The Impact of Cranberry-Derived Polyphenols on Physical Performance and Skeletal Muscle Bioenergetics

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ABSTRACT

The Impact of Cranberry-Derived Polyphenols on Physical Performance and Skeletal Muscle Bioenergetics

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Concordia University, 2024

Dietary choices have a direct impact on the gut microbiome, which in turn influences several body functions. In recent years, polyphenols, which are plant secondary compounds, have been shown to have prebiotic-like effects and associated with multiple health benefits. Cranberries are native to North America and have the highest polyphenol content and antioxidant capacity among the commonly consumed fruits and vegetables. Furthermore, cranberries stand out due to their high levels of the rare A-type proanthocyanidin (PAC-1), which is believed to be the main contributor to the beneficial effects. Recently, the concept that a link between skeletal muscle and the host's gut microbiota exists was put forward, with several research groups proposing that supplementation with polyphenols could promote improved muscle function, and consequently, improve exercise performance. One proposed mechanism for the positive effects of polyphenols on muscle function is through improved mitochondrial capacity. Mitochondria are the main producers of ATP, and their ability to generate energy as efficiently as possible is directly related to performance, especially in endurance athletes. The effect of cranberry polyphenols on exercise performance and skeletal mitochondrial function has not been explored before. This dissertation consists of five chapters. Chapter 1 introduces key concepts to provide background information and states the rationale, objectives, and hypotheses of the dissertation. Chapter 2 describes a rodent study that aimed to investigate the effects of cranberry A-type proanthocyanidins combined with

HIIT training on maximal running speed and skeletal muscle mitochondrial function. Chapter 3 consists of a systematic review with meta-analyses that synthesizes the current literature on the effects of polyphenol-rich berries on exercise performance, inflammation, and muscle damage. Chapter 4 is a clinical trial done with competitive/elite endurance runners that investigated the effects of a polyphenol-rich freeze-dried cranberry powder on running performance, lactate production, and skeletal muscle oxygenation. Chapter 5 is a follow-up study to the one described in Chapter 4 that aimed to explore the effects of the same cranberry powder on skeletal muscle mitochondrial capacity using near-infrared spectroscopy in healthy active adults. Finally, Chapter 6 discusses the findings from chapters 2-5 and provides general limitations and future research directions.

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Contribution of Authors

Manuscript 1:

Francis Parenteau designed the study, under the supervision of Dr. Andreas Bergdahl. Francis Parenteau trained Grabriel Malka to use the rodent treadmill for animal testing. Francis Parenteau and Gabriel Malka collected the performance data, body mass, and water consumption. Gabriel Malka was responsible for conducting the high-intensity interval training with the animals. Francis Parenteau performed all tissue extractions, experiments, and statistical analyses. Francis Parenteau wrote the first draft of the manuscript and all subsequent edits, with feedback from Dr. Andreas Bergdahl. Prior to final submission, feedback from Gabriel Malka was obtained and the final edits were made.

Manuscript 2:

Francis Parenteau created the systematic review question, search strategy, and inclusion and exclusion criteria, with help from Dr. Andreas Bergdahl. All articles were imported to Rayann by Francis Parenteau, and he and Dr. Andreas Bergdahl independently screened the titles and abstracts of the articles and came to a consensus on which ones to include. Then, Francis Parenteau and Antoine St-Amant screened the full texts of the articles and extracted all relevant data. Antoine St-Amant performed all meta-analyses and made the forest plots. Francis Parenteau wrote the first draft of the manuscript and all subsequent edits, with feedback from Dr. Andreas Bergdahl and Antoine St-Amant.

Manuscript 3:

Francis Parenteau and Veronica Furno Puglia designed the study, under the supervision of Dr. Andreas Bergdahl. Francis Parenteau and Dr. Andreas Bergdahl wrote the ethics submission. Veronica Furno Puglia was responsible for recruitment of participants. Francis Parenteau was responsible for developing the data collection sheets, informed consent, and health questionnaires. Francis Parenteau and Veronica Furno Puglia collected data with the help of three volunteers. Francis Parenteau performed all data entry and analyses. Francis Parenteau wrote the first draft of the manuscript and all subsequent edits, with feedback from Dr Andreas Bergdahl, Dr. Mary Roberts, Dr. Alain Steve Comtois, and Veronica Furno Puglia.

Manuscript 4:

Francis Parenteau designed the study, under the supervision of Dr. Andreas Bergdahl. Francis Parenteau and Dr. Andreas Bergdahl wrote the ethics submission. Francis Parenteau was responsible for recruiting participants as well as developing data collection sheets, informed consent, and health questionnaires. Francis Parenteau and Antoine Denis collected all data, with the help of a volunteer, and performed all the analyses. Francis Parenteau wrote the first draft of the manuscript and all subsequent edits, with feedback from Dr. Andreas Bergdahl, Dr. Mary Roberts, Dr. Alain Steve Comtois, and Antoine Denis.

All authors reviewed the final manuscript and approved of the contents.

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List of Abbreviations

ADP	Adenosine diphosphate
AMPK	5'-adenosine monophosphate activated protein kinase
ATP	Adenosine triphosphate
BMI	Body mass index
CK	Creatine kinase
CRP	C-reactive protein
ETC	Electron transport chain
$FADH_2$	Flavin adenine dinucleotide
HHb	Deoxyhemoglobin
HIIT	High-intensity interval training
IL-1b	Interleukin-1b
IL-6	Interleukin-6
IL-8	Interleukin-8
k	Mitochondrial rate constant
mVO_2	Muscle oxygen consumption
NADH	Nicotinamide adenine dinucleotide
NIRS	Near-infrared spectroscopy
O_2Hb	Oxygenated hemoglobin
PAC	Proanthocyanidins
PAC-1	A-type proanthocyanidins
PGC-1a	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

RCT	Randomized controlled trial
ROS	Reactive oxygen species
SM	Skeletal muscle
SMD	Standardized mean difference
SmO_2	Muscle oxygen saturation
TAC	Total antioxidant capacity
TCA	Tricarboxylic acid cycle
THb	Total hemoglobin
TNF-alpha	Tumor necrosis factor alpha
TT	Time trial
VL	Vastus lateralis
VO_{2max}	Maximal oxygen consumption
WD	Western diet

Chapter 1: Introduction

1.1 Background

The human gut microbiota refers to the complex community of microorganisms, including bacteria, viruses, fungi, and archaea, that resides in the gastrointestinal tract. This community of microorganisms plays a crucial role in maintaining human health and has a profound impact on various physiological processes (Clemente et al, 2012). The composition of the gut microbiota is determined mainly by the host's diet and levels of exercise (Przewlocka et al, 2020). For example, diets that are high in saturated fat and refined sugar can shift the composition of the gut microbiome and lead to negative systemic effects, whereas diets rich in whole foods and fiber can positively alter the gut microbiome (Yan et al, 2016). Polyphenols are plant secondary compounds that have been shown to have prebiotic-like effects and thus induce various beneficial health effects in humans (Vandrame et al, 2011; Gonzalez-Sarrrias et al, 2018). The term polyphenol refers to a group of compounds characterized by aromatic rings carrying one or multiple hydroxyl groups (Mojzer et al, 2016). They can be separated into three main categories: phenolic acids, flavonoids, and non-flavonoids (see **Figure 1**). Each category can be further divided into subclasses, for which catechins, anthocyanins, and proanthocyanidins have the most established prebiotic effect (Alvez-Santos et al, 2020). The effects on the gut following polyphenol supplementation are related to the promotion of the microbiota abundance of beneficial such as Lactobacillus and Bifidobacterium, and the decrease the presence of pathogenic species such as Clostridium species (Ma and Chen, 2020).

Cranberries are rich in polyphenols, phytonutrients that positively affect the gut, including proanthocyanidins (PACs), anthocyanins, flavanols, and flavonols (Mathison et al, 2019). In fact, cranberries have the highest total polyphenol content among the most commonly consumed fruits

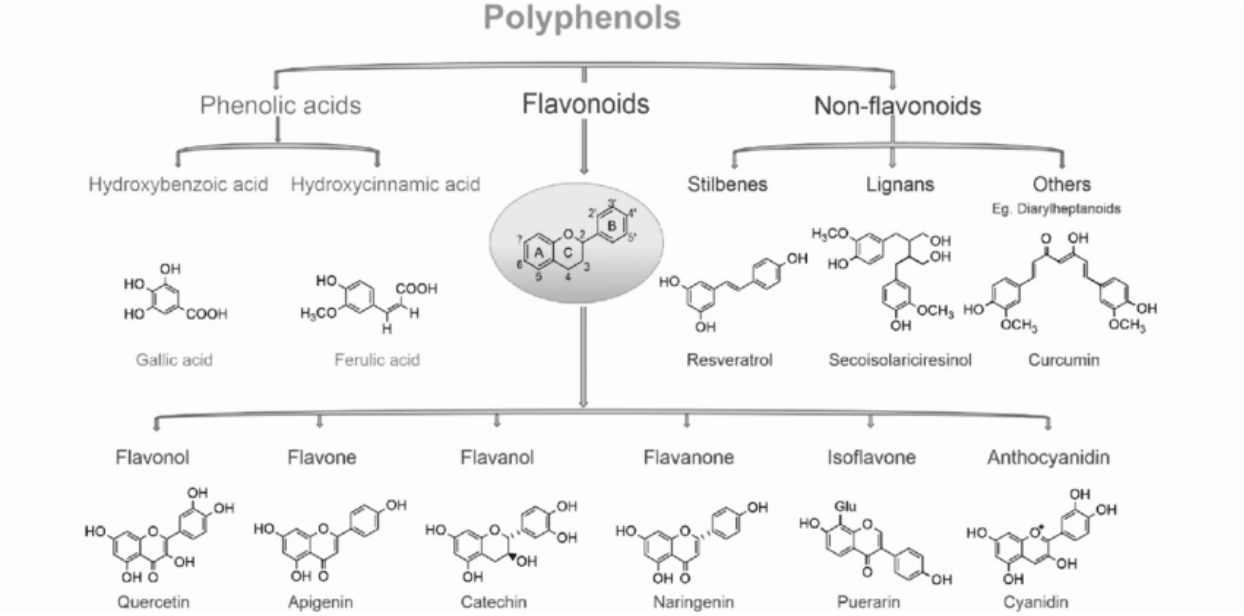

Figure 1. Polyphenol classes and subclasses (Rambaran, 2020)

and vegetables in the American diet (Flammer et al, 2013), while also having one of the highest antioxidant capacities among all fruits and vegetables (Bean et al, 2010). Furthermore, cranberries' polyphenol content stands out due to their high concentration of the rare A-type PAC (PAC-1), a member of the Flavanol sub-class which is believed to be the main contributor of the beneficial effects (Jiao et al, 2017). Per 100 g of dry mass, cranberries contain up to 3936 mg of total phenolic compounds, including 860-1283 mg of PACs, 695-1716 mg of anthocyanins, 643-1088 mg of flavonols, and 20-30 mg of quercetin (Oszmiański et al, 2017; Oszmiański et al, 2018).

The concept that a link between gut microbiota and skeletal muscle (SM) exists has been put forward in recent years, with physiological evidence supporting the existence of a "gut-muscle" axis (Przewlocka et al, 2020). Polyphenols, which have known prebiotic functions, have also been associated with improvements in SM mitochondrial function. For example, pomegranate ellagitannins undergo acid hydrolysis to ellagic acid in the stomach and are then further metabolized by gut bacteria into Urolithin A, which is bioavailable and was shown to increase

mitochondrial density in SM (Yang et al, 2020). Similarly, resveratrol, a polyphenol found in grapes and berries, was shown to directly improve mitochondrial capacity when combined with exercise training (Polley et al, 2015). When looking at overall exercise performance, a 2017 systematic review of randomized controlled trials by Sommerville et al. examined the effect of polyphenol supplementation (minimum of 7 d) on exercise performance in healthy individuals. They measured performance as the total power output calculated from either time trial or time to exhaustion and concluded that polyphenols supplementation improved exercise performance moderately by 1.9%. Half of the studies (n = 7) included in the meta-analysis used quercetin, which demonstrated greater effects on performance (2.82% vs. 1.90%) than other sources (Sommerville et al, 2017). Such effects are relevant to trained endurance athletes competing in races, where every second gained matters and can make the difference between stepping on the podium or not.

SM provides the body with structure and strength and represents roughly 40% of the total body weight in a healthy human (Manickam et al 2020). It is organized in muscle fibers, which are comprised of smaller units known as myofibrils (see **Figure 2**). These myofibrils are made of thin

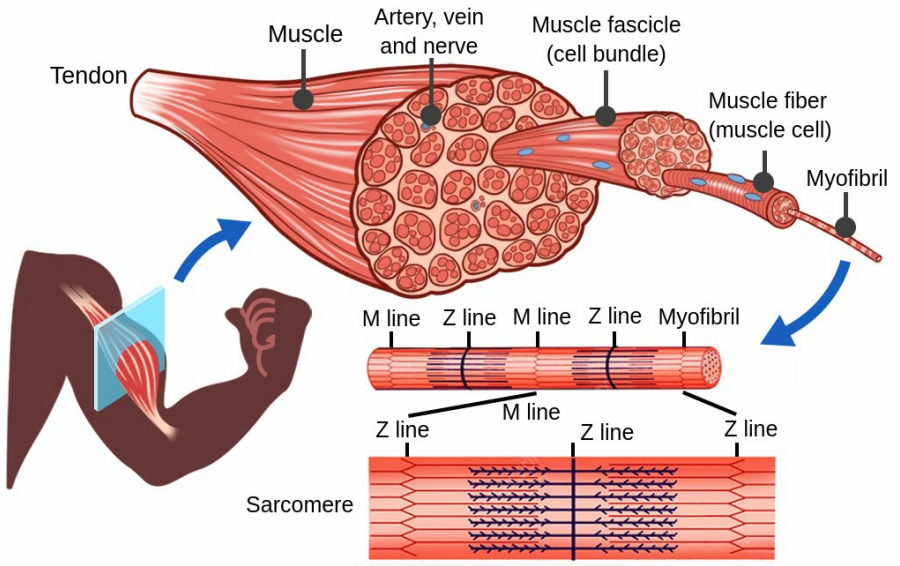

Figure 2: Structure of skeletal muscle (Frontera, 2015)

and thick filaments that run parallel to each other and are organized in a longitudinal manner to form sarcomeres. The thin filaments are made of actin, tropomyosin, and troponin, while the thick filaments contain myosin. The actin filaments are covered with tropomyosin, which blocks their interaction with myosin, while the troponin group – comprised of troponin I, T, and C – is located along the actin filaments.

The process of excitation-contraction coupling starts the moment an action potential leads to an increase in the myocyte membrane potential. The depolarization is spread to the entire muscle fiber, resulting in the opening of the plasma membrane calcium channels and, in turn, the release of calcium by the sarcoplasmic reticulum within the muscle cells. Calcium binds to troponin C, which leads to the shifting of tropomyosin, thus allowing the myosin heads to attach to the previously blocked actin filaments. Through this process, a cross-bridge is formed, allowing the cycling to begin. At rest, the myosin head contains adenosine diphosphate (ADP) and inorganic phosphate. When myosin binds to actin, the inorganic phosphate is released, which contributes to the pivoting of the myosin head and, therefore, the moving of the thin filaments. Then, adenosine triphosphate (ATP) binds to an ATP-binding domain on the myosin head, which breaks the cross-bridge and returns myosin to its resting state. One of these so-called power strokes is very small, and multiple cycles are needed to result in a perceivable muscle contraction. As long as calcium is present in the cytoplasm, the cross-bridge cycling will go on and muscle contraction will occur. When calcium removal from the cytoplasm into the sarcoplasmic reticulum exceeds the calcium release, muscle relaxation happens (Hopkins, 2006; Gash, 2023).

Consequently, SM contraction is highly dependent on ATP availability. There are three main energy systems that are responsible for synthesizing ATP: (1) the phosphagen system, (2) the glycolytic system, and (3) mitochondrial respiration. The phosphagen system, although comprised

of three reactions, relies mostly on creatine kinase. Phosphocreatine stores within skeletal muscle allow for its hydrolysis by creatine kinase and production of ATP in a near instantaneous manner. This combined with its rapid rate of ATP regeneration makes this the dominant system during maximal efforts of 5-10 s (Greenhaff et al, 1994; Greenaff et al, 1998). The glycolytic system acts through the energy derived from blood glucose and SM glycogen stores (Pilegaard et al, 1999). Glycogen and glucose undergo a series of transformations are eventually reduced to pyruvate, producing ATP in the process. Glycolysis produces its maximum rate of ATP regeneration when an individual is working above their maximum oxygen uptake (i.e. VO_{2max}), which for a trained athlete is possible to sustain for 2 to 3 minutes (Medbo, 1989). During high-intensity exercise, pyruvate accumulates in the cytosol, which has the potential to inhibit glycolysis. Therefore, pyruvate is converted to lactate via the lactate dehydrogenase reaction, which enables glycolysis to keep on generating ATP at a relatively fast rate (Bigland-Ritchie and Woods, 1984). This process is crucial, as without conversion of pyruvate to lactate, glycolysis would be inhibited within a matter of seconds (Baker, 2010). The third system is mitochondrial respiration. This process can utilize various sources of energy, including blood glucose, SM glycogen, and free fatty acids. In the case of glucose and glycogen, they first undergo glycolysis to pyruvate, which is then converted to acetyl CoA to kickstart the tricarboxylic acid (TCA) cycle. Fatty acids also undergo several transformations in the cytosol before being transported into mitochondria where they are converted into the same acetyl CoA (Baker, 2010). The TCA cycle generates NADH and $FADH_2$, which are required to transfer hydrogens to the electron transport chain (ETC). The ETC is responsible for most of the production of ATP during mitochondrial respiration. It is composed of 5 complexes (CI-V) organized in a precise manner to form a supercomplex (Guo, 2017). Electrons obtained from NADH and $FADH_2$ are passed through CI/III/IV or CII/III/IV into the intermembrane space,

creating at proton gradient which leads to the combination of H+ electrons with O_2 molecules to form water, releasing energy in the process and driving ATP synthesis at CV (Alberts, 2002). **Figure 3** summarizes the process of mitochondrial respiration. This process, also known as oxidative phosphorylation due to the need for oxygen for ATP to be synthesized, allows for submaximal efforts to be sustained for long periods of time.

