Syndrome*, n.d.). As for any history of IDA among the athletes, one reported having a previous history of IDA in May of 2022 that was treated with ferrous sulfate supplementation. According to the blood test from our study, this athlete currently shows normal levels of iron (hemoglobin of 135g/L, serum ferritin of 58ug/L, transferrin saturation of 38%). Another athlete who reported having previously low ferritin levels still has insufficient ferritin

(18 ug/L) as well as low transferrin saturation (17%) according to Dynacare reference values, but normal hemoglobin (128 g/L). Both these athletes are not mostly plant-based athletes.

BLOOD ANALYSIS

Objective 2: To determine the prevalence of ID and IDA among participants.

Objective 3: To determine the prevalence of vitamin D insufficiency and deficiency among participants.

Table 2 demonstrates the athlete's hematology for iron and vitamin D status in 19 female athletes. An additional two athletes are included in this table since they had completed the blood test component of the study, however, did not complete the entire study. In general, the mean values for each biochemical variable among participants were within reference ranges.

Table 2

Indices of iron and vitamin D status in 19 female athletes

Variables	Units	Mean Values±SD	Reference Values
Iron	umol/L	21.37±7.02	6-27
TIBC*	umol/L	64.00±10.61	45-77
Transferrin Saturation	%	0.34±0.13	0.20-0.50
Ferritin	ug/L	52.53±26.23	12-105
25(OH)D	nmol/L	77.31±26.17	76-250
Hemoglobin (Hb)	g/L	131.16±7.22	110-147
Hematocrit (Ht)	%	0.40±0.02	0.33-0.44

*Total Iron Binding Concentration

Table 3 provides the proportion of female athletes who had either insufficient or deficient levels of vitamin D and iron.

Table 3

Prevalence of iron and vitamin D insufficiency and deficiency

Variables	Prevalence (*n* (%))	Refences values
Vitamin D		
Insufficiency	9(47)	25-75 nmol/L
Deficiency	1 (5)	<25nmol/L
Iron		
Iron deficiency	5 (26)	SF*<35 ug/L, Hb**>115 g/L, TS*** >16%)
Iron deficiency anemia	0 (0)	SF <12 ug/L, Hb <115 g/L, TS <16%)

*Serum ferritin
**Hemoglobin
***Transferrin saturation

Our results are showing that almost half (47%) of the athletes had insufficient blood levels of vitamin D and only one was found to be deficient. Out of the nine athletes from all four sports years who had insufficient levels of vitamin D, one followed a vegetarian diet and took supplements (iron, vitamin D, vitamin B_{12}), and the remainder followed a mixed diet and took no supplements aside from one athlete who took a calcium and a multivitamin supplement. Among the athletes with abnormal vitamin D levels, six played indoors sports (volleyball, basketball, or swimming) and four (including the vitamin D deficient athlete) played outdoor sports such as soccer or rugby. Moreover, considering that limited sun exposure in the winter months has an impact on vitamin D status, we observed that among the 10 athletes tested in early fall (October), three (30%) of them had insufficient levels and only one (10%) had deficient levels of vitamin D. In November, four out of six (67%) athletes tested had insufficient levels and in December, two out of three (67%) athletes tested had insufficient levels. No athlete suffered from vitamin D deficiency during the winter months.

For the iron status, no athletes suffered from IDA, but five did have ID. Out of the five athletes who had ID, one was following a vegan diet and took supplements (iron and vitamin

B_{12}), and the others followed a mixed diet and took no supplements. Moreover, out of these five athletes, two were in their 1st sports year, one was in their 2nd sports year, and two were in their 4th sports year.

FOOD INTAKE

Objective 4: To determine the proportion of female athletes who do not meet the EAR for iron and vitamin D.

Table 4 summarizes the athletes' iron and vitamin D intake from food only based on the average of two 24h recalls including one weekday and one weekend day with ASA24 web-based tool. Other nutrients of interest are also included in this table due to their direct links in iron (vitamin C) or vitamin D (calcium) metabolism. As portrayed in the table, all athletes, with the exception of one, are below the EAR for vitamin D. In contrast, all athletes, with the exception of one, were able to meet the EAR for iron with one athlete even surpassing the UL. Considering other nutrients of interest presented in the table, over 80% of the athletes were able to meet the EARs through foods.

