Cranberry-derived polyphenol supplementation to mitigate the effects of a high-fat diet on skeletal muscle contractility in rats

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Abstract

Background: Polyphenol intake is gaining focus for its vast beneficial effects on organs throughout the body. Proanthocyanidin (PAC) is a cranberry-derived polyphenol shown to contribute to improved metabolic functions. Our group has previously shown that a short-term high-fat (HF) diets influences skeletal muscle quality as indicated by altered contractility. Other polyphenols such as Urolithins and Resveratrol were previously shown to improve skeletal muscle functions. The effects of dietary PAC on skeletal muscle function have yet to be explored.

Objective: The goal of this research project is to determine whether dietary PAC supplementation can prevent the impairments of a short-term HF diet on skeletal muscle contractility in young male rats.

Methods: Thirty young male Wistar rats (100-125g; *n*=10/group) were randomly submitted to a regular chow (RC) diet, a HF diet, or a HF + PAC-supplemented diet (PAC; 0.233mg/day) for 14 days, and with weight and food intake measured every two days. After two weeks, specific force, fatigue resistance and recovery from fatigue were measured in the *extensor digitorum longus* (EDL, glycolytic) and the soleus (SOL, oxidative) muscles using an *ex vivo* muscle contractility system. Visceral and subcutaneous (SC) fat stores were collected and weighed before the rat was terminated.

Results: Body weight was similar for all diet groups at the beginning of the experimental period, and statistically higher after 14 days in the HF group compared to the RC group ($P = 0.005$), but not compared to the PAC group. The RC and PAC groups were not significantly different in terms of body weight at the end of the 14 days. Animals treated with HF and PAC diets

displayed significantly higher ($P < 0.001$) caloric intake per day than those treated with RCD. The subcutaneous (SC) fat pad, and the sum of the visceral fat deposits were both significantly heavier (SC $P = 0.002$ and 0.02 respectively; Visceral $P < 0.001$) among the HF and PAC groups compared to the RC group, but not statistically different between the HF and PAC groups. No difference was noted for specific force and muscle fatigue. Significant differences ($P < 0.001$) were detected between the HF and PAC groups for recovery from fatigue in the EDL muscle only.

Conclusion: PAC supplementation influenced force recovery in the EDL muscle, while not affecting specific force or fatigue resistance. PAC supplementation also did not benefit weight gain, or body composition in comparison to the HF group.

Résumé

Contexte: Les polyphénols sont largement étudiés pour leurs vastes effets bénéfiques sur les systèmes de l'ensemble de l'organisme. La proanthocyanidin (PAC) est un polyphénol dérivé de la canneberge pouvant contribuer à l'amélioration des fonctions métaboliques. Notre groupe a déjà démontré qu'un régime alimentaire à court terme riche en lipides (HF) influence la qualité des muscles squelettiques, ce qui se traduit par une altération de la contractilité musculaire. D'autres polyphénols tels que les Urolithines et le Resveratrol ont déjà démontré qu'ils amélioraient les fonctions des muscles squelettiques. Les effets de PAC sur les fonctions des muscles squelettiques doivent encore être étudiés.

Objectif: Le but de ce projet de recherche est de déterminer si une supplémentation alimentaire en PAC peut protéger contre les effets d'un régime HF à court terme sur la contractilité des muscles squelettiques chez les jeunes rats mâles.

Méthodes: Trente jeunes rats Wistar mâles (100-125 g; *n*=10/groupe) ont été soumis aléatoirement à un régime de moulée (RC), à un régime HF, ou à un régime HF + PAC (PAC; 0.233mg/jour) durant 14 jours, avec le poids et la consommation alimentaire mesurés tous les deux jours. Après deux semaines, la force spécifique, la résistance à la fatigue et la récupération de la fatigue ont été mesurées dans les muscles *extensor digitorum longus* (EDL, glycolytique) et soléaire (SOL, oxydatif) avec un système de contractilité musculaire *ex vivo*. Les réserves de lipides viscérales et sous-cutanées (SC) ont été prélevées et pesées avant le sacrifice de chaque rat.

Résultats: Le poids corporel était similaire pour tous les groupes au début de la période expérimentale, mais statistiquement plus élevé après 14 jours dans le groupe HF par rapport au

groupe RC ($P = 0,005$), mais pas par rapport au groupe PAC. Les groupes RC et PAC n'étaient pas significativement différents en termes de poids corporel à la fin des 14 jours. Les animaux traités avec les régimes HF et PAC présentaient un apport calorique quotidien significativement plus élevé ($P < 0,001$) que ceux traités avec le RC. Des différences significatives ($P < 0,001$) ont été observées entre les groupes HF et PAC pour la récupération de la fatigue dans le muscle EDL uniquement. Aucune différence n'a été observée entre les groupes en ce qui concerne la force spécifique ou la résistance à la fatigue dans l'un ou l'autre des muscles. Le dépôt adipeux sous-cutané (SC), et la somme des dépôts lipidiques viscéraux était significativement plus lourd (SC $P = 0,002$ et 0,02 respectivement; viscéraux $P < 0,001$) dans les groupes HF et PAC que dans le groupe RC, mais pas statistiquement différente entre les groupes HF et PAC.