Muscle mitochondrial function is important to improve exercise performance, especially in endurance athletes, as well as for healthy aging. SM mass starts to decline as early as 30 years

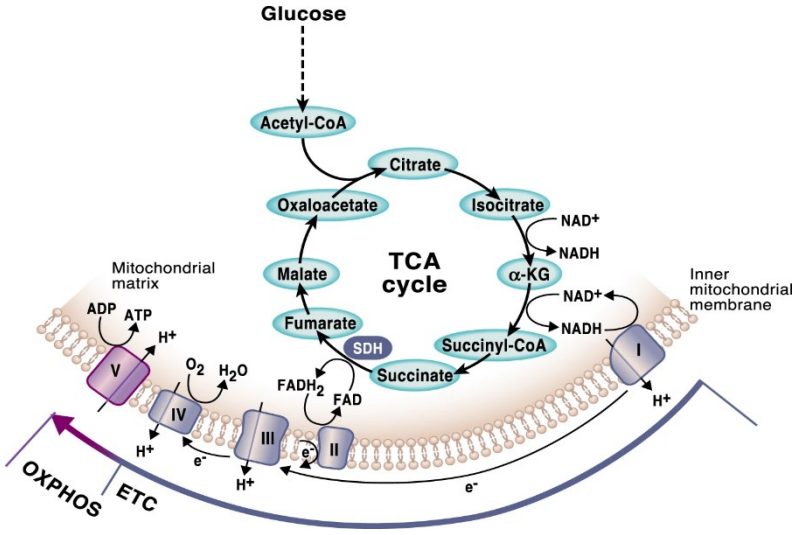

Figure 3. Oxidative phosphorylation: The TCA cycle and the ETC (Martínez-Reyes, 2020.)

of age, which is a strong predictor of all cause-mortality in women over 65 and men over 55, and when impaired has been associated with chronic and neuromuscular diseases (Nay et al, 2019; Liao et al, 2021). Similarly, mitochondrial density declines in aging, and the amount of mitochondrial DNA is directly proportional to ATP production, and consequently correlates with impaired aerobic capacity in the elderly (Short et al, 2005). On the other hand, mitochondrial enzyme activity has been shown to be positively linked with endurance performance in athletes (Irrcher et al, 2003) (see **Figure 4**). Other known adaptations of mitochondria to exercise include

increases in total mitochondrial protein (Scalzo, 2014) and in SM oxidative capacity (Pesta, 2011). In fact, mitochondrial density was shown to increase by up to 40% after 6 weeks of endurance training on a cycle ergometer in untrained individuals (Hoppeler, 1985). Therefore, measures of SM oxygenation metrics and mitochondrial capacity can be used to evaluate muscular health and aerobic function, two factors that are important in aging as well as for endurance exercise performance.

The gold standard to measure mitochondrial capacity is high-resolution respirometry. This technique allows for the ability to probe specific complexes of the ETC, which provides important information regarding the mechanisms of altered mitochondrial function (Gnaigner, 2009). However, this approach has limitations: it is invasive, requiring the participant to undergo a somewhat painful SM tissue biopsy; it has low translational significance since many experimental protocols subject the SM tissue to non-physiological conditions. Another technique that can be

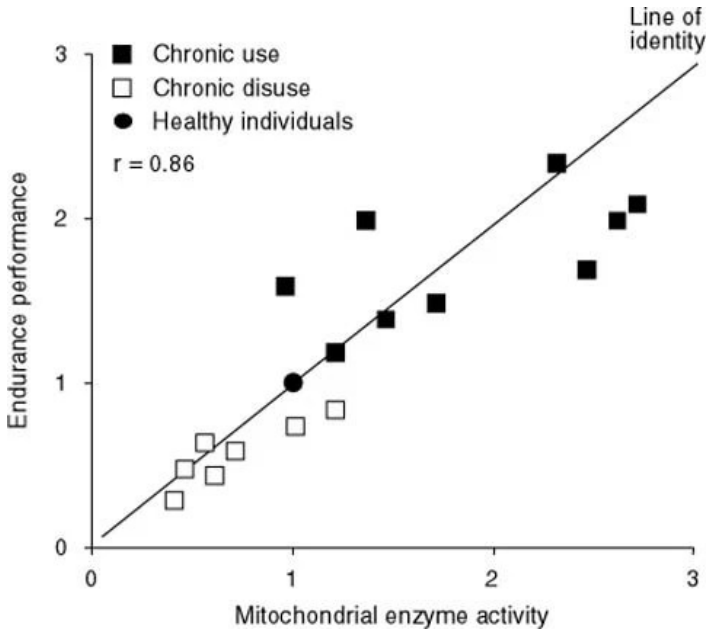

Figure 4. Relationship of changes in mitochondrial content to alterations in endurance performance. Increases in mitochondrial enzyme activity due to chronic muscle use (e.g. chronic stimulation, endurance training) lead to improvements in endurance performance. (from Irrcher et al, 2003)

used to measure mitochondrial capacity *in-vivo* is phosphorus magnetic resonance spectroscopy (31P-MRS). This technique assesses the kinetic changes in phosphocreatine during exercise and the recovery after cessation of exercise to indirectly measure mitochondrial capacity based on the assumption that the amount of creatine kinase remains constant (Chance et al. 2006; Layec et al. 2011). The main limitation of this technique is the low availability of multinuclear magnetic resonance scanners and the high cost to use them. Recently, near-infrared spectroscopy (NIRS), a non-invasive device used to measure changes in oxygenated and deoxygenated hemoglobin/myoglobin and total hemoglobin, has been gaining in popularity to measure mitochondrial capacity. The NIRS device uses multiple, continuous wavelengths in the 700–900 nm range to penetrate biological tissue. The light emitted from the device follows a semicircular curve into the tissue at a depth equal to half the distance between the light source and the detector. The protocol to assess mitochondrial capacity uses SM stimulation to increase its metabolic activity followed by repeated arterial occlusions to create recovery slopes for each occlusion (Hamaoka et al, 2011). The arterial occlusion slopes are fit to an exponential curve and a mitochondrial rate constant is calculated as a measure of mitochondrial capacity (Ryan et al, 2012). This technique was shown to have good test-retest reliability (La Mantia et al, 2018; Fennell et al, 2023) and was validated against both high-resolution respirometry (Ryan et al, 2014; Pilotto et al, 2022) and 31P-MRS (Ryan et al, 2013).

1.2 Rationale

This dissertation aims to bridge the current gap in the literature between polyphenol-rich cranberry supplementation and its potential effects on skeletal muscle mitochondrial function and exercise performance. Earlier animal and human investigations did not reveal a direct impact of cranberry supplementation on exercise performance measured as the time taken to complete a

specific event (Liburt et al., 2009; Khalikova et al., 2022; Skarpanska et al., 2017), but subsequent studies have shown that it can have positive effects on biomarkers associated with enhanced exercise performance. For example, supplementation with an acute dose of a cranberry and grape seed extract lowered the lactate response to exercise during a 3-km cycling event, suggesting a potential effect on lactate clearance rate (Labonte et al, 2013). Furthermore, cranberries have been associated with improving antioxidant capacity through increased activity levels of glutathione, superoxide dismutase, and catalase in rodents (Hussien et al, 2016; Deyhim et al, 2007; Kim et al, 2013; Elfatih, 2014; Jiao et al, 2017) and in humans (Mathison et al, 2019; Basu et al, 2011; Skarpańska-Stejnborn et al, 2017). A couple of studies also showed potential effects of cranberry on mitochondrial function by neutralizing mitochondrial reactive oxygen species (ROS) production (Denis et al, 2015) and inhibiting calcium-induced mitochondrial permeability transition pores, free radical production, and membrane lipid peroxidation (Zavodnik et al, 2019).

The type of polyphenol, the duration of treatment, as well as the dosage all vary greatly between studies. The aforementioned meta-analysis that looked at the effect of polyphenol supplementation on exercise performance included studies in which the mean daily polyphenol content was 688 ± 478 mg and the study period varied from 7 to 56 days (Sommerville et al). This stresses the fact that there is currently no consensus on what the ideal polyphenol supplementation protocol looks like, and more studies are needed to figure out whether polyphenols can improve exercise performance. This dissertation aims to add to the currently scarce body of knowledge through a series of original studies investigating the potential effects of polyphenol-rich cranberry supplementation on exercise performance and physiological markers of SM function.

Chapter 2 is a rodent study which demonstrates the effects of A-type PACs on maximal running speed and vastus lateralis mitochondrial capacity in active rodents undergoing high-

intensity interval training (HIIT). This study was the first step in identifying the potential effects of cranberry polyphenols on performance, acting as a proof of concept. The choice of a rodent model allowed us to have total control of diet as well as exercise volume and intensity. Chapter 3 is a systematic review with meta-analyses of randomized controlled trials that is currently under review in *Clinical Nutrition ESPEN*. We looked at studies that evaluated the effect of different polyphenols derived from berries on exercise performance, inflammation, and muscle damage. The goal was to further investigate if the positive effects that we observed in rodents had potential to translate to humans by looking at clinical trials that used other berries or berry-derived supplements. Chapter 4 is a human trial published in *Physical Activity and Nutrition* (Parenteau et al, 2023). In this study, we tested the effectiveness of a high-polyphenol cranberry powder at improving running performance, lactate response, and vastus lateralis oxygenation metrics during two different time trials (TT): a 400-m and 1500-m. Endurance runners were chosen as test subjects based on results from the rodent study that showed effects of cranberry supplementation on the aerobic system, more specifically mitochondrial capacity. Chapter 5 is a human trial that was accepted in *Applied Physiology, Nutrition, and Metabolism* on April 9th, 2024 (Parenteau et al, 2024). It aimed to further investigate the effects of cranberry on SM performance, this time targeting vastus lateralis mitochondrial capacity in healthy active adults. Mitochondrial function is important for performance, but also for healthy aging, and the results of this study can be applied to various populations. We used near-infrared spectroscopy (NIRS) combined with rapid cuff inflation to measure oxygen recovery curves of the vastus lateralis, an indirect measure of mitochondrial capacity.

1.3 Research Objectives and Hypotheses

The overall aim of this thesis was to determine if supplementation with a polyphenol-rich cranberry extract could have positive effects on exercise performance and skeletal muscle mitochondrial function. Chapters II, III, IV, and V each played a role in answering different portions of that question.

1.3.1 Chapter 2

Objective

- To evaluate the effect of cranberry-derived A-type proanthocyanidins on maximal running speed and vastus lateralis mitochondrial capacity in active mice performing high-intensity interval training.

Hypothesis

- A-type proanthocyanidins potentiate skeletal muscle function through improved mitochondrial capacity when combined with HIIT training, leading to performance gains.

1.3.2 Chapter 3

Objective

- To synthesize the evidence around the effects of polyphenol-rich berries on aerobic exercise performance, inflammation, and muscle damage in healthy active adults.

Hypothesis

- Supplementation with berry polyphenols improves aerobic exercise performance and lowers markers of inflammation and muscle damage.

1.3.3 Chapter 4

Objective

- To test the effect of freeze-dried cranberry powder on running performance, post-exercise lactate response, and skeletal muscle oxygenation in competitive/elite endurance runners.

Hypotheses

- The cranberry supplement improves lowers time to completion on the 400- and 1500-m time trials.
- The cranberry supplement inhibits the post-exercise lactate production during the anaerobic-focused 400-m time trial.
- The cranberry supplement improves vastus lateralis oxygenation metrics, notably deoxygenation and reoxygenation slopes as well as mean oxygen saturation during the aerobic-focused 1500-m time trial.

1.3.4 Chapter 5

Objective

- To test the effect of freeze-dried cranberry powder on vastus lateralis mitochondrial capacity in healthy active adults measured by NIRS using partial recovery curves.

Hypothesis

- The cranberry supplement improves the mitochondrial capacity constant (k).

Chapter 2: Manuscript I

Cranberry-Derived A-Type Proanthocyanidin Mitigate the Adverse Effects of a Western Diet on Exercise Capacity and Muscle Bioenergetics in Active Rodents

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Cranberry-Derived A-Type Proanthocyanidin Mitigate the Adverse Effects of a Western Diet on Exercise Capacity and Muscle Bioenergetics in Active Rodents

2.1. Introduction

Diets that are enriched in fruits, vegetables, whole grains and healthy fats are associated with increased muscle strength and better functional capacity outcomes (Bloom et al, 2018). Diet stands as the foremost environmental determinant in shaping the gut microbiota, an intricate community of bacteria that forms a diverse ecosystem comprising numerous microorganisms (Tang and Hazen, 2014; Rinella et al, 2018). The gut microbial ecosystem is the largest endocrine organ in the body, capable of producing a wide range of biologically active compounds that, like hormones, may be transported to distant sites to influence various essential physiological pathways (Tang and Hazen, 2014; Rastelli, 2019).

Poor dietary choices have been shown to impair intestinal health and promote chronic systemic inflammation via impaired gut barrier integrity (Anhê et al, 2015; Bleau et al, 2015). Transitioning from low-fat, fiber-rich food to a high-fat, high-sugar Western-style diet (WD) can induce swift alterations in the microbiome, manifesting changes in as little as a single day (Lindskog Jonsson et al, 2018). WD is associated with promoting systemic inflammation, visceral fat accumulation, and impaired skeletal muscle function (Kim et al, 2000). Skeletal muscle mass typically begins to decline as early as 30 years of age, which is a strong predictor of all cause-mortality in women over 65 and men over 55, and when reduced, has been associated with chronic and neuromuscular diseases (Nay et al, 2019; Liao et al, 2021). Muscle bioenergetics, specifically mitochondrial function, are critical for proper muscle function and have been shown to be impaired by WD (Green et al, 2015) and physical inactivity (Kenny, 2015). The amount of mitochondrial

DNA is directly proportional to ATP production, which correlates with the aerobic capacity of an individual (Short et al, 2005). Similarly, mitochondrial enzyme activity positively correlates with endurance performance in humans (Irrcher et al, 2003).

The surge in obesity rates in Westernized societies is believed to result primarily from a combination of elevated dietary fat and carbohydrates consumption and diminished levels of physical activity (Schrauwen and Westerterp, 2000). Dietary modifications have poor long-term adherence (Chao et al, 2021) and, therefore, it is important to explore new, sustainable avenues to promote improved health.

Polyphenols, including proanthocyanidins (PAC), anthocyanins, flavonols, and flavanols, are secondary plant compounds found in fruits and vegetables (Chew et al, 2019; Singla et al, 2019). Polyphenols are prebiotic-like phytonutrients shown to promote an array of health benefits by optimizing gastrointestinal functions (Vandrame et al, 2011; Gonzalez-Sarrrias et al, 2018). Cranberries contain one of the highest polyphenol concentrations among the most commonly consumed fruits in North America (Flammer et al, 2013) and its antioxidant capacity is the greatest of all fruits and vegetables (Bean et al, 2010). Furthermore, cranberries stand out due to their high concentrations of rare A-type PAC (PAC-1), a polyphenol potently associated with health benefits (Jiao et al, 2017). Cranberry extracts have been shown to positively modulate the gut microbiota while reducing cardiometabolic risk factors and chronic inflammation (Ahnê et al, 2017). The antioxidant and anti-inflammatory properties of cranberries, combined with the prebiotic effect of the polyphenols they contain, suggests that they could prevent the negative health effects caused by Western diets. Thus, this study aimed to investigate the effects of cranberry-extracted PAC-1 supplementation combined with high-intensity interval training (HIIT) on exercise performance and skeletal muscle energy capacity in a rodent model fed a high-fat, high-sugar diet.

2.2. Methods

Animals and experimental design

Male C57Bl/6 mice, 3-4 months old, were obtained from the Concordia University breeding colony and randomly assigned to either control (CON), high fat diet (HFD), or high fat diet supplemented with PAC-1 (PAC) groups. Mice were housed in a thermoneutral environment at 22℃ and kept on a 12 h light - 12 h dark photoperiod. All procedures were approved by the Animal Ethics Committee of Concordia University (#30000259) and were conducted in accordance with guidelines of the Canadian Council on Animal Care.

Once randomly assigned to the three treatment groups, mice were fed regular chow for the first two weeks. During this period, acclimation to the treadmill and baseline exercise testing were performed together with measurements of body mass and water consumption. Afterwards, mice were given free access to water and their respective treatment diet for the remaining four weeks of the study. Exercise testing was repeated at week 2 and 4 and body mass was recorded weekly. At the end of the study, mice were euthanized by CO_2 asphyxiation.

Diets

The control group's diet consisted of a regular animal chow (5075 Charles River autoclavable Rodent Diet). The high-fat diet was prepared from purified food-grade elements according to a commercial diet (Research Diets D12492; see **Table 1**). The HFD-PAC diet was elaborated from the HFD and supplemented with a purified PAC-1 polymer (200 mg/day/kg of body mass).

Exercise Training and Testing

HIIT Protocol

An adapted rodent treadmill (Bouganim and Bergdahl, 2017) was used for all exercise training and testing. Acclimation to the treadmill consisted of two sessions performed on separate days. During

the first session, mice were put on the treadmill and speed was first set to 8 m/min for a 10-min warm up. Then, speed was increased to 15 m/min for 15 minutes. Finally, the treadmill was set back to 8 m/min for a 10-min cooldown. On the second session, interval training was introduced to the animals. After the same 10-min warm up, mice performed five sets of 1-min intervals at a speed of 17 m/min, with a 2-min rest at 12 m/min between each set. After the intervals, a 10-min cool down was performed.

After the acclimation period, the HIIT protocol began. Mice were trained three days per week for a month. On each training day, mice performed a 5-min warm up followed by five sets of 1-min at more than 75% of their max running speed, with 2-min active recovery bouts at 12 m/min. After the intervals, a 5-min cool down was performed. The treadmill was set at a 5-degree incline for the whole training period. During the testing, mice were removed from the treadmill if they reached a level of exhaustion, which was determined when a mouse failed to exit the back of the belt despite tactile motivation for 10 or more seconds.

Maximal exercise capacity test

Maximal exercise capacity testing on treadmill was used to measure improvements from training and diets. The protocol consisted of an incremental test similar to a VO_{2max} treadmill protocol for humans. Mice were placed on the treadmill and did a brief 3-min warm up at 8 m/min. Then, the speed was increased to 13 m/min for 1 minute. From there, an increase of 3 m/min was done every minute until the animal reached exhaustion. The score on the test is reported as the maximal velocity the animal was able to maintain for the full minute.

Protein extraction, immunoblotting, and immunofluorescence

Vastus lateralis cell lysates were extracted in lysis buffer containing (in mmol/L) NaCl 250, HEPES 50, glycerol 10%, Triton X-100 1%, $MgCl_2$ 1.5, EGTA 1, $Na_4P_2O_7$ 10, NaF 1, Na_3VO_4 800 mol/L, pH 7.5 and centrifuged at 12 000g for 10 min. Fifteen micrograms of lysates were

separated on a 12.5% SDS–PAGE and transferred to a nitrocellulose membrane (0.45 mm, 162-0115, Bio-Rad) using 10 mmol/L sodium tetraborate buffer. The membranes were blocked in 5% BSA in TBS-T buffer (10 mmol/L Tris–HCl, pH 7.5, 150 mmol/L NaCl, 0.05% Tween 20) for 1 h at room temperature followed by overnight incubation at 4 °C with anti-VDAC antibody (ab15895; Abcam). The blots were washed, incubated with horseradish-peroxidase-conjugated secondary antibodies (anti-mouse, ab6728; Abcam), and visualized with a chemiluminescence system (Immun-Star Chemiluminescent; 1705070; Bio-Rad, Mississauga, Ontario, Canada). The bands were analyzed using the ImageJ software.

Mitochondrial respiratory measurements

Measurements of oxygen consumption were performed in MiR05 at 37 °C using a polarographic oxygen sensor (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria). Approximately 2.0–2.5 mg of muscle tissue (wet mass) was placed in either chamber in a cross-sectional design. O_2 flux was resolved by DatLab by converting nonlinear changes in the negative time derivative of the oxygen concentration signal. All experiments were carried out in hyperoxygenated levels to avoid O_2 diffusion limitations. The protocol consisted of a sequential addition of mitochondrial substrates to target the different components of the electron transport system and stimulate OXPHOS capacity. We used an adapted version of a previously published protocol (Long et al, 2019). First, state 2 respiration (absence of adenylates) was assessed by addition of malate (2 mmol/L), pyruvate (0.5 mmol/L), and glutamate (10 mmol/L). Then, ADP (5 mmol/L) was added to reach state 3 respiration for Complex I. This was followed by the addition of succinate (10 mmol/L), resulting in maximal coupled state 3 respiration through Complex I and Complex II.