Table 4

Female athlete's iron and vitamin D nutritional intake in comparison with Dietary Reference Intakes (DRI)

Measures	Units	Mean values±SD	EAR*	UL**	Prevalence of females below EAR *n* (%)	Prevalence of females exceeding UL *n* (%)
Iron	**mg**	**21.5±9.8**	**8.1**	**45**	**1 (6%)**	**1 (6%)**
Vitamin D	**IU**	**224±162**	**400**	**4000**	**16 (94%)**	**0 (0%)**

Vitamin C	mg	184±106	60	2000	2 (12%)	0 (0%)
Calcium	mg	1387±724	800	2500	2 (12%)	2 (12%)

*Estimated Average Requirements used to assess the adequacy of nutrient intake of groups
** Upper Limit

Table 5 shows the athlete's mean intake of iron, vitamin D, vitamin C, and calcium as supplements based on the average of two 24h recalls including one weekday and one weekend day with the ASA24 web-based tool. As portrayed in the table, the supplements most frequently used were iron and vitamin D, followed by vitamin C, and lastly calcium.

Table 5

Female athlete's supplement intake of iron, vitamin D, vitamin C and calcium

Supplement	Units	Number of athlete's reported using	Mean intake±SD
Iron	mg	4	38±17
Vitamin D	IU	4	1350±790
Vitamin C	mg	3	702±1124
Calcium	mg	1	600±0

Table 6 summarizes the athletes' food and supplement intake combined based on the average of two 24h recalls including one weekday and one weekend day with the ASA24 web-based tool. As shown in this table, the use of vitamin D supplements helped more athletes attain the EAR. In contrast, for iron, the use of supplements increased the number of athletes surpassing UL to 4 (23%). No changes were noted for vitamin C and calcium.

Table 6

Female athlete's food and supplement intake combined in comparison with Dietary Reference Intakes (DRI)

Measures	Units	Mean values±SD	EAR*	UL**	Prevalence of females below EAR *n* (%)	Prevalence of females exceeding UL *n* (%)
Iron	**mg**	**28.1±17**	**8.1**	**45**	**1 (6%)**	**4 (23%)**
Vitamin D	**IU**	**383±369**	**400**	**4000**	**12 (70%)**	**0 (0%)**
Vitamin C	mg	247±266	60	2000	2 (12%)	0 (%)
Calcium	mg	1404±713	800	2500	2 (12%)	2 (12%)

*Estimated Average Requirements used to assess adequacy of nutrient intake of groups

** Upper Limit

CHAPTER 6

DISCUSSION

The main objective of this study was to examine the nutritional status of both iron and vitamin D in female Gee-Gees varsity athletes and to explore the possible relationship with the athlete's dietary patterns (i.e., mixed diet and plant-based diets).

Our findings do confirm that high-level women athletes are at a high risk of developing suboptimal nutritional iron and vitamin D status that could eventually lead to deficiencies. Accordingly, among the 19 athletes, as much as 47% (9/19) of them had vitamin D insufficiency, and 26% (5/19) had ID, however, only one participant had vitamin D deficiency, and no one suffered from IDA.

Vitamin D status and intake

As outlined in the literature, low vitamin D status is prevalent in the general population as well as in athletes, in part due to limited exposure to sun-induced vitamin D throughout the winter months (Palacios & Gonzalez, 2014; Wacker & Holick, 2013). A meta-analysis and systematic review, including 23 studies with 2,313 athletes (including both sexes) from the UK, Ireland, Spain, France, Australia and other Middle Eastern countries, found that 44-67% of athletes had vitamin D insufficiency (serum 25 (OH) <80 nmol/L), the risk significantly increasing during winter season (Farrokhyar et al., 2015). Based on the literature, it was hypothesized that the prevalence of vitamin D deficiency would be negligible, but that the prevalence of vitamin D insufficiency would be around 50%. Indeed, our study found that almost half of the female athletes had insufficient levels of vitamin D and only one athlete (5%) suffered from vitamin D deficiency. Similarly, a cross-sectional study providing a comprehensive assessment of nutritional status in 34 junior elite Canadian female soccer players from Victoria, British Columbia found that 50% of the athletes had suboptimal vitamin D levels (<75 nmol/L), but none of the athletes had vitamin D deficiency (<25 nmol/L) (Gibson et al., 2011a). It is important to emphasize that most participants in our study reported having fair skin compared to dark skin, which is known to better absorb the sun-induced vitamin D (Wacker & Holick, 2013). The risk of vitamin D inadequacy has been found to increase in athletes who play indoor sports (Farrokhyar et al., 2015). Therefore, it was hypothesized that suboptimal vitamin D status would be more prevalent in the athletes playing indoor sports compared to those from outdoor sports that can benefit from higher sun exposure (Nair & Maseeh, 2012; Wacker & Holick, 2013). In contrast to previous findings, we found that among 13 athletes playing indoors, six athletes (46%) had suboptimal vitamin D levels compared to four out of six (67%) athletes practising their sports outdoors. Due to our small sample size, it was not possible to determine if these differences in proportion are statistically significant. Lastly, our study was conducted in the late fall and winter, a time when the absorption of sun-induced vitamin D is highly reduced. The prevalence of athletes having compromised vitamin D status would likely have been less if our study had taken place during the summer season.