Conclusion: La supplémentation en PAC a influencé la récupération de la force dans le muscle EDL, tout en n'affectant pas la force spécifique ou la résistance à la fatigue. La supplémentation en PAC n'a pas non plus influencé la prise de poids ou la composition corporelle par rapport au groupe HF.

Contribution of Authors

Samantha Quinn, B.Sc: Conception, performance of studies, data acquisition, analysis and preparation of manuscript

David Andrich, PhD: Performance of studies, data acquisition, analysis

Roxane St-Amand, B.Sc: Performance of studies

Alain Steve Comptois, PhD: Conception, analysis

Gawiyou Danilou, PhD: Data acquisition technique instruction

Andreas Bergdahl, PhD: Conception, analysis

David St-Pierre, PhD: Conception, data acquisition, analysis, drafting and review of article

Celena Scheede-Bergdahl, PhD: Conception, analysis, drafting and reviewing of article

List of Abbreviations

AG	Arachidonylglycerol
AMPK	Amp-activated protein kinase
APP	Apple polyphenol
CIPA	Comité Institutionnel de Protection des Animaux
CK	Creatine kinase
COX2	Cyclooxygenase-2
CSA	Cross-sectional area
EDL	Extensor digitorum longus
GF	Germ-free
GI	Gastrointestinal
HF	High fat
IBD	Inflammatory bowel disease
L_O	Optimal length
LPS	lipopolysaccharide
MYHC	Myosin heavy chain
NFκB	Nuclear factor-κB
PAC	Proanthocyanidin
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator 1 α
PPARγ	Peroxisome proliferator-activated receptor gamma
RC	Regular chow
Res	Resveratrol

ROS	Reactive oxygen species
SOL	Soleus
T2D	Type 2 diabetes mellitus
TLR	Toll-like receptor
TNFα	Tumor necrosis factor α
UQAM	Université de Québec à Montréal
Uro	Urolithin
UroA	Urolithin A

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CHAPTER 1: Literature Review

The digestive tract is important for more than just digestion. It is a crucial bridge between the diet and the health status of various organs throughout the body, influencing metabolism in tissues within the viscera and the periphery. The gut environment can be significantly impacted by the types of foods consumed; diets that are high in fat have been shown to have harmful effects that can be detected at various organs throughout the body (Fang, Chen, Wang, & Liang, 2018; Forkosh & Ilan, 2019; Yan et al., 2016). Other dietary sources can have positive effects. Those that support a healthy gut environment are commonly referred to as nutraceuticals, and can include a variety of macro and micronutrients. One group of micronutrients commonly found in fruits and vegetables are polyphenols. Recently, there has been growing interest as to how polyphenols act upon digestion to promote a healthy gut environment (Anhê et al., 2015). This sheds light on how what we consume has effects on organs such as the gut, the pancreas, the liver and the heart (Adolph, Grander, Moschen, & Tilg, 2018; Fang et al., 2018; Forkosh & Ilan, 2019; Pinent, González-Abuín, Blay, & Ardévol, 2016). Less known are the effects of the gut status and polyphenol consumption on skeletal muscle. Preliminary evidence shows that polyphenols influence local anti-inflammatory and anti-oxidant activity in skeletal muscle, tissue repair, muscle soreness, insulin sensitivity, fiber typing, energy metabolism and overall exercise tolerance, and maintain important prebiotic activity within the gut (Larrosa et al., 2010; Rodriguez et al., 2017; Sung, Byrne, et al., 2017; Sung, Kim, et al., 2017; Trombold, Barnes, Critchley, & Coyle, 2010). Contractility is an important aspect of skeletal muscle quality and includes the functions force generation, fatigue resistance, and recovery. However, the effects of polyphenols on skeletal muscle contractility remain to be elucidated. The goal of this investigation is to explore the effects of cranberry-derived polyphenol on skeletal muscle contractility when administered as a supplementation to a short-term high-fat (HF) diet.

Nutrition and systemic health

Important advances in nutritional sciences and practical dietary recommendations have occurred over recent years. However, it is evident that much work remains to improve human diets as indicated by the ever-growing population of chronic disease aggravated by poor or malnutrition. Ideally, differences in gender, genetics (Bush et al., 2020; Nielsen & El-Sohemy, 2012), health status, gut microbial properties (Bindels & Delzenne, 2013), lifestyle and age (Erdos et al., 2011; Phillips, Chevalier, & Leidy, 2016) require different dietary approaches in order for the body to function optimally. The gut microbial properties are a particularly interesting target for nutritional science research, as the gut interacts directly with the available dietary components, influences whole-body metabolism, and has recently been linked to a number of widespread health conditions such as systemic inflammation (Fang et al., 2018), atherosclerosis, congestive heart failure (Forkosh & Ilan, 2019), inflammatory bowel disease (IBD), obesity, type 2 diabetes mellitus (T2D; Delzenne & Cani, 2011), hepatic diseases (Adolph et al., 2018), and cancer (Bindels & Delzenne, 2013).

A growing area of interest in the area of nutritional sciences and gut (patho)physiology is the importance of prebiotic nutrients. Prebiotics are a category of food that provide beneficial effects on the gut by improving the status of the bacterial communities within the gastrointestinal (GI) tract. Prebiotic nutrients include fermentable fibers (not reviewed within the scope of this work) and the micronutrient polyphenols. Polyphenols have been shown to maintain vast and widespread benefits within the body, including at the level of both the gut and organ function (Chiva-Blanch & Visioli, 2012).