Data Analysis

Data are presented as means +/- SEM for immunoblotting and as means +/- SD for all other parameters. Repeated measures one-way ANOVA with Tukey post-hoc test was utilized to evaluate the effects of supplementation on body mass and maximal running capacity. For the results of the immunoblotting assays and the mitochondrial measurements, a one-way ANOVA with Tukey *post-hoc* test was used. Normal distribution was confirmed using the Kolmogorov-Smirnov goodness-of-fit test. An alpha-level of $P<0.05$ was set to detect a statistical effect of time and/or treatment for each outcome. All analyses were done using SPSS Statistics (v29, IBM Corp., Armonk, NY, USA).

2.3. Results

Body Mass

Measurements of body mass were taken weekly. No group effect was detected at any of the time points. A significant time effect ($p<0.001$) was observed for HFD between weeks 1 and 3 (26.9 (3.1) g versus 28.2 (3.3) g), 1 and 4 (26.9 (3.1) g versus 29.2 (3.4) g), and 2 and 4 (27.5 (3.3) g versus 29.2 (3.4) g) and for PAC between weeks 1 and 4 (27.35 (3.3) g versus 26.6 (5.1) g). **Figure 2** depicts the changes in body mass over time for each group.

Maximal running capacity

To evaluate the effects of treatment on aerobic capacity, mice underwent an incremental treadmill test at baseline, week 2, and week 4 (see **Figure 3**). The maximal running capacity did not improve for any of the groups following HIIT training, and no group effect was observed. Interestingly, the HFD group showed a significant decline in performance, with a time effect occurring between baseline and week 4 (34.7 (3.6) m/min versus 23.2 (7.2) m/min; $p<0.05$).

Mitochondrial respiration

Oxygen consumption rates using an Oxygraph were used to determine the changes in respiratory capacity of vastus lateralis between each condition. Mitochondrial capacity was measured by the sequential addition of substrates to target different components of the electron transport system and stimulate OXPHOS capacity (see **Figure 4**). Respiration for CON and PAC was significantly greater than that of HFD for state 2 respiration (CON: 24.3 (7.5) pmol/s/mg versus PAC: 27.1 (7.3) pmol/s/mg versus HFD: 17.3 (6.5) pmol/s/mg; p=0.02), state 3 respiration of Complex I (CON: 97.0 (24.2) pmol/s/mg versus PAC: 96.4 (17.6) pmol/s/mg versus HFD: 72.5 (19.2) pmol/s/mg; p=0.03), and maximal coupled state 3 respiration through Complex I and Complex II (CON: 134.4 (42.3) pmol/s/mg versus PAC: 127.1 (28.2) pmol/s/mg versus HFD: 92.0 (28.8) pmol/s/mg); p=0.03).

Mitochondrial density and subunits

Mitochondrial density was measured by immunoblotting with an antibody specific for the voltage-dependent anion channel (see **Figure 5**). This indicated similar levels between groups (CON: 100% (14.9%) versus PAC (112% (11.8%) versus HFD: 113.6% (19.8%); p>0.05). Normalized to total amount of proteins, there were no differences in the expression of the mitochondrial subunits (see **Figure 6)**.

2.4. Discussion

The main findings of this study are that cranberry supplementation high in PAC-1 appears to prevent declines in maximal running capacity and vastus lateralis bioenergetics caused by a high-fat diet in active mice. More specifically, vastus lateralis mitochondrial respiration at Complex I and II was significantly reduced by the high-fat diet, but PAC-1 seemed to reverse those effects. Interestingly, PAC-1 did not prevent weight gain associated with HFD.

This is the first study to investigate the effects of cranberry polyphenols on mitochondrial capacity in skeletal muscle. Mitochondrial enzyme activity is known to be positively linked with endurance performance (Irrcher et al, 2003), which aligns with our results. Evidence from the supplementation with other phenolic sources such as resveratrol (Zheng, 2012; Wang, 2018; Ryan, 2010), urolithin A (Liu, 2022), and quercetin (Davis, 2009) also points to the potential enhancement of mitochondrial function. Zheng and colleagues (Zheng, 2012) showed that resveratrol increased activity levels at Complex I and III, which they associated with its antioxidative effects since these complexes are the main mitochondrial producers of ROS (Chen, 2010; Koopman, 2010). Another group showed that resveratrol neutralizes superoxide in skeletal muscle following isometric contractions, which contributed to improved performance (Ryan, 2010). PAC-1, with a different chemical structure than resveratrol, has been associated with antioxidant properties as well (Jiao, 2017), which could explain why we observed restoration of Complex I respiration. Furthermore, Complex II is known to be impaired in rodents consuming a WD due to the increased presence of the H_2O_2 ROS (Sverdlov, 2015; Hawkins, 2010). This furthers strengthens the hypothesis that PAC-1's antioxidant properties are responsible for maintaining the mitochondrial capacity in our study.

HFD was used to recreate the dietary habits of the average North American. This study shows that exercise alone is not enough to prevent the negative effects of HFD. At first sight, the decline in exercise performance could be attributed to weight gained as a result of HFD, but the PAC and HFD groups had similar gains in body weight. HFD has been shown to impair voluntary exercise in mice compared to regular chow (Clayton, 2022), and it is therefore possible that the PAC mice were simply more active. This could account for potential differences in body composition, with the HFD group potentially having a larger percentage of adipose tissue when

compared to the PAC group. Furthermore, cranberry PACs have previously been associated with preventing weight gain cause by a HFD by increasing energy expenditure through enhanced thermogenesis (Zhou et al, 2020), which further strengthens the hypothesis that there were differences in body composition. HFD is composed of long-chain triglycerides which are thought to be responsible for the negative effects of this diet. When compared to a high-fat ketogenic diet (i.e. enriched in medium-chain fatty acids), HFD was shown to impair mitochondrial adaptations to exercise, a result that is associated with impaired respiration at complex II (Hyatt, 2016). We also observed diminished respiration at Complex II, as well as at Complex I, a finding that can be explain by the fact that three weeks of HFD consumption is enough to downregulate genes necessary for OXPHOS and mitochondrial biogenesis (Sparks, 2005).

The choice of not including a sedentary control was based on previous work showing that polyphenol supplementation improves mitochondrial capacity only when combined with exercise training (Polley et al, 2015). As mentioned above, exercise alone is not enough to offset the declines in muscle bioenergetics caused by HFD. Our choice of HIIT as a training modality, three times per week, was based on its time-efficiency and its capacity to induce comparable adaptations to those achieved through significantly longer bouts of constant-moderate intensity running (Martinez, 2019).

Based on our result, it is likely that body composition was different between the groups, since weight gain was similar between HFD and PAC, but performance was different. Hence, some of the limitations of this study are that body composition and caloric intake were not measured. Mice were provided ad libitum access to food, raising the possibility that the PAC group exhibited increased activity and consumed more calories than the HFD group, potentially accounting for

their similar weight gain. Future studies should investigate if polyphenol supplementation affects voluntary exercise and/or caloric intake.

In conclusion, the findings of this study suggest that a 28-day supplementation of cranberry-derived PAC-1 effectively mitigates running performance declines induced by HFD, possibly by preserving mitochondrial capacity levels.

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2.6. Table

Table 1: High-fat diet composition

Ingredient	**g/Kg of diet**
Maltodextrin	161.46
Sucrose	88.87
Casein	258.33
L-cystine	3.88
Soybean oil	32.29
Lard	316.46
Cellulose	64.58
Calcium Carbonate	7.1
Dicalcium phosphate	16.79
Potassium Citrate	21.31
Choline bitartrate	2.58
Mineral mix	35
Vitamin mix	10

2.7. Figures

Figure 1. Diagram of the study protocol

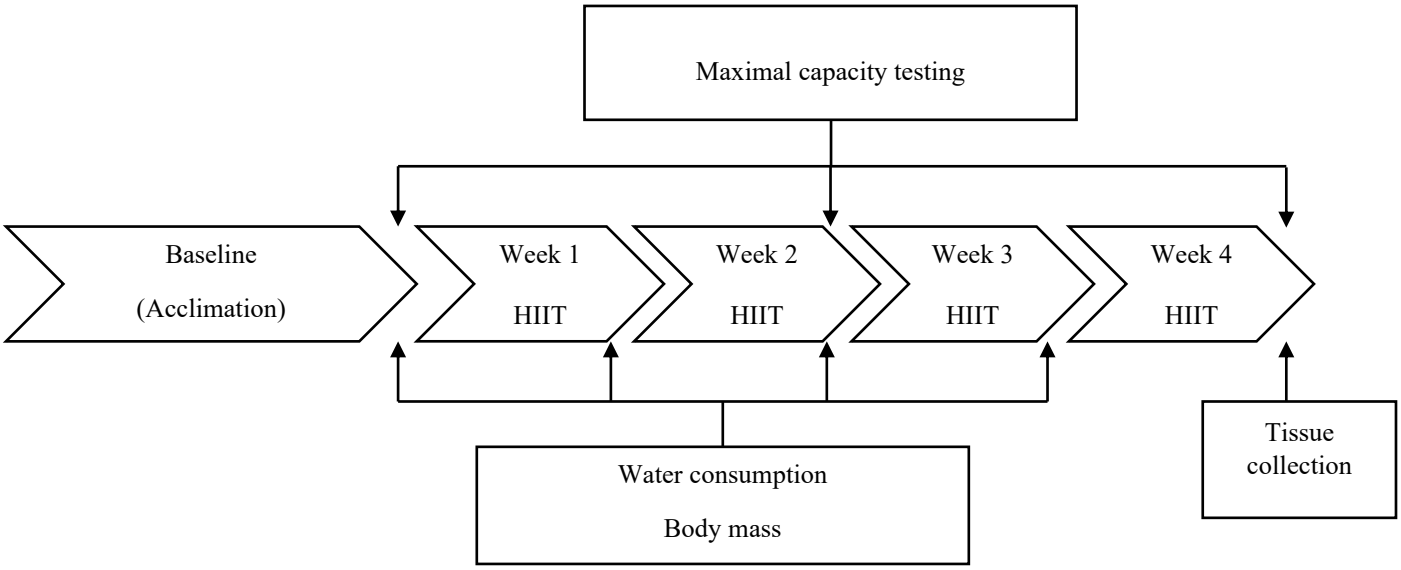


Figure 2. Weekly progression of body mass. * $p < 0.05$ versus week 1. § $p < 0.05$ versus week 2.

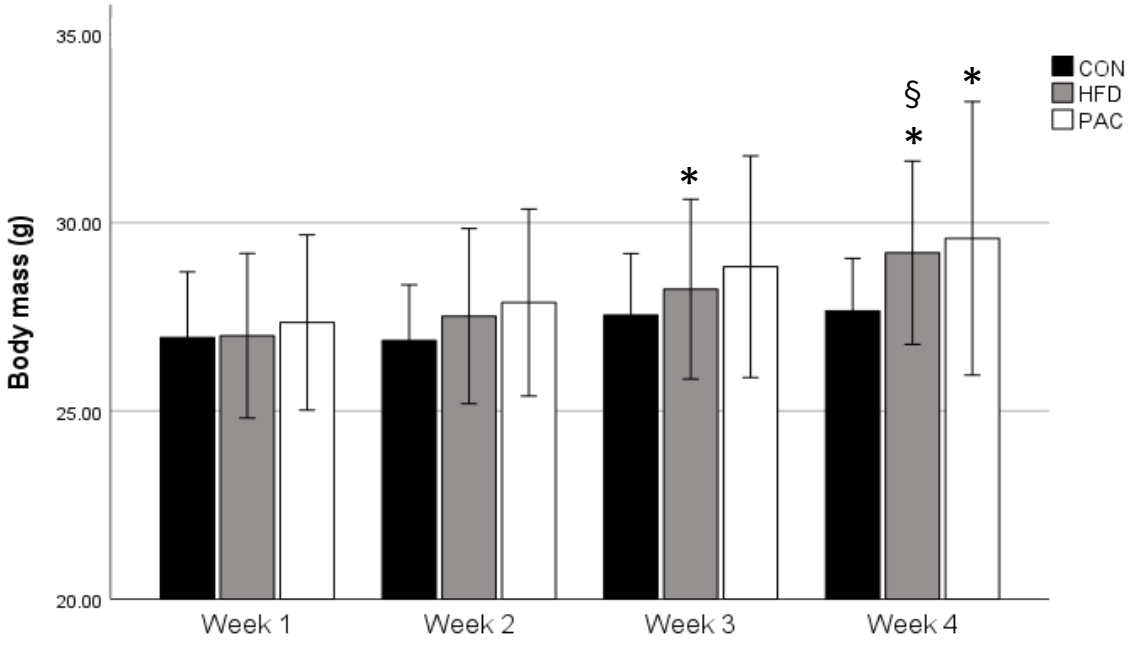


Figure 3. Maximal running capacity at baseline, week 2, and week 4. * $p < 0.05$ versus baseline.

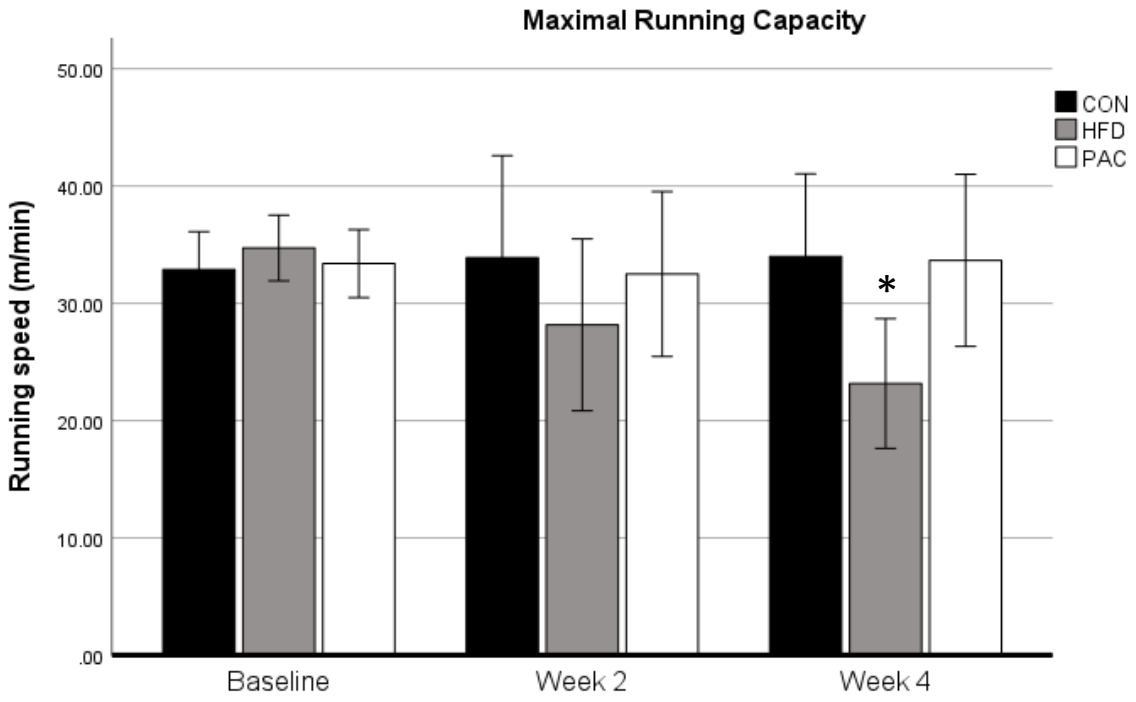


Figure 4. (A) Respiration following the activation of Complex I in the absence of ADP. (B) State 3 respiration at Complex I after the addition of ADP. (C) Maximal coupled state 3 respiration through Complex I and Complex II. * $p < 0.05$

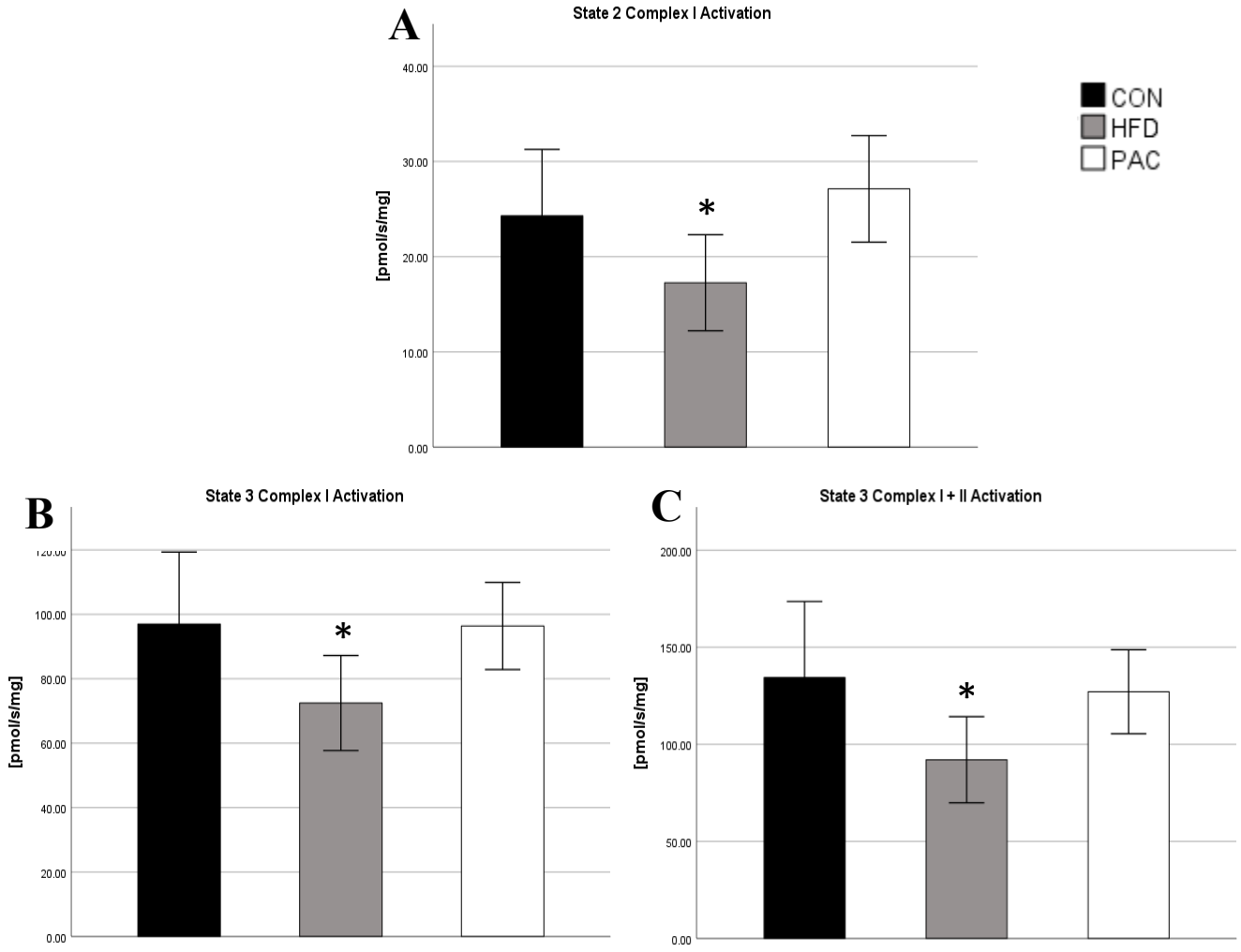
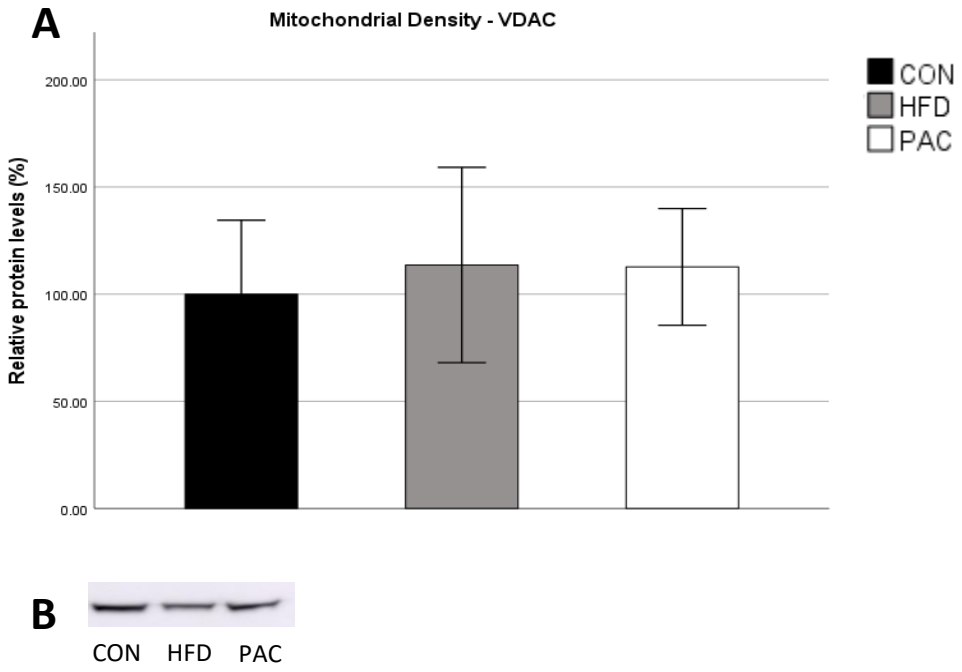


Figure 5. (A) Mitochondrial density in the vastus lateralis, measured by the abundance of the voltage-dependent anion channel. (B) Representative blots.