In terms of intake, the vast majority of athletes, regardless of their dietary eating pattern, did not meet the EAR for vitamin D, which is more than our initial hypothesis. Surprisingly, 94% of athletes did not attain the EAR (400 IU) from food alone. The mean intake was 224 IU. In agreement with our results, an observational study investigating athlete's vitamin D intake and awareness found a mean intake of 263 IU among collegial female athletes which is also below the EAR of 400 IU (Leitch et al., 2021). Gibson et al. (2011) and Vermeulen et al. (2021) also reported that none of the athletes were meeting the EAR. The intake of vitamin D supplements helped athletes meet the requirements which coincides with previous findings from Nebl and colleagues (2019). Accordingly, based on the 2007 CFG, the recommended servings of milk and alternatives were the hardest to attain among our participants. Parnell et al. (2016) obtained similar findings with several female athletes aged 14-18 years old not meeting the recommended servings for the milk and alternative food group also based on the 2007 CFG. As previously discussed, dairy products are good sources of calcium and vitamin D, two nutrients that are crucial for optimal bone health (Holick, 2009), and thus are important for athletes. A great deal of controversy over dairy products has been observed in the media in the past few years, which could provide some explanation as to why individuals tend to consume less of these products. A cross-sectional study evaluated the trends in the consumption of dairy milk in Canadians using nationally representative data from the 2004 and 2015 Canadian Community Health Survey and found that the consumption of plain milk by Canadians has declined from 70.2% to 56.1% (Islam et al., 2021). Another potential reason could involve the new CFG, where dairy products (and substitutes) have been added to the protein foods category; our female athletes may assume that any foods available in that category can act as a substitute for dairy products and alternatives.

Iron status and intake

As hypothesized, the prevalence of IDA was negligible in our female athletes and the prevalence of ID was slightly below a third. Other studies have also identified little to no athletes with IDA, but a higher prevalence of ID. Gibson and colleagues (2011) found that among 34 junior elite Canadian female soccer players, 89% had ID (SF<35 ug/L, Hb >117 g/L) and only one athlete (3.6%) presented with IDA (SF<12 ug/L, Hb <117 g/L). Another research among top-level female athletes from various sports (handball, soccer, tennis, skating, wrestling) with an age-matched group of female nonathletes identified 52% (30/57) of athletes with ID compared to only 8.6% (5/57) of athletes with IDA (Sandström et al., 2012). Lastly, a study assessing the micronutrient status of recreational vegan, vegetarian, and non-vegetarian runners identified approximately 30% of runners with ID in each diet group and no athlete presenting with IDA (Nebl et al., 2019).