What are polyphenols?

Polyphenols are a group of biologically active compounds found in what are commonly classified as "functional foods". This notion is grounded in the idea that biological activity extends beyond basic nutrition, which is represented by energetic nutrients. Polyphenols are divided into four classes: Flavonoids, Stilbenes, Lignans, and Phenolic Acids, and are often simply referred to by the names of the common dietary sources (Table 1). The Flavonoid and Phenolic Acid classes also include subclasses, for which the titles are at times referred to in research as direct indications of polyphenols (Table 1). Much like vitamins, polyphenols are a

Table 1: Polyphenol classes include Flavonoid, Stilbene, Lignan and Phenolic Acids. The Flavonoid and Phenolic Acids classes include subclasses. Polyphenols are commonly identified by the name of the common dietary form, as well as the name of the subclass

POLYPHENOL CLASSES								
Flavonoid			Stilbene	Lignan	Phenolic Acids			
Subclass		Common dietry forms	Common dietary forms	Common dietary forms	Subclass			Common dietary forms
Simple Subclasses	Flavan-3-ol	catechin, epicatechin, epigallocatechin	resveratrol	secoisolariciresinol	Free forms	Benzoic acids	hydroxybonzoic acid	hydrolyzable tannins (gallotannins, ellagitannins) gallic acid
	Flavone							
	Flavanone							
	Isoflavone							
	Flavanol	quercetin, kaempferol				Cinnamic acids	hydroxycinnamic acid	caffeic acid ferulic acid sinapic acid
	Anthocyanin							
Complex Subclasses	Condensed tannins (proanthocyanidins)	procyanidins	curcumin	matairesinol	Bound forms	quincic acid, shikimic acid, tartaric acid		
	Derived tannins	theaflavins				chlorogenic acid (caffeic+quincic)		

group of compounds that are consumed from food sources and pass through the digestive tract in order to enter the body to exert their unique effects, such as the modulation of pro-inflammatory gene expressions (Carmela Santangelo, Rosaria Varì & Roberta Di Benedetto, 2007), induction of muscle hypertrophy (Rodriguez et al., 2017), decreasing hepatic and intestinal oxidative stress and inflammation, and improving insulin sensitivity (Anhê et al., 2015), as well as radical scavenging activity, lending to important antioxidant effects (Gülçin, 2006). However, unlike

vitamins, the specific effects of each type of polyphenol, the mechanism of action through which each polyphenol exerts its effects, and the recommended daily intakes have yet to be fully elucidated. This paucity of knowledge is largely due to the novelty of polyphenols, their low bioavailability, rapid metabolism, as well as the complexity of the mechanistic pathways through which they exert their biological functions.

The digestive tract is an important aspect for the ensuing effects of polyphenols as food sources of polyphenols must be consumed and digested in order for them to enter the body. Interestingly, one of the targets of beneficial biological activity of certain polyphenols is the gut itself.

What is the Gut Microbiota?

The human GI tract refers to the esophagus, stomach, small and large intestine. The GI tract harbors a microbiome, which includes all microorganisms, bacteria, viruses, and fungi, present within the GI tract (Federico, Giorgio, & Miele, 2017). Specifically, the gut microbiota refers to the microbial flora that reside in the GI tract and exercises crucial functions both inside the intestines and beyond. The microbiota is both acquired and conditioned with a personal microbial profile at birth, however age, genetic background, treatments with antibiotics, physical activity and diet are factors that can modulate an individual's microfloral composition and, by extension, GI health (Bleau, Karelis, St-Pierre, & Lamontagne, 2015). Accordingly, the relationship that exists between the gut microbiota and the host is biological mutualism. Dysbiosis then, is a term that defines changes in the composition and/or activity of the gut microbiota in association with various pathological features (Delzenne & Cani, 2011). Dysbiosis

is a critical element among the mechanisms of a number of pathologies, especially metabolic disorders.

The regulation of polyphenol-driven benefits on the gut microbiome

An important aspect of GI tract health status is prebiotic and probiotic activity. Prebiotics serve as food supplements for the microorganisms in the gut, which contribute to improved microbial balance (Gibson & Roberfroid, 1995). As previously mentioned, prebiotics include non-digestible carbohydrates, fermentable fibers and nutraceuticals such as polyphenols. As recently reviewed by our group, polyphenols exert positive outcomes on the gut microbiota and its host (Lupien-Meilleur et al., 2019). Probiotics are live microorganisms and specific strains of bacteria that are administered to promote positive effects on GI function. Probiotics may be bacteria that are already present in the intestine, but also often refers to exogenous consumption of bacteria through pharmaceuticals, yogurts with live cultures, and other similar means. Evidently, both probiotics and prebiotic food confer health benefits for the host (Bindels & Delzenne, 2013; Delzenne & Cani, 2011).