Transition to Chapter 3

In Chapter 2, we established that cranberry PACs influence maximal running speed and mitochondrial capacity in active mice consuming a HFD. This study served as a proof of concept to move forward with subsequent human trials. Prior to designing a human study, we decided to perform a systematic review of randomized controlled trials with meta-analysis to explore the effects of polyphenol-rich berries on aerobic exercise and biomarkers of performance. Chapter 3 presents that manuscript, which is currently under review in *Clinical Nutrition ESPEN*.

Chapter 3: Manuscript II

Effects of polyphenol-rich, berry supplementation on exercise performance: a systematic review and meta-analysis

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Under review in*: Clinical Nutrition ESPEN* (March 2024)

Effects of polyphenol-rich, berry supplementation on exercise performance: a systematic review and meta-analysis

3.1. Abstract

Background & Aims: Polyphenols are plant secondary compounds that possess antioxidant properties associated with preventing inflammation-mediated ailments such as cardiovascular disease and cancer. Recent studies hint at their capacity to enhance exercise performance. Berries, in addition to containing high amounts of essential vitamins and minerals, are extremely rich in polyphenols. Therefore, the aim of this systematic review and meta-analysis was to compile relevant human randomized controlled trials exploring the potential of polyphenol-rich berries to enhance exercise performance and associated biomarkers. **Methods:** The PubMed, Web of Science, and SPORTDiscus databases were searched using keywords related to berry supplementation, exercise performance, and biomarkers of performance. In total, 2374 articles were screened and 14 were included in the analysis. **Results:** The results indicate no statistically significant effect of berry supplementation on exercise performance and its associated biomarkers. However, there is a trend towards a positive pooled effect size of berry supplementation on time to exhaustion (SMD: 0.57, Z: 1.51, p-value: 0.13). Furthermore, all pooled effect sizes favor berry supplementation. **Conclusions:** Due to variations in testing protocols and biomarkers of interest among the studies included, no more than 7 articles were included for any given outcome measure. This underscores the necessity for additional high-quality randomized controlled trials (RCTs) to strengthen the evidence and allow for recommendations to be made. This systematic review and meta-analysis was registered on Open Science Framework (DOI 10.17605/OSF.IO/NCAVJ).

Keywords

Berry, Polyphenol, Exercise, Inflammation

3.2. Introduction

The surge in dietary supplement consumption among athletes in recent years has become a prevalent phenomenon, with gravitation towards natural health products for their potential to optimize exercise performance[1]. Commonly embraced approaches encompass a range of supplements, such as multi-vitamins, protein powders, creatine, and caffeine. Nutraceuticals, compounds derived from whole foods and renowned for their therapeutic and nutritional properties, present a promising avenue for athletes seeking to enhance performance[2]. Within the vast array of nutraceuticals, polyphenols emerge as a prominent category. These plant-derived compounds, responsible for the vibrant pigmentation in fruits and vegetables, encompass anthocyanins, flavanols, and flavonols. They not only possess therapeutic properties against inflammation-mediated diseases (i.e. cardiovascular disease, neurodegenerative diseases, and cancer), but also offer significant nutritional benefits[3,4]. In addition, polyphenols have recently garnered attention for their potential impact on exercise performance.

Berries, in particular, are a noteworthy source of polyphenols, featuring abundant phenolic compounds, vitamins, and minerals[5]. High in calcium, potassium, sodium, and iron, berries also serve as a vital reservoir of essential vitamins[6]. Moreover, scientific studies have established a connection between the consumption of berries and a decreased occurrence of disorders induced by reactive oxygen species (ROS), including cardiovascular issues, cancer, and inflammatory processes[7]. While berries do contain antioxidant vitamins such as A, C, and E, their exceptional antioxidant capacity predominantly stems from the abundance of phenolic compounds, notably anthocyanins[8]. Anthocyanins have demonstrated efficacy in lowering inflammatory markers such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) at specific daily doses[9,10].

During strenuous exercise, the elevated production of ROS is a consequence of increased oxidative metabolic demands, which can pose a challenge to achieving optimal muscle function[11]. ROS, influence contractile force production in a biphasic manner, with an optimal level required for peak contraction[12]. Beyond this threshold, a decline occurs, compromising the muscle's ability to generate force[13]. This impaired muscle function causes damage to the tissue and is accompanied by elevated levels of the lactate dehydrogenase enzyme (up to 8 days following the exercise bout) and creatine kinase (up to 6 days following the exercise bout)[14]. In this context, the potential of berry anthocyanins to serve as a natural antioxidant defense against exercise-induced free radical over-production holds promise for enhancing overall performance. Therefore, the objective of this systematic review and meta-analysis was to identify randomized control trials examining the impact of berry polyphenol supplementation on exercise performance, exercise-induced inflammation, and muscle damage.

3.3. Methods

Search Strategy

This systematic review with meta-analysis was registered on the Open Science Framework (DOI 10.17605/OSF.IO/NCAVJ) and adheres to the guidelines outlined in the Cochrane Handbook for Systematic Reviews[15]. All articles investigating the effect of berry polyphenols on aerobic exercise performance as of January 31st, 2024, were screened from PubMed, Web of Science, and SPORTDiscus using the following keywords: '(berry OR berries OR tart cherry OR haskap berry OR blueberry OR blackberry OR raspberry OR strawberry OR cranberry OR PAC OR A-type proanthocyanidin OR PAC-1 OR proanthocyanidin OR ellagitanin) AND (athletic performance OR exercise performance OR sport performance OR aerobic endurance OR aerobic power OR aerobic performance OR muscular endurance OR muscular power OR muscular performance OR

training performance OR sport OR athlete) AND (RCT OR randomized controlled trial OR randomized clinical trial OR clinical trial).' Google Scholar was also searched using a combination of terms such as “Polyphenols” and “Exercise”, to identify any articles that may have been missed. The reference lists of the selected articles were reviewed to identify any additional titles for inclusion, and the search was not restricted by language or year of publication.

Study Selection and Eligibility Criteria

The articles obtained from the search were imported into the web-based software Rayyan[16]. FP and AB screened the titles and abstracts for inclusion, and duplicates were deleted. Subsequently, FP and ASA assessed the full-text articles based on the predetermined search criteria. We included studies that met the following criteria: (i) were designed as blinded (single or double) randomized controlled trials (RCTs), (ii) involved healthy participants aged 18 years or older with a body mass index less than 30, (iii) utilized an aerobic exercise testing protocol lasting more than 30 seconds, and (iv) provided a berry-derived supplement for at least 4 days. The primary outcomes were exercise performance metrics and biological markers of performance, including exercise time to fatigue, time to complete a given distance, any graded aerobic exercise test score, maximal oxygen uptake (VO_2), markers of muscular damage and inflammation, and post-exercise blood lactate response. Studies were excluded if: (i) participants were given multiple supplements simultaneously, (ii) participants had any medical conditions or were taking medication, or (iii) polyphenol supplement came from a non-berry source. Two authors (FP and ASA) evaluated the inclusion of studies based on these criteria, with any conflict settled by a third author (AB). **Figure 1** illustrates the selection process.

Data Extraction and Quality Assessment

Data extraction was conducted independently by FP and ASA and entered in an Excel spreadsheet. Information collected included: (i) participant characteristics (age, sex, BMI, location, and fitness level), (ii) study design (duration, setting, and characteristics), (iii) details of the supplementation protocol (berry type, polyphenol content, timing), (iv) testing protocol utilized, (v) markers of performance assessed (time to exhaustion, time trial, VO_2, inflammatory and muscle damage marker, blood lactate), and (vi) assessment of bias. Attempts were made to contact two authors to obtain missing data[17,18].

Statistical Analysis

Statistical analyses were conducted using R version 4.3.2[19], with the meta package[20]. A meta-analysis statistic was conducted for all outcome measures with at least two studies, but forest plots were only generated when at least three studies were available for a single outcome measure, including the examination of the effects of berry or berry-derived supplementation on changes in time to exhaustion, VO2max, C-reactive protein, creatine kinase, and lactate dehydrogenase. For each outcome of interest, the effect was converted to the standardized mean difference (SMD) with standard deviation and 95% confidence interval, using a random effects model (Dersimonian-Liard). In studies where the standard deviation of change (SDchange) was not provided, the value was calculated using the formula outlined in the Cochrane Handbook[15]. This calculation either utilized a correlation coefficient from a similar study or assumed a conservative value of 0.5. Heterogeneity was assessed using the I-square statistic (I^2), with values of of 25%, 50%, and 75% considered as indicating low, moderate, and high heterogeneity, respectively[20]. Additionally, effect sizes of 0.2, 0.5, and 0.8 were considered as indicating small, medium, and large effects, respectively[22].

FP and ASA independently completed a risk of bias assessment for each article based on the Cochrane Handbook tool[15]. Five domains were assessed: (i) randomization process, (ii) deviations from intended interventions, (iii) missing outcome data, (iv) measurement of the outcome, and (v) selection of the reported result.

3.4. Results

Study Selection

The titles and abstracts of 2374 articles were obtained and screened for inclusion, with duplicates identified and deleted. Following the screening process, 18 full-text articles remained. The reference list of the selected articles was assessed, resulting in an addition of 8 more studies. Subsequently, the 26 articles were evaluated based on the predetermined search criteria, and 11 articles were excluded based on various reasons stated in **Figure 1.** As a result, 14 studies were included in the final meta-analysis.

Study Characteristics

A summary of the characteristics of the studies included in this meta-analysis is provided in **Table 1**. The studies included 309 participants (264 males, 45 females) with fitness levels ranging from recreationally active to high-level athletes. All studies were placebo-controlled, with 10 opting for a parallel design. One study was non-blinded[23], while two were single-blinded[18,24] and the remaining were double-blinded. All studies used berry concentrates, powders, or juices, except for three that used a single polyphenol, quercetin, extracted from the Fava D'Anta berry[25-27]. The average daily anthocyanin consumption varied from 27.58 mg to 552 mg. Six studies provided a different polyphenol than anthocyanin, including quercetin (n=3)[25-27], punicalagin (n=1)[17], proanthocyanidin (n=1)[28], and total polyphenol (n=1)[29]. Only one study reported no information regarding polyphenol content[30].

Risk of Bias

The category of "deviations from intended interventions" presented the highest risk of bias, with two studies categorized as high risk[18,24] and seven others showing some concerns. No other category exhibited a high risk. The "bias from the randomization process", "missing outcome data", and "reported results" had five, nine, and six studies showing some concern, respectively. The "measurement of the outcome" had low risk of bias for all but two studies[18,24] (see **Figure 2**).

Effects on Exercise Performance

Exercise performance metrics included time to exhaustion, power output, and VO_{2max}. Although some studies included a time trial, none provided baseline time trial values. The analysis for time to exhaustion included four studies with 108 participants, with all but one study using a parallel design[25-26,29,31] (**Figure 3**). The results show a strong trend toward positive pooled effect size of berry supplementation (SMD: 0.57, Z: 1.51, p-value: 0.13), with the studies having moderate-high heterogeneity (I^2 = 69%). For VO_{2max}, seven studies were identified, with a total of 186 subjects and 5 parallel RCTs[17,25-27,30-31] (**Figure 4**). Berry supplementation had non-significant positive pooled effect size on VO_{2max} (SMD: 0.12, *Z*: 0.38, *p*-value: 0.70). The heterogeneity was moderate-large (I^2 = 71%). Finally, only two studies reported maximal power as their main performance outcome[30,32]. These studies included 28 participants, showed a non-significant positive pooled effect size on maximal power output (SMD: 0.32, *Z*: 0.95, *p*-value: 0.34), and had low heterogeneity (I^2 = 0%) All other studies incorporated in this systematic review included a form of aerobic testing that was either unique or did not have pre- and post-test values.

Effects on Inflammatory Markers

To investigate the effect of berry polyphenols on inflammation, the included studies looked at C-reactive protein, IL-6, IL-1b, and IL-8. The inflammatory marker that was discussed in the most

studies was C-reactive protein, with a total of five studies and 100 subjects[17-18,28,32-33] (**Figure 5**). The results of the meta-analysis show a non-significant negative pooled effect size on this marker (SMD: -0.09, *Z*: -0.48, *p*-value: 0.63) and low heterogeneity (I^2 = 0%). Only two studies included values for each of the other inflammatory markers, all showing non-significant negative pooled effect sizes (IL-6: SMD: -0.56, *Z*: 1.35, *p*-value: 0.18; IL-1b: SMD: -0.03, *Z*: -0.10, *p*-value: 0.92, IL-8: SMD: -0.32, *Z*: -1.03, *p*-value: 0.30)[28,32,34]. Heterogeneity was moderate for IL-6 (I^2 = 44%) and low for both IL-1b and IL-8 (I^2 = 0% for both).

Effects on Creatine Kinase

Creatine kinase was chosen as a marker of exercise-induced muscle damage (**Figure 6**). Seven studies with a total of 155 subjects included this marker[17,23-24,28,31-32,34]. The results show a non-significant negative pooled effect size (SMD: -0.16, *Z*: -1.03, *p*-value: 0.31). The studies presented low heterogeneity (I^2 = 0%).

Effects on Lactate Dehydrogenase

In total, five studies were included to explore the effects of berry supplementation on lactate dehydrogenase (**Figure 7**). These studies included 120 participants and we found a non-significant negative pooled effect size (SMD: -0.016, *Z*: -0.09, *p*-value: 0.93) with low heterogeneity (I^2 = 0%)[23-24,28-29,31].

3.5. Discussion

This systematic review and meta-analysis aimed to investigate the potential ergogenic effects of polyphenol-rich berry supplementation. Specifically, the main outcomes measures were aerobic exercise performance, inflammation, muscle damage, and lactate response to exercise. Although the results did not demonstrate statistical significance for any of the parameters included in the

meta-analyses, the pooled effect sizes did exhibit a trend in favor of berry supplementation for time to exhaustion (p=0.13)

Similar meta-analyses have been conducted in recent years, but none have specifically examined the effect of multi-day polyphenol supplementation from berries on performance, inflammation, muscle damage, and lactate response to exercise. Overall, results of previously published systematic reviews and meta-analyses regarding the ergogenic effects of polyphenols on exercise performance vary greatly, with some showing positive outcomes[35,36] and others showing negative effects[37,38].

A 2017 systematic review with meta-analysis explored the effects of polyphenols on exercise performance and found a statistically significant effect in favor of polyphenols[35]. Five studies included in that review overlapped with the studies in our review[25-27,29,31], the highest overlap among all meta-analyses looking at polyphenols and performance. The authors adjusted the exercise data to a common power metric to account for variations in exercise protocols, which may explain them reaching statistical significance. In our analysis, for time to exhaustion, VO_{2max}, and maximal power, we were only able to compare four, seven, and two studies, respectively, and all had a non-significant positive pooled effect. The limited number of studies suggests that more research is needed to strengthen the evidence of any effect of berries on performance metrics. Notably, there was a trend toward a positive pooled effect for time to exhaustion, with the four studies included having a positive mean difference. Exhaustion is multifactorial and occurs when an individual can no longer meet the requirements of a given exercise[39]. We included biomarkers of muscle fatigue in this review – i.e. lactate and interleukins – but none showed significant effects of berry supplementation. Another factor in muscle fatigue is oxidative stress[40]. During high-intensity aerobic exercise, ROS production is increased and antioxidant production decreases,

which has been shown to correlate with the training load[41]. Polyphenols are known to have antioxidative effect, which could potentially explain the observed trend towards a positive effect on time to exhaustion. Six of the studies we included investigated oxidative stress, with three showing improvements in total antioxidant capacity (TAC)[23,30-31]. One noted no difference in TAC[34] and two observed improvements in other markers of oxidative stress[24,28].

The results for VO_{2max} showed no significant effects of berry polyphenols. There is evidence of a genetic "ceiling" for VO_{2max} in highly trained individuals, whereby improvements in aerobic capacity eventually plateau despite continuous training, at which point other physiological adaptations are required to improve performance[42,43]. Since all participants in this review were active and several studies included high-level athletes, the lack of observed effect on VO2max could be attributed to this phenomenon.

Six out of the 14 studies included in this analysis utilized a form of aerobic testing that was either unique or lacked pre- and post-test values. Among these, four studies used a time trial – 5km to marathon length – but did not provide baseline values[23,28,33-34]. Levers and colleagues observed a significant improvement in half marathon times among participants consuming Montmorency cherry supplement, whereas the other three studies found no difference in time to completion[34]. Carvalho and colleagues investigated the effect of an acai beverage on running time to exhaustion at 90% VO_{2max} and observed significantly higher times in the treatment group when compared to the placebo[24]. Unfortunately, they did not have baseline measurements available, precluding the inclusion of their results in the meta-analysis. Finally, Quinlan et al. reported lower sprint times following a modified version of the Loughborough Intermittent Shuttle Test, which correlated with lower levels of CRP and CK[18]. This performance metric was unique and therefore could not be included in meta-analysis.

Some limitations need to be considered when interpreting the results. The small number of RCTs included in the meta-analyses prevented subgroup analysis, which means that no conclusions could be drawn regarding the source of moderate to high heterogeneity between studies for certain outcomes[15]. Also, we included only active participants, with five studies involving highly trained athletes. Individuals performing at a high level are more likely to be at or close to their genetic ceiling and, therefore, fail to show significant improvements in performance and biomarkers. Finally, although all studies investigated aerobic exercise performance, there was a wide range of protocols. This is why we did not have many studies included in the meta-analysis for each outcome and could have affected the results.