In terms of iron intake from food only, all athletes, aside from one, were able to meet the EAR for iron which is much higher than anticipated and contradicts the literature. The tool ASA24 does not consider physical activity which is known to increase the iron needs of athletes (Whiting & Barabash, 2006). While there is a set EAR for vegetarians of 14.6 mg iron (1.8 times the requirements), there is no EAR for female athletes (Alaunyte et al., 2015; Whiting & Barabash, 2006). In comparison, diet assessment in young athletic female populations from older studies found higher proportions of athletes not meeting the iron requirements (Dwyer et al., 2012; Gibson et al., 2011b; Koehler et al., 2012; Papadopoulou et al., 2002). More recent studies found that as much as 100% of female athletes were not respecting the iron requirements (Condo et al., 2019) or 80% of athletes not respecting the minimum requirements (Gibson-Smith et al., 2020). Both recent studies did not take into consideration if women were taking a contraceptive method known to reduce menstruation, and thus iron loss for which almost half the athletes from our study reported using a contraceptive method. Another study considering the influence of a low-dose oral contraceptive on menstrual blood loss in 20 young women nonathletes found that there was approximately 50% less blood loss in women taking the oral contraceptive compared to the women that were not and that the oral contraceptive increased serum ferritin levels by about 25% in participants that had low SF levels (<10 ug/L) (Larsson et al., 1992). When considering the intake from food and supplement combined, 29% of athletes are not respecting the DRIs (one being below the EAR and four surpassing the UL). Although iron supplementation has been shown to improve aerobic capacity in athletes with IDA (S.-R. Pasricha et al., 2014;

Zourdos et al., 2015), the literature states that iron supplementation does not benefit athletes with normal iron status and may lead to exceeding UL as observed in our study (Nichols et al., 2023).

Other micronutrients

In terms of other nutrients of interest in our study, 88% of athletes were able to meet the EAR for vitamin C with and without the use of supplements. These results are similar to another study using the same dietary assessment tool (ASA24) but among Australian female football players. They found that 83% were able to meet the minimum vitamin C requirements (Condo et al., 2019). Moreover, similarly to our study, Vermeulen et al. (2021) identified 83% of athletes meeting the vitamin C requirements. For calcium, 88% were able to meet the minimum requirements with two athletes surpassing UL with foods only. This contradicts the literature as other studies indicate 65.5% (Condo et al., 2019) and 78% not meeting the recommendations for calcium. This could be in part because these studies used higher recommendations as reference (1000 mg as opposed to 600 mg) and due to our small sample size.

Supplement intake

Almost half of the athletes (41%) did report taking supplements. This is lower than previous findings indicating that 87-100% of Canadian athletes are taking supplements (Kristiansen et al., 2005; Lun et al., 2012; Parnell et al., 2016; Wiens et al., 2014). The literature states that athletes tend to take more supplements to meet their needs and enhance their performance (Knapik et al., 2016; Krzywański et al., 2020; Wiens et al., 2014). It is important to note that the above Canadian studies, with larger sample sizes, included both sexes and that men are known to consume more supplements compared to women (Aguilar-Navarro et al., 2021), thus possibly explaining the larger prevalence of supplement usage. Finally, except for vitamin D, most athletes regardless of dietary eating pattern, were able to meet their nutrition needs simply through foods. Accordingly, research indicates that vitamin or mineral supplements are often not needed if adequate energy from a variety of foods is consumed to maintain an adequate body weight (Lukaski, 1995; Parnell et al., 2016; Rodriguez et al., 2009).

Plant-based athletes and their nutritional iron and vitamin D status and intake

It was hypothesized that a quarter of the athletes would have adopted a mostly plant-based diet. Although the increasing popularity of this dietary preference among athletes and the health benefits outlined in previous literature, fewer female athletes than anticipated adopted a plant-based diet (Melina et al., 2016; Rogerson, 2017; Sisay et al., 2020). Out of the 63 interested athletes, only three (5%) adopted a vegan diet and two (3%) a vegetarian diet. This demonstrates that the vegan/vegetarian diet may not be as popular as predicted among the Gee-Gees varsity female athletes. Although some athletes are beginning to include more plant-based meals into their diets, it does not appear that many have completely converted to this dietary eating pattern. To the best of our knowledge, there are no Canadian studies that have looked at the proportion of female athletes adopting a vegan/vegetarian diet. Most studies simply consider this type of dietary pattern for athletes along with recommendations (Fuhrman & Ferreri, 2010; H. Lynch et al., 2018; Rogerson, 2017; Vitale & Hueglin, 2021; K. C. Wirnitzer, 2018). In fact, a recent study mentions that no nationally representative Canadian studies have described the prevalence of excluding animal-based foods. Therefore, they aimed to estimate the prevalence of Canadians (nonathletes), aged two and above (n= 20 477), who adhere to plant-based diets using nationally representative data from the 2015 Canadian Community Health Survey. As a result, they found that approximately 5% of Canadians reported adhering to any type of plant-based diet (vegan, vegetarian, pescatarian, or red meat excluder). More specifically, 1.3% reported being vegetarian and 0.3% reported being vegan. The plant-based diet was most reported by individuals of South

Asian cultural identity and individuals with higher educational attainment (Valdes et al., 2021). The data from this study does date from eight years ago, therefore the proportion of Canadians adopting a plant-based diet in 2023 is likely to be different.