Nutraceuticals such as polyphenols continue to be explored for their positive influence on biological functions. Since the gut microbiota represents the first decisive interaction between the diet (macronutrients and nutraceuticals) and the host, the gut is being widely investigated for its role in affecting chronic dietary- and lifestyle-related disorders (Yan et al., 2016). It has been suggested that the composition of an individual's gut microbiota determines the systemic concentrations and bioavailability of polyphenols and their bioactive derivatives (Tomás-Barberán, García-Villalba, González-Sarrías, Selma, & Espín, 2014). This has important

consequences on how the gut environment affects the health and function of organ systems in each individual, and supports the link between the gut microbiome and health status.

Polyphenols have recently been explored as bioactive compounds as they maintain important prebiotic, anti-oxidant, anti-inflammatory, and anti-atherogenic properties, which are established in the intestinal/colonic environment (Anhê et al., 2015; Eid et al., 2014; Gunawardena, Govindaraghavan, & Münch, 2014; Kim, Jung, Kim, & Kwak, 2008; Larrosa et al., 2010; Prasain & Barnes, 2014; Rosenblat, Volkova, Borochov-Neori, Judeinstein, & Aviram, 2015; Taleb, Maddocks, Morris, & Kanekanian, 2016). Evidently, the status of the gut remains a key component in the steps between polyphenol consumption and the benefits at the various organ levels.

Polyphenol consumption

Sources of polyphenols are found among a variety of highly common food (Table 2). Presently, many of the compounds presented in Table 1 and exemplified in Table 2 have not been tested in purified forms, however, Resveratrol (Res) is one of the most extensively studied polyphenols since the beginning of the past decade. Ellagitannins (Urolithin; Uro), caffeic acid, turmeric, quercetin and Proanthocyanidin (PAC) have also all been tested in purified forms.

i. Resveratrol (Res)

Res is a naturally occurring polyphenol abundant in blueberries, peanuts, and in the skin of red grapes and which remains intact in red wine (Manach, Scalbert, Morand, Rémésy, &

Jiménez, 2004), white wine and grape juice (Balentine et al., 2015). Res is found in very small quantities in the diet however, so the protective effects from normal nutritional intake are arguable (El Gharras, 2009; Manach et al., 2004). Nonetheless, Res has been shown to act as an exercise mimetic by preventing wasting disorders in the hindlimb of rats (Momken et al., 2011). Res largely arrives at the colon unmetabolized, where metabolites Res sulfate and Res glucuronide are formed (King, Bomser, & Min, 2006). Res has been reviewed for its ease of absorption and accumulation, and for a high absorption of Res sulfate to be readily detected in the plasma (King et al., 2006). Within the gut, Res interacts with the microbiota, influencing marked changes in the composition (Sung, Kim, et al., 2017). Given the low bioavailability of Res from the diet, the alteration of the gut microbiota has been proposed as a crucial component for the numerous and powerful effects that have been observed, including anti-inflammatory, antioxidant, and according synergistic antidiabetic effects (Sung, Kim, et al., 2017).

ii. Urolithin (Uro)

When a source of Uro is ingested, such as with pomegranate (Table 2), it is catabolized in the gut and first yields a class of compounds called tannins. Ellagitannins, one of the tannin groups, consist of punicalagins and granatins. Punicalagins are quickly hydrolysed by the gut microbiota to release ellagic acid, which is further metabolized to yield a variety of urolithins. The first product of ellagic acid catabolism is Urolithin D, and successive loss of hydroxy groups results in the formation of metabolites Urolithin C, A and B (Espín et al., 2007). The presence of various bacterial species within the gut microbiota may yield

different concentrations of each urolithin, with individuals showing different conversion rates of ellagic acid (Begoña Cerdá, Francisco A. Tomás-Barberán, & Espín, 2004; Tomás-Barberán et al., 2014)(Begoña Cerdá et al., 2004; Tomás-Barberán et al., 2014). Pomegranate extract and its derivatives ellagitannin and Uro have been reviewed for ameliorative actions towards metabolic syndrome (Medjakovic & Jungbauer, 2013) and important anti-inflammatory effects within the gut (González-Sarrías, Larrosa, Tomás-Barberán, Dolara, & Espín, 2010; Larrosa et al., 2010) .

iii. Caffeic acid

Caffeic acid is regarded as the most abundant phenolic acid as it represents between 75% and 100% of the total hydroxycinnamic content among fruit sources (El Gharras, 2009). Coffee is a source of chlorogenic acid, which is an ester of caffeic and quincic acids (Table 1). Both caffeinated and decaffeinated coffee have been associated with a reduced risk of developing T2D as evidenced by beneficial effects on glucose homeostasis (Tsuda et al., 2012). In terms of absorption, one study reported that around one-third of orally ingested chlorogenic acid and 95% of caffeic acid was absorbed from the small intestine in humans, and suggested that degradation into smaller compounds likely occurred post-absorption (Olthof, Hollman, & Katan, 2001).

iv. Curcumin (Turmeric)

Turmeric is a common spice which contains caffeic acid, and whose main component is curcumin, the principle yellow pigment (Maiti & Dunbar, 2018). Curcumin is another natural polyphenol with low oral bioavailability. One report described oral curcumin undergoing intestinal absorption, then immediately entering the liver via the portal vein, where is it then degraded into metabolites curcumin glucuronide and curcumin sulphate (Maiti & Dunbar, 2018). Another report described the degradation of curcumin similarly to Res, where oral curcumin is degraded to a number of metabolites within the GI tract, which interact with the gut microbiota to both effect the health protective actions of curcumin (Jin, Song, Weng, & Fantus, 2018). Important anti-inflammatory activity along with neuroprotective (antiamyloidogenic) properties have supported promising effects against neurodegenerative diseases (Maiti & Dunbar, 2018). Further, curcumin has been reported for important anti-obesity and anti-diabetic actions as a result of widespread influence of inflammation, oxidative stress, insulin sensitivity and intestinal wall permeability (Jin et al., 2018).