3.6. Conclusion

In conclusion, the efficacy of polyphenol-rich berry supplementation in enhancing exercise performance or positively affecting biomarkers of performance remains unclear. The optimal dosage and duration remain unknown, and the testing protocols among studies varies greatly. Still, the results of the meta-analyses show non-statistically significant pooled effect sizes in favor of berry supplementation for all outcome measures, which indicates that more quality RCTs are needed before any recommendations can be made.

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Conflict of Interest

Authors declare no conflict of interest.

Author Contributions

Francis Parenteau: conceptualization, methodology, investigation, data curation, writing – original draft, writing – review and editing.

Antoine St-Amant: investigation, data curation, formal analysis, writing – review and editing.

Andreas Bergdahl: conceptualization, investigation, writing – review and editing, supervision.

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3.8. Figures

Figure 1. The preferred reporting items for systematic reviews with meta-analysis (PRISMA) flowchart

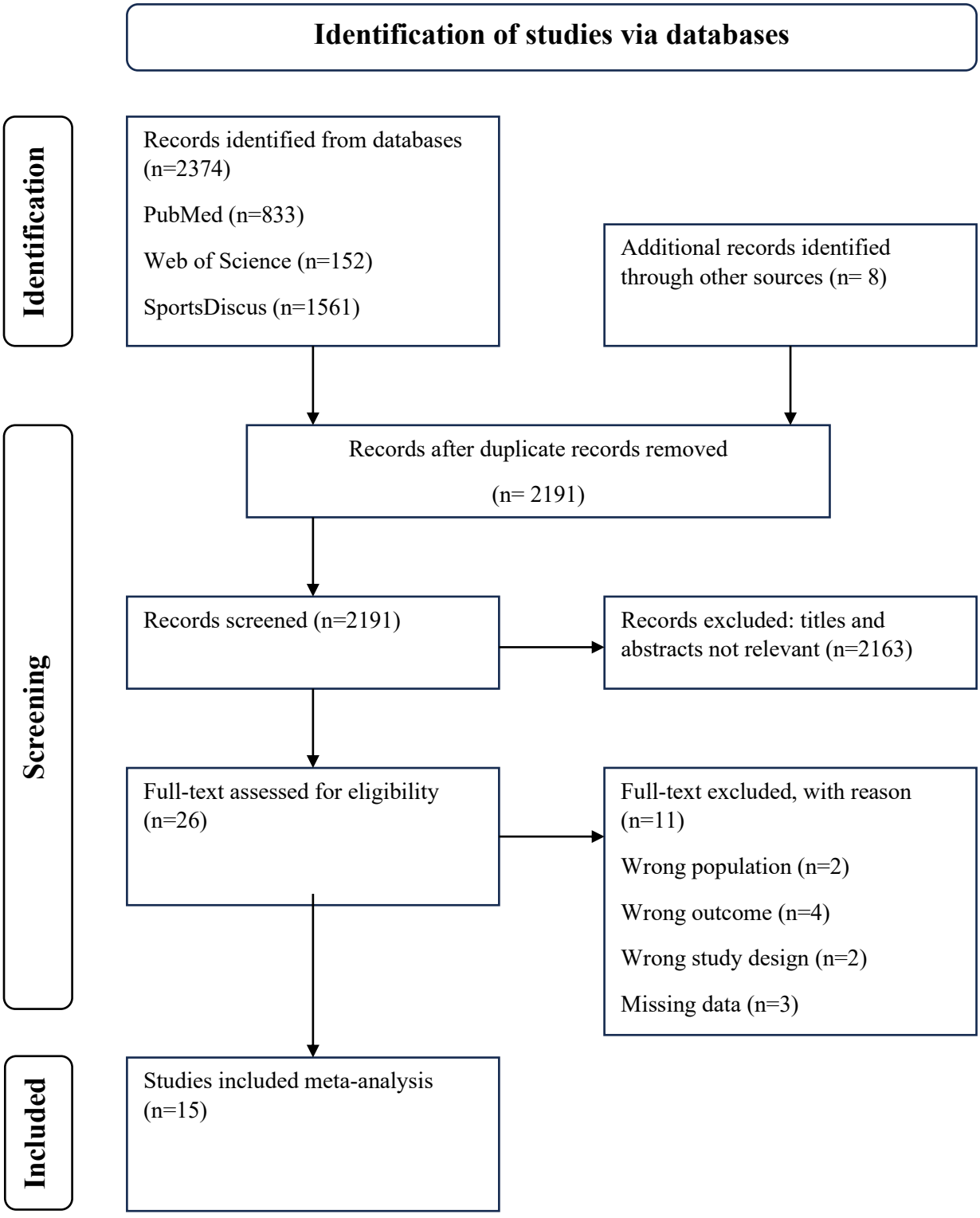


Figure 2. Risk of Bias Assessment

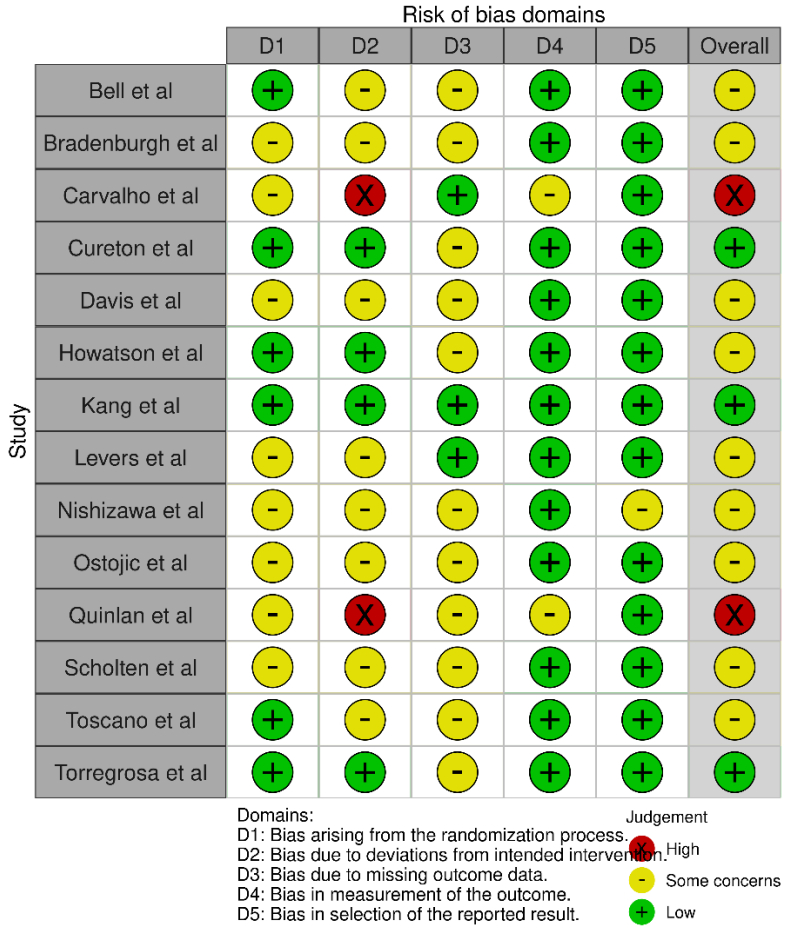


Figure 3. Forest Plot Illustrating the Impact of Berry Extracts on Time to Exhaustion in Studies of Interest.

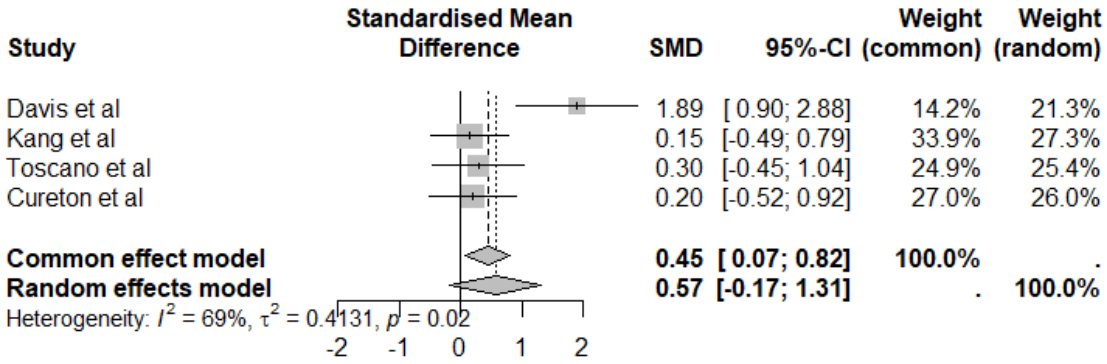


Figure 4. Forest Plot Illustrating the Impact of Berry Extracts on VO2 in Studies of Interest.

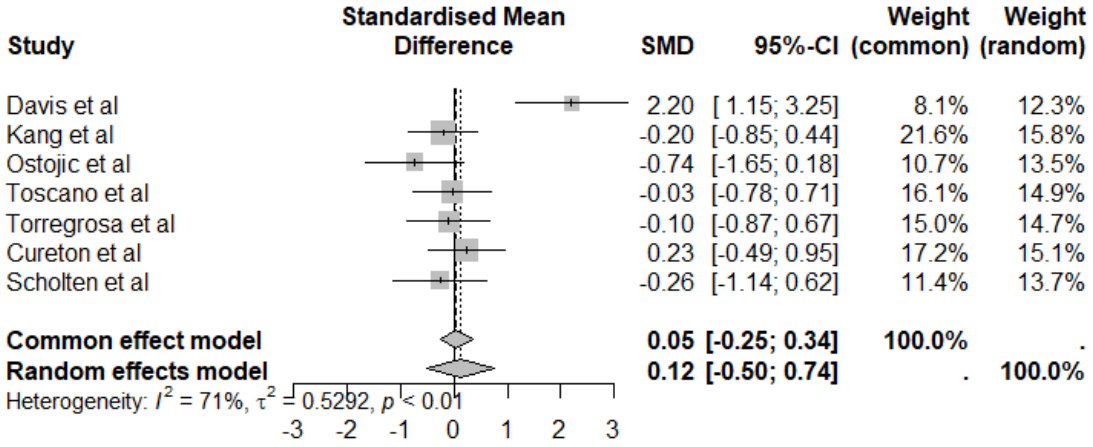


Figure 5. Forest Plot Illustrating the Impact of Berry Extracts on C-Reactive Protein in Studies of Interest.

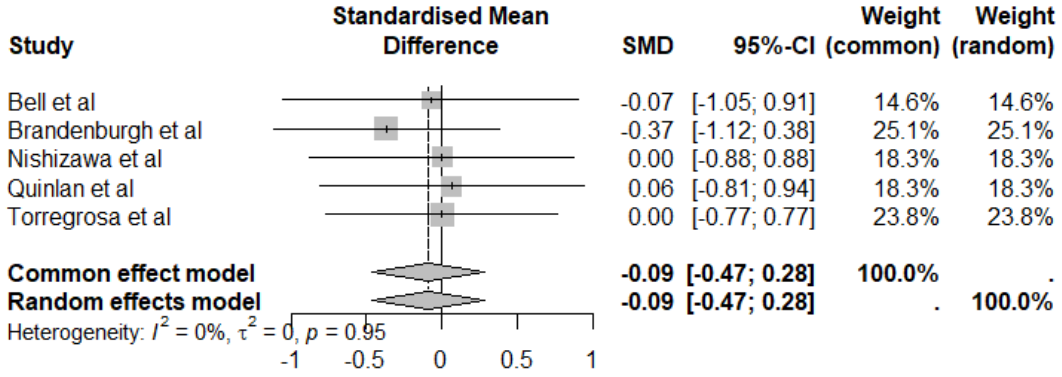


Figure 6. Forest Plot Illustrating the Impact of Berry Extracts on Creatine Kinase in Studies of Interest.

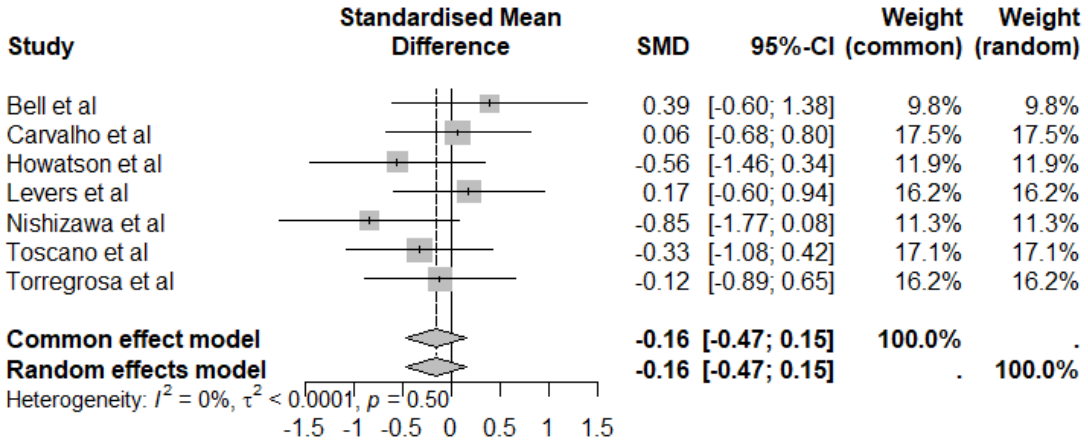
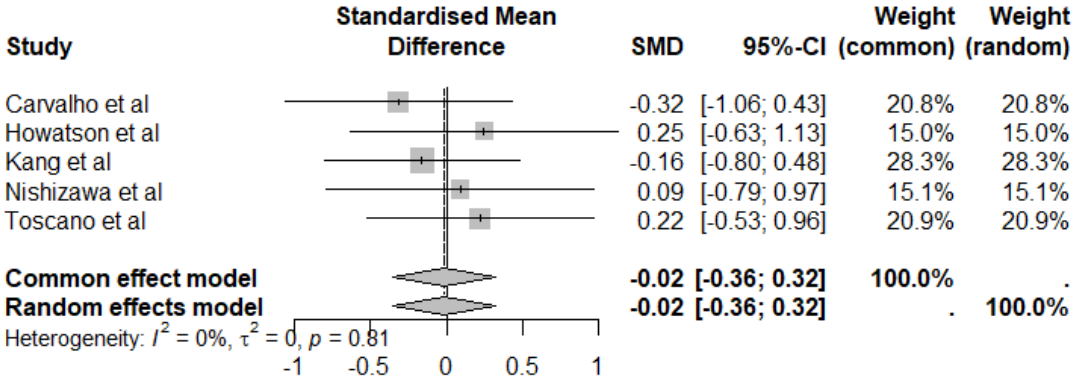


Figure 7. Forest Plot Illustrating the Impact of Berry Extracts on Lactate Dehydrogenase in Studies of Interest.



3.9. Table

Table 1. Summary of studies

Article	Country	Study Design	Participants	Berry Type	Supplementation protocol	Duration	Testing Protocol
Bell et al., 2016	England	double-blind, placebo-controlled parallel RCT	16 semi-professional male soccer players	Montmorency cherry	30 mL of concentrate, twice daily. 552 mg total anthocyanins daily	8 days	Adapted version of the LIST
Bradenburgh et al., 2019	Canada	double-blind, placebo-controlled crossover RCT	14 healthy participants with running experience	Blueberry	24 g of powder, three times per day. 316 mg total anthocyanins daily.	4 days	8-km TT on non-motorized treadmill
Carvalho et al, 2015	Brazil	single-blind, placebo-controlled crossover RCT	14 aeronautical pentathlon, running, and sprinting male athletes	Acai	300 mL beverage, once daily. 27.58 mg total anthocyanins daily.	4 days	Graded VO2max test on treadmill. Time to exhaustion test at 90% VO2max
Davis et al, 2010	USA	double-blind, placebo-controlled crossover RCT	12 active participants (7 males, 5 females)	Fava d'Anta	500g quercetin powder, twice daily.	7 days	Graded VO2max test on cycle ergometer. Time to exhaustion test at 75% VO2max
Howatson et al, 2010	UK	Non-blinded, placebo-controlled parallel RCT	20 recreational runners (13 males, 7 females)	Montmorency cherry	236 mL tart cherry juice, twice daily. 80 mg total anthocyanins daily.	8 days	Time to completion of the London marathon
Kang et al, 2012	Japan	double-blind, placebo-controlled parallel RCT	38 active males	Lychee	2 50 mg capsules, twice daily. 66 mg total polyphenols daily.	30 days	Time to exhaustion test on treadmill at 80% maximal HR
Levers et al, 2016	USA	double-blind, placebo-controlled parallel RCT	27 endurance trained runners or triathletes (18 males, 9 females)	Montmorency cherry	1 capsule daily. 66 mg total anthocyanins daily	10 days	Time to completion of a half-marathon
Nishizawa et al, 2011	Japan	double-blind, placebo-controlled parallel RCT	20 University male endurance runners	Lychee	Two 50 mg capsules daily. 58.6 mg proanthocyanidins	60 days	5-km running TT
Ostojic et al, 2008	Serbia	double-blind, placebo-controlled parallel RCT	20 active participants (14 males, 6 females)	Coffeeberry	Two 400 mg doses per day. Polyphenol content not mentioned	28 days	60-sec vertical jump test followed by a shuttle run test
Quinlan et al, 2020	UK	single-blind, placebo-controlled parallel RCT	20 team-sport players (8 males, 12 females)	Montmorency cherry	30 mL of concentrate, twice daily. 552 mg total anthocyanins daily	8 days	Adapted version of the LIST

Toscano et al, 2015	Brazil	double-blind, placebo-controlled parallel RCT	28 recreational runners (22 males, 6 females)	Grape	10 mL/kg/day of juice, divided in two servings daily. 52.58 mg/L of juice total anthocyanins.	28 days	Graded VO2max test on treadmill. Time to exhaustion test on treadmill at the anaerobic threshold
Torregrosa et al, 2019	Spain	double-blind, placebo-controlled crossover RCT	30 amateur male endurance cyclist	Pomegranate	375 mg capsule, twice daily. 225 mg punicalagins daily.	15 days	Incremental exercise test to exhaustion on road bicycle
Cureton et al, 2009	Georgia	double-blind, placebo-controlled parallel RCT	30 recreationally active males	Fava d'Anta	1000 mg quercetin in 946 mL sports beverage, divided in four servings daily	7-16 days	Graded VO2peak test on cycle ergometer. 10-min cycling performance test
Scholten et al, 2015	USA	double-blind, placebo-controlled parallel RCT	20 physically active males	Fava d'Anta	1000 mg quercetin capsule daily.	8 weeks	Graded VO2max test on treadmill. 5-km TT

Transition to Chapter 4

Chapter 3 contributed to shine a light on the potential ergogenic effects of polyphenol-rich berry supplementation. Although no marked effect was observed for any of the outcome measures included, we gained some insight for the potential of polyphenols to positively modulate certain markers of performance. The scarce body of research highlighted the need for more human trials to widen the wealth of knowledge around polyphenol supplementation.

Chapter 4 aimed to elucidate the potential effects of cranberry supplementation on running times and markers of performance in endurance runners. The manuscript presented in Chapter 4 is published in *Physical Activity and Nutrition*.

Chapter 4: Manuscript III

Cranberry supplementation improves markers of performance in trained runners.

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Cranberry supplementation improves physiological markers of performance in trained runners

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[Purpose] Cranberries have the highest polyphenol and antioxidant capacity among fruits and vegetables and may protect against exercise-induced free radical production, consequently improving performance. This study aimed to investigate the effect of polyphenol-rich cranberry extract (CE) on time-trial performance and lactate response following exercise.