As only two of the five plant-based athletes completed our study, the results do not allow us to draw any valid conclusions as to whether or not the plant-based diet increases the risk of vitamin D and iron insufficiency or deficiency among female athletes.

The main limitation of this study is the small sample size, thus reducing external validity and limiting the statistical analyses performed. Although, we recruited 63 athletes who were willing to participate, only 17 completed all the necessary steps of the study. One possible explanation for this very low participation rate could be related to the fact that the current study was conducted among high-performance athletes during competition and training season with the intent to obtain their iron and vitamin D status in an active state. However, this proved to be disadvantageous as athletes were less available due to their extremely busy schedules (sport- and academic-related commitments) and increased winter-related illnesses such as cold and flu, leading to early dropout rates. Moreover, there are some limitations around the estimations of dietary intakes using a 24-hour recall. The food recalls require athletes to remember all food consumed in the previous 24 hours and require precise estimations of food portions so that the data analysis can accurately represent the athlete's intake. This can be challenging especially when eating take-out foods. In addition, the average over two days do not accurately represent individual intakes. Therefore, more sophisticated methods based on sophistical modelling should be used to mitigate some of the limitations of the 24-hour recall by removing the effects of within-person variation (Dodd et al., 2006). Furthermore, through any diet assessment method, there is the risk of false declaration of what one is truly eating when under evaluation, also known as the Hawthorne effect. As a result, this could lead to underestimations or overestimations of some foods reported. Lastly, the databases on most software, such as ASA24, are incomplete. Therefore, when items are not found or present within the database, the item is either omitted or substituted by another similar option which reduces the accuracy and validity of the actual intake and analysis.

CONCLUSION

This study was the first to investigate the nutritional status of iron and vitamin D and assess the possible relationship according to participants' diet, including a mixed diet or plant-based diet, in female varsity athletes from the University of Ottawa. To our surprise, very few athletes reported adopting a plant-based diet. In fact, only five followed a plant-based diet: three participants followed a vegan diet and two followed a vegetarian diet. Out of those five athletes, one vegan and one vegetarian athlete completed our study in its entirety.

Our findings suggest that the prevalence of IDA and vitamin D deficiency were very modest among this small group of female athletes compared to suboptimal levels of vitamin D and ID that were much more prevalent. For instance, nine (47%) athletes had insufficient vitamin D levels, five (26%) had ID, only one (5%) had vitamin D deficiency and no female athletes suffered from IDA. Moreover, suboptimal vitamin D levels were prevalent in athletes playing indoor sports as well as in athletes playing outdoor sports. This could in part be because the study was conducted in the late fall and winter when the absorption of the sun-induced vitamin D is minimal and since we had almost double the number of athletes playing indoor sports compared to outdoor sports. Due to the limited number of plant-based participants analyzed in this study, it was not possible to establish if insufficiencies or deficiencies were more prevalent in the athletes who adopted a plant-based compared to a mixed dietary pattern. In addition, 94%

of athletes, did not meet the EAR for vitamin D through foods and were only able to meet their requirements through supplementation. In contrast, as much as 94% of athletes were able to meet the EAR for iron and iron supplements caused four athletes to exceed the UL. Finally, considering 94% of athletes were able to meet the iron recommendations, there was still a decent proportion of athletes with ID (26%) emphasizing that iron absorption is limited.

This research project emphasizes the importance of including vitamin D blood level analyses in the pre-routine testing for female Gee-Gee varsity athletes since almost half the athletes had suboptimal levels. Currently, vitamin D tests are not conducted mainly because they are not covered by provincial medical insurance. An alternative would be to have all athletes take supplements as a preventative measure, especially during the late fall and winter months. Our results also support that iron status, including iron stores (ferritin levels), should be continuously monitored throughout all four years instead of only being tested in the first year since one athlete reported having low ferritin levels in the first year, which remained an ongoing issue. Moreover, five athletes in their 1st, 2nd, and 3rd year had ID. Other colleges and universities not currently conducting any testing for their high-performance female athletes should highly consider including these tests due to the negative implications a lack or deficiency could have. They should also be followed by a dietitian, which is normally the case, but include specific education regarding the best sources of iron and vitamin D, as well as how to optimize iron absorption.