v. Quercetin

Quercetin is present in a number of fruits and vegetables, but most notably onion. Murakami, Ashida, and Terao (2008) reviewed that substantial amounts of dietary quercetin are absorbed from the digestive tract, and then converted into metabolites. Similar to other polyphenols, it is the widespread effects of the metabolites that act synergistically to represent powerful anti-carcinogenic actions for quercetin. Further, many of the metabolites are returned to the digestive tract, as quercetin has been shown to modulate gut bacteria

(Etxeberria et al., 2015; Murakami et al., 2008). Interestingly, the lymphatic system also contributes to the transport of quercetin metabolites throughout the body (Murota & Terao, 2005)

vi. Proanthocyanidins (PAC)

Cranberries are rich in flavonoids and are mostly noted for containing important concentrations of PAC (Table 2*;* Prasain & Barnes, 2014). PACs are group of polyphenols with particularly strong evidence supporting bioactive effects and influence on gut health (Anhê et al., 2015)(Anhê et al., 2015). The exact mechanism of intestinal absorption and transcellular transport is unknown (Prasain & Barnes, 2014), however the molecular size, lipophilicity, solubility and excretion routes are relevant (Rasmussen, Frederiksen, Krogholm, & Poulsen, 2005). Intact PAC with higher-molecular weights have poor absorption from the GI tract, and arrive at the colon. PAC is metabolised by gut microbiota, and much like Uro, can be influenced by an individual's bacterial profile resulting in various degrees of degradation, bio-availability and -activity (Prasain & Barnes, 2014). The prevailing belief in the literature concerning the mechanism of absorption is that the degraded metabolites of PAC (gut-derived monomeric forms and phenolic acid) are reabsorbed through the colon to enter systemic circulation, then excreted in the urine, but not before exerting diverse bioactive and physiological effects, which will be explored (Manach et al., 2004; Nieman, Mitmesser, Nieman, & Mitmesser, 2017; Rasmussen et al., 2005)(Manach et al., 2004; Nieman et al., 2017; Rasmussen et al., 2005).

Table 2: Common food sources of polyphenols, identified by the class, and flavonoid subclass if applicable

Food Source	Phenolic Compound	
	Flavonoid subclass	Other classes
Cocoa	Flavan-3-ol	
	Proanthocyanidin	
Tea	Flavan-3-ol	
	Flavanol	
	Proanthocyanidin	
	Derived tannins (black and oolong)	
Grapes and grape products	Flavan-3-ol	Resveratrol
	Anthocyanin	
	Proanthocyanidin	
Apple and apple products	Flavan-3-ol	Phenolic acids
	Flavonol	
	Proanthocyanidin	
Citrus fruits and juices	Flavanone	
	Flavones	
Cranberries	Anthocyanin	
	Proanthocyanidin	
Raspberries/Blackberries/ Blueberries/Black currant	Anthocyanin	Phenolic acids
	Flavanols	Stilbenes
Strawberries	Anthocyanin	Stilbenes
	Proanthocyanidin	Phenolic acids
Stone fruits (peaches, pears, plums, prunes)	Flavan-3-ol	Lignans
	Proanthocyanidin	Phenolic acids
Apricot	Flavan-3-ol	
	Flavanol	
Cherry	Flavan-3-ol	Phenolic acids
	Flavanol	
	Anthocyanin	
Rhubarb	Anthocyanin	
Date fruit and date fruit products	Flavanols	Phenolic acid
	Anthocyanin	
Kiwi		Phenolic acids
Pomegranate		Phenolic acids (hydrolyzable tannins)
Tomato	Flavanone	Phenolic acids
	Flavanol	
Peanuts		Stilbenes

Linseed (flax)		Lignans
Chestnut/Walnut		Phenolic acids (hydrolyzable tannins)
Hazelnut/pecan/almond	Proanthocyanidin	
Nut skins	Proanthocyanidin	
Soybeans/soy-based foods	Isoflavone	
Leguminous plants	Flavan-3-ol	Lignans
	Isoflavone	
	Proanthocyanidin	
Cereals/ refined- and whole-grain wheat	Flavones	Lignans
		Phenolic acids
Barley	Proanthocyanidin	
Potatoes		Phenolic acid
Beans (green and white)	Flavan-3-ol	
	Flavanol	
Spinach/lettuce/globe artichoke heads/broccoli	Flavonol	
Celery/parsley	Flavones	
Onion/kale/leek	Flavonol	
Cloves/peppermint/sage/rose-mary/spearmint/thyme		Phenolic acids
Cinnamon	Proanthocyanidin	
Olives (green and black)/Olive oil	Flavones	Lignans
	Anthocyanin	Phenolic acid
Beer	Isoflavone	Phenolic acids
	Proanthocyanidin	
Wine	Flavan-3-ol	Resveratrol
	Flavanol	
	Flavanone	Phenolic acids
	Anthocyanin	
	Proanthocyanidin	
Coffee	Proanthocyanidin	Phenolic acid