[Methods] A total of 14 trained runners were tested at i) baseline, ii) 2 h following an acute CE dose (0.7 g/kg of body mass), and iii) 4 weeks after daily supplement consumption (0.3 g/kg of body mass). At each time point, runners performed a 1500-m race followed by a 400-m race where the live vastus lateralis oxygenation changes were determined by near-infrared spectroscopy and blood lactate was measured at rest and 1 and 3 min after each trial. The Shapiro-Wilk test and repeated-measures analysis of variance were used to establish significance ($P < 0.05$).

[Results] Cranberry supplementation over 28 d improved aerobic performance during the 1500-m time trial, whereas the acute dose had no effect. More specifically, muscle reoxygenation rates were significantly faster after 28 d compared to baseline ($P = 0.04$; $\eta^2 = 0.29$), and a trend towards slower deoxygenation rate was observed ($P = 0.13$; $\eta^2 = 0.20$). Chronic CE consumption also buffered the post-exercise lactate response for the 400-m race ($P = 0.01$; $\eta^2 = 0.27$), while no effects were seen for the longer race.

[Conclusion] Our results suggest that cranberry supplementation may have ergogenic effects, as it improves physiological markers of performance during short- and long-distance running.

[Keywords] polyphenol, proanthocyanidins, NIRS, muscle oxygenation, oxygen consumption, blood lactate

INTRODUCTION

Polyphenols, including proanthocyanidins (PAC), anthocyanins, flavonols, and flavonols, are secondary plant compounds found in fruits and vegetables[1,2]. Cranberries have the highest total polyphenol content among the most consumed fruits in the American diet, and one of the highest antioxidant capacities among fruits and vegetables[3,4]. Furthermore, their polyphenol content stands out because of the high concentration in rare A-type PAC, which is believed to be the main contributor to their beneficial effects[5].

The use of dietary supplements is growing among athletes, and many are turning to natural health products to improve exercise performance[6]. Strenuous exercise significantly increases reactive oxygen species production owing to high oxidative metabolic demands[7,8]. Consequently, polyphenols may offer natural antioxidant defense against exercise-induced free radical production.

During operation, the contribution of the aerobic and anaerobic energy systems depends on the relative intensity. Blood lactate can be measured during or at the termination of exercise to provide insight into the anaerobic capacity of an athlete, and has been associated with relative performance during shorter high-intensity events, such as the 400-m and 800-m time trials (TT)[9]. Maximal oxygen consumption (VO_{2max}) testing is the gold standard for measuring aerobic capacity but is not practical for field testing. Conversely, live muscle oxygenation can be measured using near-infrared spectroscopy (NIRS), a non-invasive technique that is associated with pulmonary VO_2 and has been used in various athletic populations, such as cyclists and runners[10-12]. NIRS devices are portable and can provide muscle-specific information, such as reoxygenation metrics, which offer a reliable way to measure post-exercise recovery[13].

Previous clinical studies have demonstrated the ergogenic effects of cranberries on cycling and rowing, which are notably related to lowering inflammatory markers and buffering lactate[14,15]. To our knowledge, the effects of cranberries on running performance remain unknown. This study aimed to investigate the effect of cranberry supplementation for 28 d on performance, lactate production, and muscle oxygenation when running 1500-m (aerobic) and 400-m (anaerobic) TT.

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METHODS

Participants

A total of 14 trained endurance athletes (8 males and 6 females) were recruited from local varsity cross-country teams and running clubs. All athletes were 18–40 years old and performed at least 5 h of endurance training per week. Table 1 lists the participants' basic characteristics. The exclusion criteria were as follows: (i) smoking or vaping, (ii) use of ergogenic aids or drugs, (iii) injuries or physical limitations that could impair proper running mechanics, and (iv) cardiovascular or metabolic disease. The protocol (#30016555) was approved by the Human Research Ethics Committee of Concordia University. All participants signed a consent form before the start of the study.

Table 1. Participants Characteristics.

Variable	Mean (SD)
Age (years)	28 (6)
Height (cm)	169.4 (8.1)
Weight (kg)	62 (8.1)
Status	
Elite	5
Competitive	7
Recreative	2
Event	
Cross-Country	7
Half Marathon	2
Marathon	2
Other	3
Berry Consumption	
Low	7
Moderate	3
High	4

Study Design

The study utilized a repeated-measures design with case-control matching, comprising three separate visits during which athletes performed a 1500-m TT followed by a 400-m TT. The athletes were instructed to perform their pre-race warm-up before testing. A 10-min static break was provided between the two TTs to ensure full recovery. Each athlete completed an online health questionnaire before their first visit, which included questions related to their health, dietary habits, exercise, and history of injury. Baseline measurements were obtained during the first visit (baseline). At the second visit, 1 week after baseline, participants consumed an acute dose of CE 2 h before testing (SD-CE). The third visit was conducted 28 d after CE consumption to test for chronic effects (28-CE). The tests were performed on a local 200-m indoor track. Figure 1 shows the breakdown of the study population. The discontinuation of participation was not related to any aspect of the supplementation or testing protocols.

Dietary Intervention

The athletes were instructed to maintain their usual diet throughout the study period and on the testing days to avoid confounding factors. Table 1 presents information on berry consumption that we collected. CE comprised a lab-grade freeze-dried powder containing 7.2–10% PACs (Fruit D'Or Inc., Villeroy, QC). The participants were instructed to keep the supplement in a cool, dry place away from sunlight, as per the manufacturer's recommendations. In preparation for their second visit, the participants consumed an acute dose representing 0.7 g/kg of body mass, 1–3 h before testing. For chronic testing, a dose representing 0.3 g/kg of body mass was consumed daily for 28 d.

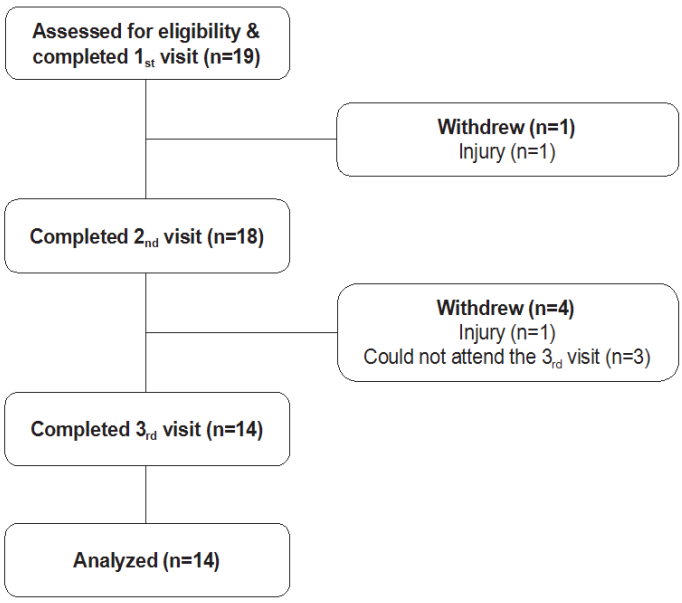

Figure 1. Consort diagram breakdown of the subject population from recruitment to data analysis.

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Muscle Oxygenation

NIRS monitors (Moxy monitors, 5th generation; Fortiori Design, Minnesota, USA) were used to assess changes in muscle oxygenation by utilizing four wavelengths of near-infrared light (680, 720, 760, and 800 nm), with a source-detector spacing of 12.5 and 25.0 mm[16]. Because oxygenated and deoxygenated hemoglobin have different absorbance spectra, they reflect light differently. Consequently, the Moxy device can report changes in both the total tissue hemoglobin concentration ([THb]) and oxygenated hemoglobin expressed as a percentage of the total hemoglobin (SmO_2).

Skinfold measurements were performed at the location of the right vastus lateralis, 10 cm above the patella, using skinfold calipers (Harpenden, UK). For the data to be considered viable, the skinfold measurements should be less than half the distance between the emitter and detector. The NIRS device was placed on the right vastus lateralis muscle and secured using medical tape and HypaFix. The black self-adhesive tape was wrapped around the leg to ensure no movement of the device and to block any extraneous light. The raw SmO_2 and [THb] signals were collected at a frequency of 0.5 Hz with data smoothing performed by the Moxy software. The deoxyhaemoglobin concentration ([HHb]), representing muscle O_2 extraction, was computed from SmO_2 and [THb] using the following equation:

(i) $SmO_2 = 100 \times [O_2Hb]/[THb]$, and
(ii) $[THb] = [O_2Hb] + [HHb]$.[17]

Baseline SmO_2 and [THb] were computed as a 2-min average when athletes sat still, before the 1500-m TT. Peak deoxygenation (SmO_{2min}) was calculated for both TTs as the lowest 5-s SmO_2 average minus the baseline SmO_2. Deoxygenation and reoxygenation rates were calculated as the linear regression of the first 12 s of each TT and the first 12 s following the completion of each TT, respectively[16].

Blood Lactate

Blood was collected from the non-dominant hand using a finger prick with a portable lactometer (Lactate Plus, Nova Biomedical, Waltham, MA, USA). The finger was cleaned using 70% ethanol wipes and soapy water on the fingertip and blood collection was done using disposable lancets. Blood lactate was collected before the 1500-m TT to obtain a baseline measurement (< 2 mmol/L) and at 1- and 3-min marks after each TT.

Data Analysis

A Shapiro-Wilk test of normality was performed for each parameter. Repeated-measures analysis of variance (RMANOVA) was performed for the TT, lactate, and NIRS data, and the Bonferroni post-hoc test was used when statistical significance was detected. Effect sizes are reported as partial eta squared, where: $\eta^2 \leq 0.06$ = small effect; η^2 between 0.06 and 0.13 = medium effect; $\eta^2 \geq 0.14$ = large effect. Repeated measures correlation (rmcorr) was performed to investigate the relationship among running time, lactate production, and peak muscle deoxygenation[18,19]. All data are presented as means ± standard deviation. Data were analyzed using SPSS version 29. Figures were created using R studio version 2023.06.1. Before all the analyses, the significance level was set at 0.05.

RESULTS

Table 2 presents a summary of these data. CE consumption for 28 d demonstrated a trend toward improving running times for the 1500-m TT ($P = 0.10$; $\eta^2 = 0.15$), but not for the 400-m TT ($P = 0.39$; $\eta^2 = 0.07$). Although not statistically significant, the faster running time during the 1500m

Table 2. Summary of results for the performance metrics.

Variable	Group	Mean (SD)	η^2	p-value
Time Trials (s)				
1500m	Baseline	321.36 (45.34)		
	SD-CE	322.24 (43.96)	**0.15**¥	0.10
	28-CE	307.93 (30.69)		
400m	Baseline	73.47 (10.99)		
	SD-CE	73.82 (10.21)	0.07	0.39
	28-CE	70.4 (7.76)		
Muscle Oxygenation Metrics				
Baseline SmO_2 (%)	Baseline	81.8 (6.8)		
	SD-CE	79.8 (8.2)	**0.2**¥	0.14
	28-CE	80.4 (8.4)		
Baseline THb (AU)	Baseline	12.5 (0.5)		
	SD-CE	12.4 (0.4)	0.03	0.71
	28-CE	12.5 (0.4)		
1500m				
Deoxy Rate (%/s)	Baseline	-4.6 (2.6)		
	SD-CE	-4.8 (1.5)	**0.2**¥	0.13
	28-CE	-4.1 (1.8)		

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Variable	Group	Mean (SD)	η^2	p-value
Peak Deoxy (Δ%)	Baseline	-59.9 (12,5)		
	SD-CE	-61.3 (6.8)	0.07	0.55
	28-CE	-57.6 (20.7)		
Mean SmO_2 (%)	Baseline	30.0 (11.4)		
	SD-CE	28.2 (6.8)	0.02	0.82
	28-CE	28.4 (14.0)		
400m				
Deoxy Rate (%/s)	Baseline	-5.1 (2.6)		
	SD-CE	-4.8 (1.1)	0.005	0.95
	28-CE	-5.0 (2.3)		
Peak Deoxy (Δ%)	Baseline	-63.6 (15.0)		
	SD-CE	-68.2 (8.2)	0.01	0.90
	28-CE	-63.4 (21.2)		
Mean SmO_2 (%)	Baseline	27.3 (9.6)		
	SD-CE	26.6 (9.5)	0.01	0.89
	28-CE	26.0 (11.5)		

Mean data expressed as means ± SD. Repeated measures ANOVA p-levels and partial ETA squared (η^2) listed for each variable. ¥ represents a large effect size ($\eta^2 \geq 0.14$); * represents $p<0.05$. *SmO_2* muscle oxygen saturation, *THb* total hemoglobin, *Deoxy* muscle deoxygenation.

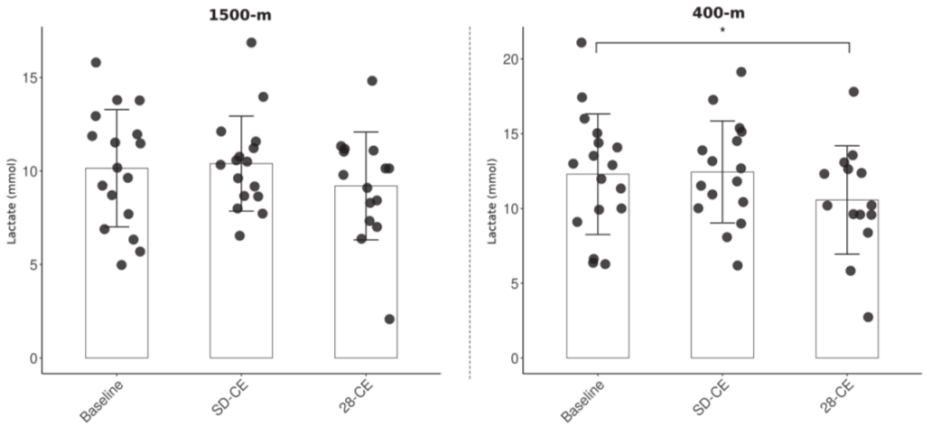

Figure 2. Post-exercise lactate response following the 1500-m and 400-m time trials. * p < 0.05.

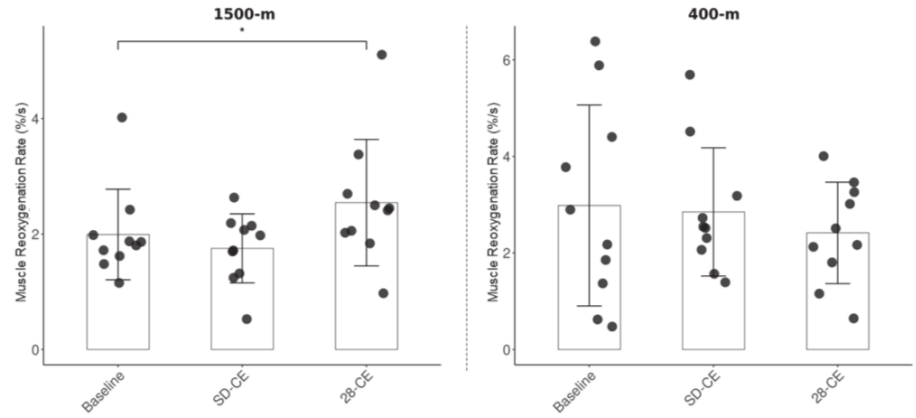

Figure 3. Peak muscle deoxygenation during the 1500-m and 400-m time trials. * p < 0.05.

TT translated to a 1.5% mean increase in running speeds. Baseline lactate levels were the same at all time points (P = 0.27; η^2 = 0.11). CE significantly buffered the lactate peak at 1 min post-run for the 400-m TT (P = 0.01; η^2 = 0.27), but not for the 1500-m TT (P = 0.33; η^2 = 0.08) (Figure 2).

There was no effect of CE supplementation on baseline SmO_2 (P = 0.14; η^2 = 0.2) or THb (P = 0.71; η^2 0.2) between the three conditions, although a trend towards a lower SmO_2

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was observed in the acute dose condition. For the 1500-m TT NIRS data, the muscle reoxygenation rate was significantly faster in the CE-28 condition compared to the baseline ($P = 0.04$; $\eta^2 = 0.29$) (Figure 3). Furthermore, a trend towards a slower deoxygenation rate was observed ($P = 0.13$; $\eta^2 = 0.20$). Peak muscle deoxygenation was similar across all time points ($P = 0.55$; $\eta^2 = 0.07$). For the 400-m TT, no differences were observed for the deoxygenation rate ($P = 0.95$; $\eta^2 = 0.005$), the reoxygenation rate ($P = 0.66$; $\eta^2 = 0.05$), or the peak deoxygenation ($P = 0.90$; $\eta^2 = 0.01$). There were no statistically significant effects of SD-CE on any test parameters.

A correlation between lactate production and running time was observed during the 400-m TT ($P = 0.02$; $r_{rm} = -0.43$) but not for the 1500-m TT ($P = 0.91$; $r_{rm} = 0.02$). Conversely, there was an association between peak muscle deoxygenation and running time for the 1500-m TT ($P = 0.07$; $r_{rm} = 0.40$) but not for the 400-m TT ($P = 0.38$; $r_{rm} = 0.21$). When comparing the relationship between lactate production and peak muscle deoxygenation, we observed only weak associations at both 1500 m ($P = 0.12$; $r_{rm} = 0.27$) and 400 m ($P = 0.28$; $r_{rm} = 0.26$).

When each parameter was normalized to sex, training status, main event, and berry consumption, no statistically significant differences or trends were observed (data not shown).

DISCUSSION

The main finding of this study was that cranberry supplementation for 28 d appeared to improve running speed as well as aerobic performance in trained runners during a 1500-m TT. More specifically, the muscle reoxygenation rates were significantly faster and were accompanied by enhanced running times. The faster time to completion of the 1500-m TT was associated with a 1.5% increase in speed, which is important for competitive runners. For the 400-m TT, CE buffered the post-exercise lactate response, although it did not affect the other parameters.

Few studies have examined the effects of cranberries and cranberry polyphenols on exercise performance. Our results for the acute dose are consistent with the findings of previous animal studies[20,21], in which cranberry did not improve running performance. Similarly, Skarpanska et al. found that 6 weeks of CE supplementation did not affect the 2000-m rowing TT or post-exercise lactate response, which agrees with our findings[14]. Conversely, an acute cranberry and grape seed extract dose lowered the lactate response following a 3 km cycling TT[15]. We observed a similar effect in the 400-m TT, but not during the 1500-m TT. The different testing modalities (running vs. cycling) could explain why such results were observed in a longer cycling TT, as it has been shown that submaximal and maximal cycling requires higher muscular power output, which produces blood lactate concentrations that are larger as compared to running[22].

This is the first study to evaluate the effect of cranberries on muscle oxygenation using an NIRS device, although a few studies have used the same technique to examine the effects of polyphenols from other sources. Similar to our observations, a study on flavonol-rich dark chocolate supplementation for 2 weeks found no effect on baseline muscle oxygenation and peak muscle deoxygenation[23]. Conversely, studies on Montmorency cherry supplementation have shown higher baseline muscle oxygenation, but no effect during a moderate- or severe-intensity exercise bout[24,25]. Similarly, polyphenol supplementation for 7 d was not sufficient to affect any muscle oxygenation metric during a 4-km cycling TT[26]. When looking at the maximal muscle deoxygenation during exercise, a study on the polyphenols mangiferin and luteolin found that they significantly lowered muscle O_2 levels during a 30-s Wingate test after consumption for 48 h and 15 d, which they attributed to an improved ability of the muscle to extract oxygen[27], conversely to what we observed. No studies have evaluated the muscle deoxygenation and reoxygenation rates after cranberry supplementation.