To conclude, future studies must investigate the nutritional status of high-performance female athletes using a larger sample to increase validity and generalizability to a larger population. In addition, to properly assess the impact of following a vegan-based diet, it is necessary to recruit more athletes who follow this dietary pattern. Lastly, some strategies such as offering an incentive must be developed to increase participation and prevent dropouts in busy varsity athletes to increase the sample size once again.

Appendix A - Survey Monkey Questionnaire

Iron and vitamin D status in female Gee-gee's varsity athletes and impact of plant based diet / Statut nutritionnel en fer et vitamine D chez les athlètes féminines Gee-gee's et l'impact d'un régime à base de plantes

This questionnaire was designed to help us get to know you better. Your answers will remain confidential and only researchers will have access to the answers. Please answer the questions honestly. This should take approximately 5 minutes to complete. Thank you.

Ce questionnaire a été conçu pour nous aider à mieux vous connaître. Vos réponses resteront confidentielles et seules les chercheurs auront accès aux réponses. Veuillez répondre honnêtement aux questions. Cela devrait prendre environ 5 minutes pour le remplir. Merci.

* 1. What is your age?
Quel est votre âge?

☐

2. What is your current weight?
Quel est votre poids actuel?

☐

3. What is your current height?
Quelle est votre taille actuelle?

☐

* 4. In what sport are you currently enrolled in at the University of Ottawa?
Dans quel sport êtes-vous inscrit présentement à l'université d'Ottawa?

- ☐ Soccer
- ☐ Basketball
- ☐ Volleyball
- ☐ Hockey
- ☐ Rugby
- ☐ Swimming / Natation
- ☐ Track & cross country / Athlétisme
- ☐ Other (please specify) / Autre (s.v.p spécifier):

* 5. In what sport year are you?
Dans quelle année sportive êtes-vous?

- [ ] First / Première
- [ ] Second / Deuxième
- [ ] Third / Troisième
- [ ] Fourth / Quatrième

* 6. Do you have any children?
Avez-vous des enfants?

Yes, specify amount / Oui, spécifier le nombre: ____________

No / Non ____________

* 7. How would you describe your menstrual cycle in general?
Comment décririez-vous votre cycle menstruel en générale?

- [ ] Regular / Régulier
- [ ] Irregular / Irrégulier
- [ ] Light / Léger
- [ ] Medium / Médium
- [ ] Heavy / Lourd
- [ ] I do not have my period / Je n'ai pas de menstruation

* 8. Are you currently on an oral contraceptive pill or an intrauterine device (IUD)?
Prenez-vous une pilule contraceptive ou utilisez-vous un stérilet/appareil intra-utérin?

- ○ Yes / Oui
- ○ No / Non

* 9. Do you have any medical conditions (e.g., Gastrointestinal disease, blood disorder)?
Aviez-vous une condition médicale (p.ex. maladies gastro-intestinales, troubles sanguins)?

Yes, specify / Oui, s.v.p spécifier: ____________

No / Non ____________

* 10. What is the pigmentation of your natural skin? Choose the type (below) that best suits the colour of your skin.
Quelle est la couleur de votre peau naturel ? Choisir le type (ci-dessous) qui correspond le mieux à la couleur de votre peau?

- ☐ Extremely fair skin / Peau très pâle
- ☐ Fair skin / Peau pâle
- ☐ Medium skin / Peau médium
- ☐ Olive skin / Peau olive
- ☐ Dark skin / Peau foncé
- ☐ Very dark skin / Peau très foncé

* 11. Have you ever suffered from iron deficiency anemia?
Avez-vous déjà souffert d'une anémie par manque de fer?

Yes, specify / Oui, spécifiez:

i) The date (month and year) of diagnosis / La date (mois et année) du diagnostic:

ii) Treatment received (medical and/or nutritional) / Le traitement reçu (médicale et/ou nutritionnel) : ____________

No / Non ____________

* 12. S.V.P indiquez votre nom au complet / Please indicate your full name

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