Based on Balentine et al., 2015; El Gharras, 2009; Gunawardena et al., 2014; Pérez-Jiménez, Neveu, Vos, & Scalbert, 2010; Tresserra-Rimbau et al., 2013

Many organs and tissues within the body remain at the mercy of the metabolic effects of foods consumed. Polyphenols maintain highly beneficial and health-protective effects. They are currently being broadly investigated for their effects against a number of diseases including T2D, cancer and neurodegenerative diseases (Jin et al., 2018; Maiti & Dunbar, 2018; Murakami et al., 2008; Spagnuolo et al., 2016; Sung, Kim, et al., 2017; Tsuda et al., 2012). Further, numerous axes have been drawn between the gut microbiota and commonly studied organs, such as the liver (Adolph et al., 2018; Fang et al., 2018), and the heart (Forkosh & Ilan, 2019). Another end-target organ for gut microbiota-polyphenol effects is skeletal muscles (Yan et al., 2016). However, given the vast and diverse amount of actions of this organ, numerous mechanisms and effects remain under defined.

The link of the gut to skeletal muscle function

One underexplored organ system when considering the gut's systemic connections to the host is skeletal muscle. Despite the seemingly lack of evident relationship, recent work has established an axis that links the gut microbiota and skeletal muscle (Bindels & Delzenne, 2013; Yan et al., 2016). Termed "The Gut Microbiota-Muscle Axis", this relationship establishes the modulatory effects that the gut microbiota has on skeletal muscle physiology, and provides a platform on which to explore dietary substances' implications as they extend beyond basic nutrition.

Skeletal muscle represents between 40 and 50% of body mass in adults, and is responsible for 20-30% of resting oxygen consumption (Stump, Henriksen, Wei, & Sowers, 2006). Mammalian skeletal muscle fibres can be classified according to their characteristics:

Type I slow-twitch fibers, and Type II fast-twitch fibers (see Table 3). Fast-twitch fibers are further subcategorized into type IIa and IIx with a third type, type IIb found mainly in animal models. Fiber type is important for differences in metabolism and energy consumption, as well as mechanical responses. Slow-twitch, type I fibers are oxidative, have low fatigability, and low twitch-tension production. They are well suited for prolonged, low-intensity work. The soleus (SOL) muscle is represented by mostly type I fibers. Fast-twitch, type II fibers are oxidative-glycolytic (type IIa) and glycolytic (type IIx and IIb). Glycolytic fibers have high twitch-tension production and high fatigability. Oxidative-glycolytic fibers are considered intermediate as they can sustain activity, or contract with high tension and then fatigue. The Extensor digitorum longus (EDL) muscle consists predominantly of type II fibers. The spectrum of morphological, metabolic and contractile properties of fiber-type distribution (Type I – Type IIa – Type IIx – Type IIb) facilitates converting the portion of fiber types within a muscle (Gundersen, 2011).

The primary function for skeletal muscle is contractile activity. This includes force generation, resistance to fatigue, and recovery from fatigue. Other vital functions for skeletal muscle include hypertrophic and regenerative capacities. Skeletal muscle maintains a crucial role in metabolism regulation, as it remains the major tissue in glucose metabolism (Wei et al., 2008), and pivotal for lipid metabolism by way of triacyl glyceride uptake, oxidation, and subsequent fatty acid storage (Andrich, Ou, et al., 2018; Kiens, 2006). Skeletal muscle secretes important myokines, which are analogous to adipokines (Pedersen et al., 2003), and generates specific reactive oxygen species (ROS) as a result of stimulation. These species are reviewed by Jackson (2008) for maintaining roles in the adaptation and optimization of contractile properties. Skeletal

muscles are spread around the whole body, so given their vast variety of functions, they maintain important implications for overall health.

Table 3: Mammalian muscle fiber types and their function

	Type I	Type II		
		Type IIa	Type IIx	Type IIb
Metabolism	Oxidative	Oxidative/glycolytic	Glycolytic	Glycolytic
Twitch duration	Slow	Medium	Fast	Fast
Endurance	High	Medium	Low	Low
Shortening velocity	Slow	Medium	Fast	Fast
Energy efficiency	High	Medium	Low	Low
Fatigability	Low	Medium	High	High
Cross-sectional area (CSA)	Small	Medium	Large	Large
Innervation	Small motor unit One nerve innervates few muscle fibers	Medium-size motor unit One nerve innervates fewer muscle fibers	Big motor unit One nerve innervates many muscle fibers	Big motor unit One nerve innervates many muscle fibers

From: Gundersen, 2011

Skeletal muscle function is a crucial component in measures of quality of life and performance, and has recently been shown to be associated with the state of the gut. Evidence for the existence and development of the gut microbiota-muscle axis model involved the transfer of the gut microbiota, via fecal suspension, from obese and lean model pigs into germ-free (GF) mice (Yan et al., 2016). The mice developed muscular phenotypes and body compositions that trended towards those of their donors (Yan et al., 2016). More specifically, what was found in terms of effects on the skeletal muscles was 3-fold, and striking. First; a trend was observed for the gastrocnemius muscle of lean mice to have a larger cross-sectional area (CSA) than in the obese-model mice, which is similar to the trend in the difference between the lean and obese pigs. Second; differences in the expression levels of myosin heavy-chain (MYHC) isoforms were

observed. There was a higher expression level for slow-twitch type I fibers, and a lower expression level for fast-twitch type IIb fibers in both the obese-model donors and recipients. Third; enhanced lipid deposition in the gastrocnemius muscle was consistent between the obese pig and mice recipient models. Taken together, these results elegantly show that the gut microbial composition influences muscle hypertrophy, fiber type distribution, and lipid metabolism (Yan et al., 2016).