Several studies have investigated the effects of polyphenols from sources other than cranberries on exercise performance. A recent systematic review of randomized controlled trials by Sommerville et al. examined the effect of polyphenol supplementation (minimum of 7 d) on exercise performance in healthy individuals[28]. They measured performance as the total power output calculated from either time trial or time to exhaustion testing and concluded that supplementation with polyphenols improved exercise performance moderately but warranted further investigation to determine the optimal dose[29]. Half of the studies (n = 7) included in the meta-analysis used quercetin, which demonstrated greater effects on performance, notably peak power production[29]. Interestingly, cranberries contain 20–30 mg of quercetin per 100 g fresh weight[30], which may have played a role in our results. The duration, treatment, and supplement dosage also differed from those used in the aforementioned meta-analyses. The mean daily polyphenol content was 688 ± 478 mg and the supplementation period varied from 7 to 56 d. For our study, 0.3 g/kg of body weight/day was administered for 28 d, which represents a mean of 1,664 mg of PACs daily, based on our participants' average body weight, which was 62.0 kg. The notable discrepancy in dosages and polyphenol types across the studies provided a trivial means of comparison.

We also observed a moderate correlation between lactate production and running time for the 400-m TT as well as between peak muscle deoxygenation and running time for the 1500m TT. These results are in agreement with previous findings[8,9] and attest to the quality of the data collected. Furthermore, these correlations indicate better oxygen extraction by the muscle, resulting in less lactate production and greater deoxygenation. With further stratification of our data based on participant sex, training status, main event, and berry consumption, we observed no statistically significant differences or trends.

Some of the limitations of this study were that a placebo powder was not developed, and the plasma polyphenol levels were not measured. The cranberry extract powder used

had a unique formula enriched in polyphenols, making it hydrophobic, chalky in texture, and tart in taste. Therefore, it was not possible to formulate a placebo powder with the same appearance and taste. If we had been able to measure the plasma polyphenol levels in our participants, we could have gathered some insight into the bioavailability of cranberry polyphenols as well as a direct measure of the compliance of each participant. Future studies should seek to develop a viable placebo for this cranberry supplement and perform blood tests to examine its bioavailability.

In summary, these results show that CE supplementation for 28 d, but not enough to lower the time to completion, improved physiological markers of performance that may help postpone the onset of muscle fatigue during both a 400-m TT and a 1500-m TT.

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Transition to Chapter 5

Our first human study identified marked ergogenic effects of cranberry supplementation, specifically related to muscle oxygenation metrics, and buffering of the exercise-induced lactate response. The effects on muscle oxygenation warranted further investigation to determine if the improved reoxygenation rates we observed post-exercise were related to improved mitochondrial capacity.

Chapter 5 aimed to investigate the effect of cranberry supplementation on muscle mitochondrial capacity using a non-invasive technique. The manuscript was accepted in *Applied Physiology, Nutrition, and Metabolism* on April 9th, 2024.

Chapter 5: Manuscript IV

A polyphenol-rich cranberry supplement improves mitochondrial capacity in healthy adults

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A polyphenol-rich cranberry supplement improves mitochondrial capacity in healthy adults

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5.1. Abstract

Cranberries are rich in polyphenols, have a high antioxidant capacity, and may protect against exercise-induced free radical production. Mitochondria are known producers of free radical in skeletal muscle, and preventing overproduction of radicals may be a viable approach to improve muscle health. This stud aimed to investigate the effect of a polyphenol-rich cranberry extract on muscle oxidative capacity and oxygenation metrics in healthy active adults. 17 participants (9 males, 8 females) were tested at: i) baseline, ii) 2 hours following an acute CE dose (0.7 g/kg of body mass), and iii) after 4 weeks of daily supplement consumption (0.3 g/kg of body mass). At each time point, muscle oxidative capacity was determined using near-infrared spectroscopy to measure the recovery kinetics of muscle oxygen consumption following a 15-20 s contraction of the vastus lateralis. Cranberry supplementation over 28 days significantly improved muscle oxidative capacity (k-constant, 2.8 ± 1.8 vs. 3.9 ± 2.2; $p = 0.02$). This was supported by a greater rate of oxygen depletion during a sustained cuff occlusion (-0.04 ± 0.02 vs. -0.07 ± 0.03; $p = 0.02$). Resting muscle oxygen consumption was not affected by cranberry consumption. Our results suggest that cranberry supplementation may play a role in improving mitochondrial health, which could lead to better muscle oxidative capacity in healthy active adult populations. The study protocol was registered with ClinicalTrials.gov (#NCT06186297)

Keywords: Polyphenol, proanthocyanidins, NIRS, rapid cuff inflation, muscle oxygenation, oxygen consumption, mitochondria.

5.2. Introduction

Polyphenols, which include proanthocyanidin (PAC), anthocyanins, flavanols, and flavonols, are antioxidant compounds found in fruits and vegetables shown to have beneficial effects on metabolic health and aging (Mathison et al, 2019; Singla et al, 2019; Scalbert et al, 2005). Cranberries have the highest polyphenol content among the commonly consumed fruits in the American diet as well as one of the highest antioxidant capacities among fruits and vegetables (Flammer 2013; Bean 2010). Furthermore, their polyphenol content stands out due to the high concentration of rare A-type PAC, a main contributor to the beneficial effects (Jiao 2017). Cranberries also contain other potent polyphenols, namely quercetin and resveratrol (Padmanabhan et al, 2016). Recent studies have reported an effect of polyphenols on mitochondrial capacity, with most looking at the effects of resveratrol in combination with exercise training (Polley et al, 2016; Menzies et al, 2013). The mitochondria play a critical role in oxidative metabolism, and mitochondrial capacity is associated with muscle function in healthy and diseased populations (Coen et al, 2013; Larsen et al, 2012; Grevendonk et al, 2021).

The gold standard for measurement of mitochondrial capacity is taking muscle biopsies, then subjecting the permeabilized tissues to high-resolution respirometry (Gnaiger, 2009). Biopsies are invasive and must be carried out in a hospital setting by a medical doctor, making recruitment of participants and feasibility difficult. Alternatively, phosphorus magnetic resonance spectroscopy (^{31}P-MRS) can be used to measure changes in phosphocreatine during exercise and recovery, but this methodology is limited due to the high associated costs (Chance et al, 2006; Layec et al, 2011; McCully et al, 1989). Recently, near-infrared spectroscopy (NIRS) has emerged as a viable, non-invasive alternative to indirectly measure mitochondrial capacity by assessing the recovery kinetics of muscle oxygen consumption (mVO_2) (Nagasawa et al, 2003; Motobe et al, 2004; Ryan

et al, 2012). NIRS computes changes in muscle oxygenation by utilizing the oxygen-dependent absorption of near-infrared light through haemoglobin and myoglobin (Jobsis, 1977). This technique has been validated against both ^{31}P-MRS (Ryan et al, 2013) and muscle biopsies (Ryan et al, 2014).

To our knowledge, the effect of cranberry polyphenols on muscle oxidative capacity has never been investigated. Consequently, the aim of this study was to determine the effects of a 28-day supplementation with polyphenol-rich cranberry extract on muscle oxidative capacity in active healthy adults. We hypothesized that cranberry supplementation would enhance vastus lateralis recovery kinetics of oxygen consumption following voluntary isometric contractions.

5.3. Methods

Participants

21 participants (10 males, 11 females) volunteered for this study. All subjects were recruited from the Concordia University community through pamphlets. To be included in the study, participants had to be 18-40 years old and exercising three or more days per week. Exclusion criteria were (i) smoking or vaping, (ii) ergogenic aid or drug use (iii) injuries or physical limitations that could impair exercising, and (iv) cardiovascular or metabolic disease. The study protocol (#30017882) was approved by the University Human Research Ethics Committee of Concordia University and registered with ClinicalTrials.gov (#NCT06186297). All participants provided informed consent prior to the start of the study.

Study Design

The study utilized a repeated-measures design with case–control matching, consisting of 3 separate visits. Prior to the first visit, participants filled out an online health questionnaire. On the first visit, resting heart rate, blood pressure, body weight and height were measured. Then, skinfold

measurements were taken at the site of right vastus lateralis (VL). Finally, baseline muscle oxidative capacity protocol was performed. The second visit was scheduled one week later, and participants were asked to consume a single dose of the cranberry extract (CE) two hours prior to testing (Control). The goal here was to add a negative control to identify if potential observed changes were random effects or true changes since a single dose taken a couple of hours prior to testing should not affect muscle oxidative capacity (Parenteau et al, 2023). The third visit occurred after 28 days of daily CE consumption and tested the chronic effects (28-CE).

Dietary Intervention

Participants were instructed to maintain their usual diet throughout the duration of the study. On testing days, they were asked to be fasted for 4 hours prior to testing and to avoid caffeine. Information on berry consumption is reported in **Table 1**. CE consisted of a lab-grade freeze dried powder containing 7.2-10.0 % PAC (Fruit D'Or Inc, Villeroy, Qc). Participants were instructed to keep the supplement in a cool, dry place and away from sunlight, as per the manufacturer's recommendation. In preparation for their second visit, participants consumed an acute dose representing 0.7g/kg of body mass two hours prior to testing. For chronic testing, a dose corresponding to 0.3g/kg of body mass was consumed daily for 28 days.

NIRS Devices

NIRS monitors (Moxy monitors, 5th generation; Fortiori Design, Minnesota, USA) were used to assess changes in muscle oxygenation by utilizing four wavelengths of near-infrared light (680, 720, 760, and 800 nm), with source detector spacing of 12.5 and 25.0 mm (Paquette et al, 2018). Since oxygenated and deoxygenated hemoglobin have different absorbance spectra, they reflect light differently. Consequently, the Moxy device can report changes in both total tissue hemoglobin

concentration ([THb]) and oxygenated hemoglobin expressed as a percentage of the total hemoglobin (SmO_2).

A skinfold measurement was taken at the location of the right VL, 10 cm above the patella, using skinfold calipers (Harpenden, UK). For viable data, skinfold measurements should be less than half the distance between the emitter and the detector, 12.5 mm in this case. The NIRS device was placed on the right VL, attached and secured with medical tape and Hypafix. Black self-adhesive tape was wrapped around the leg to ensure no movement of the device and to block extraneous light. The raw SmO_2 and [THb] signals were collected at a frequency of 2 Hz. The deoxyhemoglobin concentration ([HHb]) and oxygenated hemoglobin concentration (O_2Hb) were computed from SmO_2 and [THb] using the following equations:

(i) $SmO_2 = 100 \times [O_2Hb]/[THb]$, and

(ii) $[THb] = [O_2Hb] + [HHb]$. (Alvares et al, 2020)

Testing Protocol

Participants were seated on a chair with both legs elevated and extended horizontally. The three NIRS devices were placed on the right and left vastus lateralis and on the right medial gastrocnemius muscle. A blood pressure cuff (Hokanson E20 rapid cuff inflator, Bellevue, WA) was placed on the right thigh, proximal to the NIRS device.

A baseline measurement of muscle oxygenation was obtained for two minutes. Then, the blood pressure cuff was inflated to 250 mmHg for 30 s to measure resting mV_{O2}. The muscle was given 30-60 s of recovery, and this process was repeated a second time. Subsequently, the participant was asked to perform an isometric contraction of the right VL for 15 s, after which a series of 6 brief (5 s) arterial occlusions were applied to measure the rate of recovery of mV_{O2} back to resting

levels. This protocol was performed twice, with a 60 s break in between. Finally, to normalize the NIRS signal, a 5 s isometric contraction of the VL was performed, followed by a 3- to 6-min arterial occlusion applied to fully deoxygenate the tissue. This peak hyperemic response upon release was used to indicate 100% oxygenation. The test protocol lasted about 15 minutes. A representation of the data gathered during the testing protocol is shown in **Figure 2**.

Calculation of muscle oxygen consumption and correction for blood volume

The mV_{O2} was calculated as the slope of change in O_2Hb and HHb during the arterial occlusion using simple linear regression. O_2Hb and HHb were calculated as a percentage of the ischemic calibration and a correction for blood volume (β) was performed (Ryan et al, 2012). During arterial occlusion, it is assumed that changes in O_2Hb and HHb occur with a 1:1 ratio that represents mitochondrial oxygen consumption only (i.e. without any arterial oxygen delivery or venous return of deoxygenated blood), thus creating a closed system under the NIRS probe. Despite this assumption, a flux in blood volume occurs from the redistribution of heme between high-pressure arteries and low-pressure veins, resulting in the need to remove this blood volume change from the NIRS signal to isolate mVO_2. Blood volume corrections were performed based on the following equations:

(iii) $\Delta NIRS_{signal} = mVO_2 + \Delta$blood volume

(iv) $$\beta(t) = \frac{|O_2\text{Hb(t)}|}{(|O_2\text{Hb(t)}| + |HHb(t)|)}$$

Where $\Delta NIRS_{signal}$ represents the adjusted NIRS signal, and Δblood volume represents β variations at each time point. Once β was determined, it was applied to the raw data according to the following equations:

(v) $O_2Hb_c = O_2Hb - [tHb * (1 - \beta)]$

(vi) $HHb_c = HHb - (tHb * \beta)$

Where O_2Hb_c and HHb_c are the corrected oxygenated and deoxygenated hemoglobin signals, respectively.

Calculation of muscle oxidative capacity

The original method to measure muscle oxidative capacity required a complete recovery of oxygen to baseline levels, which needed as many as fifty 5-10 s ischemic occlusions to be attained - this is time consuming and uncomfortable for the participant. A shorter version of the original protocol was developed using partial recovery curves to extrapolate the mitochondrial rate constant using only six 5 s ischemic occlusion (McCully et al, 2020). The Mito6 protocol was used to calculate muscle oxidative capacity in the right VL by using the six ischemic cuff slopes and matching them to the following exponential equation:

(vii) $Y = End - Delta * e^{-1/k}$

Where Y represents relative mV_{O2} during the arterial occlusion, End is the mV_{O2} immediately after the cessation of exercise, Delta is the change in mV_{O2} from rest to end of exercise, and k is the mitochondrial rate constant (in min^{-1}). The Mito6 protocol was validated against the Mito22 protocol (22 ischemic occlusions) and showed repeatability, making it an effective tool to measure muscle oxidative capacity (McCully et al, 2020; Sumner et al, 2023). An Excel spreadsheet developed by Pelka et al. was used to compute the k-constant, and we followed their protocol to calculate the recovery slopes and fit them to the exponential model, using a minimum of four slopes with a threshold r^2 of 0.9 (Pelka et al, 2023).

Statistical analysis

Normality of the data was determined by Shapiro-Wilk test. A one-way ANOVA was used to compare baseline values with the control and chronic conditions. Data are presented as means ± standard deviation and SPSS version 29 was used for analysis. Figures were made using R studio version 2023.06.1. Prior to analysis, significance level was set to ≤ 0.05.

5.4. Results

17 of the 21 participants who signed up for the study were included in the analysis. One participant was excluded from the study for non-respect of pre-test conditions; 3 were excluded due to large adipose tissue thickness at the right VL. Subject population from recruitment to data analysis is presented in **Figure 1**. Compliance was determined via a mid-study check-in and a post screening questionnaire. 16 out of 17 (94%) participants consumed every dose. The other participant reported missing two doses over the 28 days. Although we did not track overall food intake throughout the study, we asked participants to report how much polyphenol-rich foods (i.e., berries and other fruits) they consumed daily, and 14 out of 17 participants reported low-to-moderate consumption of polyphenol-rich foods. **Table 1** summarizes participant characteristics.

There were no significant differences for any parameters between the baseline and the negative control condition. The mean mitochondrial rate constants (k) of the right VL significantly improved by a mean of 1.1 min^{-1} after 28 days of cranberry supplementation (2.8 ± 1.8 min^{-1} vs. 3.9 ± 2.2 min^{-1}; $p = 0.04$), which suggests more active mitochondria (see **Figure 3**). This was supported by a greater rate of oxygen depletion during the ischemic calibration (-0.04 ± 0.02 %/s vs. -0.07 ± 0.03 %/s; $p = 0.002$) (see **Figure 4**). Resting muscle oxygen consumption was similar between the groups (-0.05 ± 0.02 %/s vs. -0.05 ± 0.01 %/s; $p > 0.05$).

The left VL had no significant differences from baseline in both the slope of oxygen depletion during the isometric contraction (-0.2 ± 0.2 %/s vs. -0.3 ± 0.2 %/s; $p > 0.05$) and the recovery slope following the exercise bout (0.2 ± 0.1 %/s vs. 0.2 ± 0.1 %/s; $p > 0.05$). Further, the change in THb was the same between baseline and 28-CE (7.9 ± 4.4 vs. 8.3 ± 4.8; $p > 0.05$). No effect was observed for the resting muscle oxygen consumption of the distal right gastrocnemius between baseline and 28-CE (-0.03 ± 0.02 %/s vs. -0.04 ± 0.02 %/s; $p > 0.05$). No difference was observed in the ischemic slope (-0.06 ± 0.03 vs. -0.05 ± 0.02; $p > 0.05$).

5.5. Discussion

The main finding of this study reveals that supplementing with a polyphenol rich cranberry extract for 28 days improves muscle oxidative capacity. Furthermore, it seems that blood flow and, therefore, oxygen delivery to the muscle, are not affected by the supplement. A proposed mechanism for the observed effects of polyphenols on mitochondrial capacity is through 5'-adenosine monophosphate activated protein kinase (AMPK) signaling. AMPK regulates mitochondrial gene expression through the activation of SIRT1 and, subsequently, PGC-1A. Several studies, both in-vitro and in-vivo, have shown that dietary polyphenols can modulate AMPK activation and, subsequently, activate the downstream SIRT1-PGC-1A pathway to improve mitochondrial function [33-39]. Another possibility could be that CE affects mitochondrial dynamics directly. A study looking at the effects of pomegranate peel high in polyphenols on brown adipose tissue observed higher mitochondrial complex IV activity, which is considered a marker of improved oxidative phosphorylation system activity (Echevarria et al, 2021). In addition, a study by Polley and colleagues demonstrated that supplementation with the polyphenol resveratrol directly improves mitochondrial capacity, but only when combined with exercise training (Polley et al, 2015). Interestingly, they observed no change in mitochondrial capacity for their group that

underwent exercise training only. These results both align with ours and justify our decision to solely include participants who exercised at least three times per week.

On the second visit, we chose to have participants consume the cranberry extract two hours prior to testing. This was based on prior pharmacokinetic investigations, which found that ingestion of a cranberry juice containing 835 mg of polyphenols yielded plasma concentrations ranging from 0.56 to 4.64 nmol/L after only four hours, indicating rapid removal from the body (Milbury et al, 2010). This agrees with the Holt et al. study that showed peak bioavailability of cocoa PACs at the 2-hour mark post-ingestion (Holt et al, 2002). We used this second visit as a negative control to confirm reliability of our data. No differences in muscle oxidative capacity or other parameters were observed when compared to baseline, suggesting the MOXY monitors produced accurate data.

This is the first study to use the MOXY monitor to measure muscle oxidative capacity. Other groups have used the Oxymon NIRS device. Although the MOXY data has not been validated directly against the Oxymon data, it has been validated against its sister device, the PortaMon. Both devices were shown to produce physiologically credible total saturation indexes at rest and during exercise when a correction with ischemic calibration was performed (McManus et al, 2018), as was completed for the current study. MOXY monitors have also been investigated for relative and absolute test-retest reliability under conditions of rest, vascular occlusion, and exercise (McManus et al, 2018; Feldmann et al, 2019; Crum et al, 2017).