The physiological environment that supports optimal skeletal muscle function and maintenance is crucial, complex, and highly modifiable. In light of the recent literature, the gut microbiota appears to maintain a vast influence over total body and skeletal muscle homeostasis (Yan et al., 2016) through currently uncharacterized mechanisms. Promising data indicate that specific nutraceuticals modulate the gut microbiota to ultimately potentiate skeletal muscle function (Anhê et al., 2015; Sung, Byrne, et al., 2017). In other words, polyphenols are reported to exert prebiotic effects on the gut microbiota, and based on the connection indicated by the gut microbiota-skeletal muscle axis, the consumption of polyphenols could potentially maintain a substantial, and positive impact on skeletal muscle function and quality. In this section, some of the most commonly studied polyphenols that are known for their beneficial effects on skeletal muscle will be reviewed.

i. Resveratrol

Res has been extensively researched for its effects on whole body metabolism (Sung, Byrne, et al., 2017), exercise performance (Dolinsky et al., 2012), muscle deconditioning (Mortreux, Riveros, Bouxsein, & Rutkove, 2019) and gut microbiota composition (Etxeberria et al.,

2015; Sung, Kim, et al., 2017). Sung, Byrne, et al., (2017) investigated the effects of Res on exercise capacity, as measured by treadmill running in rodent models of heart failure. They found that two weeks of Res administration was effectively able to treat exercise intolerance, which is one of the major symptoms of heart failure disease. The authors observed changes to the gut microbial community including an increased ratio of Bacteriodetes to Firmicutes, which is important for glucose homeostasis, and increased relative abundances of bacteria parabacteroides, bilophila and Akkermansia in the Res treated heart failure mice. Credit is given to the observed alterations in the gut microbiota as a mechanism for Res to affect whole-body energy metabolism and by extension, exercise tolerance (Sung, Byrne, et al., 2017). This is substantiated by other findings from the same group upon execution of a fecal transplant from Res-fed mice to obesogenic diet-fed mice (Sung, Kim, et al., 2017). First, it was observed that mice fed a high-fat-high-sucrose diet for 8 weeks had impaired glucose clearance compared to chow-fed mice, while mice fed an obesogenic diet with Res had significantly improved glucose tolerance. Therefore, another cohort of mice fed an obesogenic diet for 5 weeks was then administered fecal microbiota transplants collected from mice fed a 0.4% Res diet. One week later, the obese recipient mice displayed substantial improvements in glucose homeostasis (Sung, Kim, et al., 2017). These findings indicate that Res consumption causes an alteration in the gut microbiota, which in turn plays a crucial role in mediating its beneficial metabolic effects on skeletal muscle.

ii. Urolithin

Localized anti-inflammatory and anti-oxidant actions, as well as increases in myocyte membrane stability are proposed for the protective effects on skeletal muscle function observed in humans supplemented with Uro (Trombold et al., 2010; Trombold, Reinfeld, Casler, & Coyle, 2011). In 2010, Trombold and colleagues explored whether dietary supplementation with ellagitannins could attenuate muscular damage after eccentric exercise, which characteristically produces delayed onset muscle soreness, as indicated by recovery of isometric elbow flexor strength among recreationally active males. In 2011, the same group repeated the study with individuals who routinely performed resistance training. In the earlier study, recovery of isometric strength in the supplementation group was greater in the 48 to 72-hour period post exercise, and in the later study, an attenuation of strength reduction was observed in the supplementation group 2 hours post exercise. This suggests that pomegranate derived polyphenols could exert beneficial effects on tissue recovery, and even exert acute ergogenic effects on muscle strength and soreness in the elbow flexors. The authors believe that the mechanistic action is via a reduction in inflammation and oxidative stress, and an increase in membrane stability among myocytes mediated by polyphenols (Trombold et al., 2010, 2011). More recently, Bellone and colleagues (2018) tested pomegranate supplementation on functional recovery following ischemic stroke, and found that stroke patients supplemented with pomegranate improved significantly more in self-care and locomotion compared to the placebo group, and showed significant improvement from baseline in self care, transfers, locomotion and social cognition. The authors attribute the findings to lesser muscle damage and soreness following bouts of rehabilitative exercise,

which would result from increased blood flow and decreased inflammation due to the actions of the polyphenols derived from the pomegranate supplement (Bellone et al., 2018). These findings speak to the preservation of muscle fiber integrity and muscle function associated with pomegranate supplementation in a situation where inflammation and catabolism would otherwise be favored, and are the first to provide clinical support that oral consumption affects whole-body inflammation and metabolism.