We excluded 3 participants due to large skinfold measurements. Based on the manufacturer's recommendation, the adipose tissue thickness should be no greater than half the distance between the emitter and the detector - i.e., less than 12.5 mm. When manual skinfold measurements were compared with gold standard ultrasound measurements, skinfold values for the mid-thigh were

observed to be an average of 2.4 times larger than ultrasound (23.4 ± 4.7 mm vs. 9.9 ± 2.2 mm, respectively) (Lewandoski et al, 2022). Based on those results, the inclusion limit for skinfold measurements was set to < 20 mm for the VL, which we considered a conservative value to ensure we did not have participants with true adipose tissue thickness above 12.5 mm.

Limitations of this study were the absence of a placebo supplement, the inability for blinding, no measure of plasma levels of polyphenols, and lack of information on participant exercise regime. The CE powder we used was a unique formula enriched in polyphenols, which made it slightly hydrophobic, chalky in texture, and tart in taste. Consequently, it was not possible to formulate a placebo that would look and taste the same. Instead, we employed the negative control. Still, participants were not randomized, which should be noted as a limitation. If we had been able to measure plasma polyphenol levels, we could have gathered insight into the bioavailability of cranberry polyphenols specifically, as well as a measure of compliance. Finally, it would have been interesting to gather more information about each participant's training regimen to see if a specific type of physical activity is superior to improve muscle oxidative capacity. Future studies should seek to develop a viable placebo for this cranberry supplement and control for training regimen to investigate which has the greatest potential for improving muscle oxidative capacity.

In summary, our results show that 28 days of CE supplementation had a direct impact on increasing muscle oxidative capacity, which could lead to improved muscle function in healthy active adult populations. Our findings also strengthen the body of literature on the validity of NIRS as a reliable non-invasive tool for measuring muscle oxidative capacity.

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Competing interests

The authors declare there are no competing interests.

Author Contribution Statement

FP: Conceptualization, Data Curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing – review & editing

AD: Data Curation, Formal analysis, Investigation, Writing – review & editing

MR: Investigation, Methodology, Resources, Visualization, Writing – review & editing

ASC: Investigation, Methodology, Resources, Visualization, Writing – review & editing

AB: Conceptualization, Investigation, Formal analysis, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing

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Data availability statement

Data generated or analyzed during this study are available from the corresponding author upon request.

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5.7. Table

Variable	*Mean (SD)*
Age (years)	23 (4)
Height (cm)	172 (10)
Weight (kg)	70 (19)
BMI (kg/m^2)	24 (5)
Resting HR (bpm)	65 (13)
SBP (mmHg)	112 (9)
DBP (mmHg)	63 (7)
Skinfold - right VL (mm)	13.1 (4.5)
Berry Consumption	
Low	9
Moderate	5
High	3

Note: all measures were taken at the beginning of the study

Table 1: Participant characteristics (n=17). Berry consumption levels are expressed as low (< once weekly), moderate (1-3 times per week) or high (> 3 times per week).

5.8. Figures

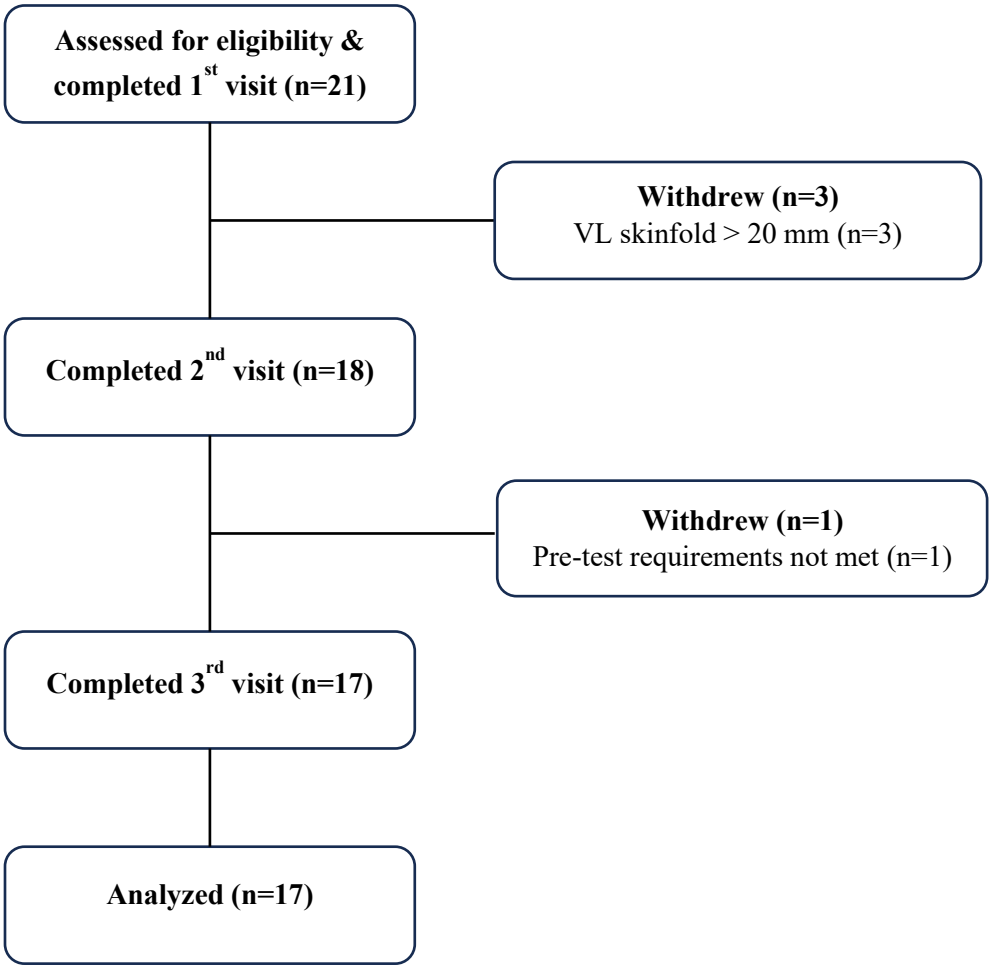

Figure 1: Consort diagram breakdown of the subject population from recruitment to data analysis

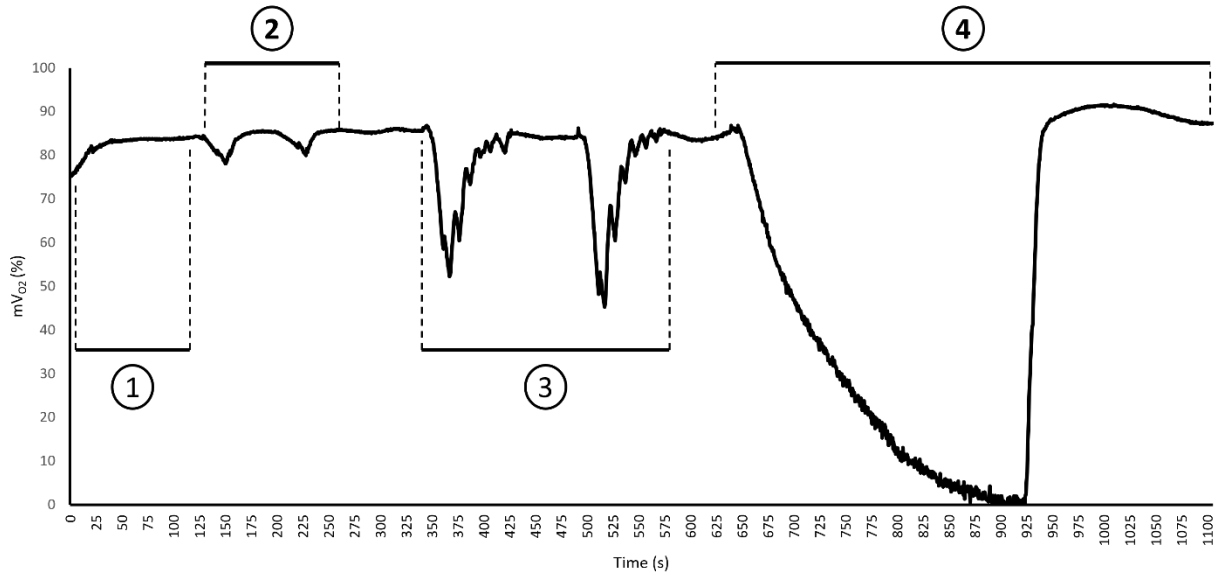

Figure 2: Experimental protocol for the right vastus lateralis. (1) Baseline muscle oxygenation was first established for 120 seconds. (2) Resting muscle oxygen consumption was then measured twice using a 30-second cuff occlusion. (3) The Mito-6 protocol was performed twice. (4) An ischemic calibration was done at the end of the protocol.

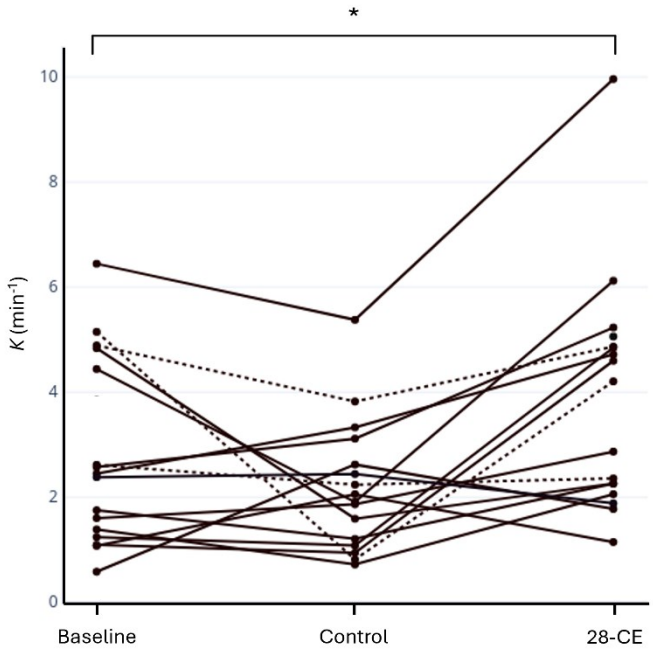

*Figure 3: Mitochondrial rate constant (k) for the recovery of muscle oxidative capacity for the baseline, negative control, and 28-day supplementation timepoints. AU: arbitrary unit. * represents $p < 0.05$.*

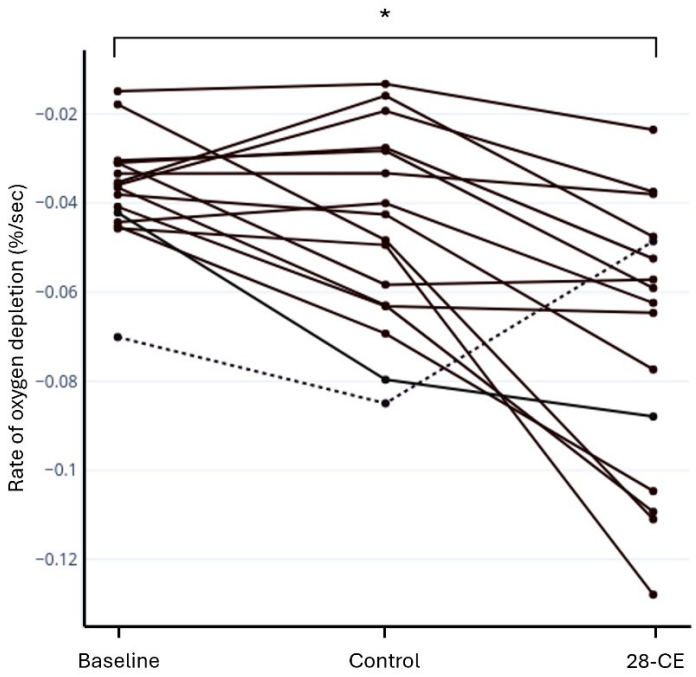

*Figure 4: Rate of oxygen depletion for the recovery of muscle oxidative capacity for the baseline, negative control, and 28-day supplementation timepoints. * $p < 0.05$*

Chapter 6: General Discussion

6.1 Synthesis of results

The aim of this thesis was to investigate the potential ergogenic effects of cranberry supplementation high in PAC-1, specifically on aerobic exercise performance and skeletal muscle bioenergetics. Chapter 2 used a rodent model as a proof of concept to determine if a purified PAC-1 extract could modulate maximal running capacity and influence mitochondrial capacity. The mice were fed a HFD to mimic the common north American dietary habits. The results show that PAC-1 supplementation for 28 days prevents the declines in running capacity caused by HFD, which correlates with improved mitochondrial capacity of the vastus lateralis, the main muscle involved in running. Considering this compelling rodent data, Chapter 3 aimed to see if the observed benefits in mice would translate to a human model. A systematic review with meta-analysis exploring the effects of polyphenol-rich berries on aerobic exercise performance and biomarkers of performance was conducted. The results expose the lack of available quality controls, confirm the incertitude regarding the ergogenic potential of polyphenol supplementation, and justify moving forward with additional human studies. Since improvements in aerobic capacity through enhanced mitochondrial function was the main take-home message from the rodent study, Chapter 4 aimed to elucidate if similar effects would occur in trained endurance runners. Supplementation with freeze-dried cranberry powder for a month led to improvements in vastus lateralis oxygenation metrics following a 1500-m TT and buffering of the exercise-induced lactate response following a 400-m TT. Although the statistical analysis showed that times were not significantly faster, a large effect size ($\eta^2 = 0.15$) in favor of cranberry supplementation was observed for the aerobic-focused 1500-m TT. Nevertheless, it was clear that there were effects on muscle oxygenation metrics and lactate production, which strengthened our hypothesis that the

benefits of cranberry supplementation are carried out through improvements in mitochondrial function. Therefore, Chapter 5's objective was to measure mitochondrial capacity of the vastus lateralis before and after cranberry supplementation with NIRS and rapid cuff inflation to calculate the mitochondrial k constant using partial recovery curves. The results showed that 28 days of cranberry supplementation significantly increases the mitochondrial k constant, which suggests improvements in overall mitochondrial capacity.

6.2 Limitations

In addition to the specific limitations addressed within each manuscript, this thesis had some general limitations. First, the cranberry supplement used in Chapter 2 was different in composition and dosage to the one used in Chapters 4 and 5. For the rodent study, a purified PAC-1 extract was obtained and a dose of roughly 200 mg/kg body weight/day was given. For a 60 kg human, that dose represents 12 g of PAC-1 per day. The freeze-dried cranberry powder that we used in Chapters 4 and 5 contained 7-10% PACs, meaning that a 60 kg participant would have needed to consume between 120 g and 170 g of the supplement. Such a dose is unrealistic due to the almost guaranteed aversion that participants would develop and does not compare to the smaller amounts used in previous studies (688 ± 468 mg/day). Thus, we chose to give 0.3 g/day of the powder, which represents 1.3-1.8 g of PACs per day. Furthermore, the freeze-dried cranberry powder tasted very tart, and it was hard to mix in water due to the fact PACs are hydrophobic, making it impossible to make a viable placebo. Consequently, we were somewhat limited when drawing conclusions as causality cannot be assumed for the two human studies.

The results of chapter 4 and 5 may not be generalizable to the overall healthy population. In Chapter 4, the participants were competitive/elite endurance runners. Athletes normally have better dietary habits and are metabolically healthier. Although this was a positive for our study,

individuals with a more controlled intake of food are not representative of the general population. In Chapter 5, the participants were healthy and active participants. Most of the participants were recruited within the Health, Kinesiology, and Applied Physiology department. Therefore, it is possible that they were not representative of the general healthy population because of their specific knowledge on topics such as exercise physiology and nutrition.

6.3 Recommendations for future research

This thesis expanded on the knowledge around the ergogenic effects of polyphenol-rich cranberry supplementation. Our goal was to gain an understanding of the physiological mechanisms involved and how they influence performance. We showed that cranberry supplementation positively affects mitochondrial capacity, both in rodents and in humans. Still, many questions remain unanswered, and additional studies in the following areas should be performed:

1. Explore the effects of cranberry supplementation on aerobic capacity and mitochondrial function in different populations. For example, elderly populations could be of interest, since there is a known decline in muscle mass and function with aging. Similarly, certain diseases, such as Alzheimer's disease, Parkinson's disease, cardiomyopathy, chronic fatigue syndrome, fibromyalgia, and diabetes have underlying pathophysiological mechanisms related to mitochondrial dysfunction via ROS production and mitochondrial DNA damage (Pieczenik et al, 2007).
2. Study the ability of PACs to turn on regulators of mitochondrial biogenesis, such as peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) and sirtuin 1 (SIRT1), and how this can affect mitochondrial DNA.
3. Study the effects of cranberry supplementation on other markers of exercise performance, such as VO_{2max}, cardiac output, and blood flow to the working muscles.

4. Develop a placebo for the freeze-dried cranberry supplement, perhaps in capsule form to avoid the tart taste and hydrophobic nature of PACs. A dye should be used to match the bright red color of the powder. A large capsule can hold roughly 1-1.5 g of powder, which would mean taking 12-18 capsules per day for a 60 kg individual if matching the dosage used in this thesis.
5. Perform a dose-response study to determine the minimal effective dose. Additionally, explore the consequence of timing of intake on markers of performance. Since polyphenols act as prebiotic agents, their effects may vary if consumed with or without food.

6.4 Conclusions

Polyphenols are plant secondary compounds associated with an array of health benefits, mainly for their antioxidant effects. This dissertation shows how cranberry polyphenols promote health via enhanced skeletal muscle mitochondrial function, leading to gains in exercise performance. Cranberries are native to North America and widely produced in Quebec, and our work opens a potential new avenue for farmers and producers to explore, although it is still to soon to affirm that cranberries have clear ergogenic effects. We laid the groundwork for future studies to gather more evidence and determine what the physiological mechanisms involved are and who, other than active young adults, may benefit from cranberry supplementation.

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Note: These references are for chapters 1 and 6 and are in order of appearance. The references for chapters 2-5 can be found at the end of each chapter to keep the integrity of the published/submitted versions.

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Appendix

Appendix for Chapter 3: Effects of polyphenol-rich, berry supplementation on exercise performance: a systematic review and meta-analysis

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Lines 2-3
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Lines 24-43
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Lines 47-74
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Lines 74-76
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Lines 96-100
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Lines 81-83
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Lines 83-92
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Lines 94-108
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Lines 106-108
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Lines 110-116
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Lines 110-116
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Lines 132-135
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Lines 118-125
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Lines 118-125
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data	Lines 125-

Section and Topic	Item #	Checklist item	Location where item is reported
		conversions.	127
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Line 118
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Lines 122-125
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Lines 128-129
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Lines 129-131
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Lines 132-135
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Lines 122-125
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Lines 138-143
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	N/A
Study characteristics	17	Cite each included study and present its characteristics.	Lines 145-154
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Lines 156-160
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Lines 163-195
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Lines 163-195
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Lines 163-195
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Lines 163-195
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Lines 163-195
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	N/A
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Lines 170-208
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Lines 197-246
	23b	Discuss any limitations of the evidence included in the review.	Lines 247-

Section and Topic	Item #	Checklist item	Location where item is reported
			255
	23c	Discuss any limitations of the review processes used.	Lines 247-255
	23d	Discuss implications of the results for practice, policy, and future research.	Lines 257-262
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Lines 79-80
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Lines 79-80
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Lines 275-276
Competing interests	26	Declare any competing interests of review authors.	Line 278
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	N/A

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

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