Carefully, and locally maintaining muscle cell quality is another mechanism through which Uro exerts beneficial effects. Mitophagy was induced in the muscles of both young and older rodents as a result of long-term oral administration of UroA (Ryu, 2016). The prevention of age-related muscle decline was explored by feeding 16-month old mice a HF diet supplemented with 50 mg/kg/d UroA for 8 months (Ryu, 2016). No changes were found in body weight gain, nor fat nor lean mass, but a substantial increase in muscle function was observed compared to the control HF-fed rats at 22 and 24 months old (Ryu et al., 2016). The group then began a trial with aged mice (22.5 months) who had been fed chow, and randomized them to 6 more weeks of either UroA or chow, and found that the Uro group achieved a 42% greater running endurance than the group who continued on chow. Finally, they repeated a similar protocol with young male Wistar rats being randomized to consume chow or UroA, and found that the UroA group achieved on average a 65% greater running capacity than the control (Ryu, 2016). When muscle fibers were examined, no substantial differences in the transcript of myosin heavy chain was found, which indicates that the improvement in muscle function is not a result of a change in muscle fiber type (Ryu, 2016), but likely an improvement of muscle cell quality. These results also show that UroA improves muscle cell quality across the stages of life.

Finally, it has been previously found that polyphenols work as modifiers of signal transduction pathways, where they exert anti-inflammatory effects by modulating pro-inflammatory gene expressions via NFκB and MAPK signaling (Anhê et al., 2015; Carmela Santangelo, Rosaria Varì & Roberta Di Benedetto, 2007). These effects have been observed for Uro in *in vitro* and in redolent models (González-Sarrías et al., 2010; Larrosa et al., 2010).

iii. Caffeic acid, Curcumin and Quercetin

Caffeic acid, which is the major metabolite of chlorogenic acid, displays anti-diabetic effects by acting directly on skeletal muscle to acutely stimulate AMPK activity, which is involved in the mechanisms governing exercise-stimulated, insulin-independent glucose transport (Tsuda et al., 2012). Caffeic acid is also a powerful antioxidant (Gülçin, 2006). Similarly, anti-hyperglycemic effects, and a reduction of insulin resistance in peripheral tissues have been reviewed for curcumin (Ghorbani, Hekmatdoost, & Mirmiran, 2014). In its own light, quercetin has been shown to lessen high-fat-sucrose diet induced dysbiosis, attenuate the Firmicutes/Bacteroidetes ratio, and inhibit the growth of bacterial species within the gut associated with diet-induced obesity among Wistar rats (Etxeberria et al., 2015). In the same study, quercetin reduced serum insulin levels and insulin resistance (Etxeberria et al., 2015). These polyphenols maintain especially important implications for glucose homeostasis, and skeletal muscle quality among individuals at risk for developing T2D, who have developed T2D, or who have an impaired exercise capacity.

iv. Proanthocyanidins, date fruit and procyanidin/apple polyphenol

One of the important, diverse effects of PAC is causing an alteration in energy metabolism. In one study, lard oil containing grape seed PAC extract was administered to fasted male Wistar rats (Pajuelo et al., 2011). After 5h, the concentrations of plasma triglycerides, free fatty acids, glycerol and urea were significantly lower in the PAC group, and mitochondrial oxygen consumption using pyruvate as the substrate, was increased in skeletal muscle. PAC also increased the expression of genes involved in energy metabolism such as PPARγ and PGC1α (Pajuelo et al., 2011). This shows that the oxidative capacity of skeletal muscle is increased rapidly after acute supplementation. Given that the oxidative capacity was increased via the use of pyruvate as a substrate, which promotes glycosidic metabolism (Pajuelo et al., 2011), it is possible that the anaerobic threshold might be increased.

Additional diverse effects of PAC within the body include improved processes of muscle repair, and changing muscle-fiber types. Among rats submitted to contusion-induced injury in the gastrocnemius muscle, those administered proanthocyanidolic oligomer for 2 weeks prior to injury as well as continued administration thereafter, exhibited an earlier peak of activation of satellite cell response, a decrease in neutrophil elevation, and an earlier macrophage response (Myburgh, Kruger, & Smith, 2012). Muscle fiber regeneration was complete after 14 days in the PAC group, but not in the control. This shows that PAC administration supports muscular repair from localized muscle tissue injury. Moreover, eight weeks of low-dose (0.5% w/w) apple polyphenol (APP) supplementation (prepared from unripe apples) was sufficient to cause a shift from fast to intermediate/slow muscle fiber types, indicated by a significant up-regulation of myoglobin protein expression, I and IIa

myosin heavy-chain (MyHC) isoforms in the EDL, IIx in the plantaris, and a compensatory significant decrease of IIb MyHC in the plantaris in 12-week old male Sprague Dawley rats (Mizunoya et al., 2017). Similar effects were observed from supplementation with quercetin (Mukai et al., 2010), which is a minor component of APP. Overall, bio-activity affecting shifts towards fatigue resistant muscle fiber types carries important implications for the skeletal muscles in atrophy-inducing conditions.

Finally, the prebiotic effects from this class of polyphenols are crucial steps in the mechanisms for their benefits at the level of skeletal muscles. Date fruit, which is high in anthocyanidins (Eid et al., 2014), flavonoids and flavanols (40.5mg/100g and 31.7mg/100g respectively; Taleb et al., 2016), exert prebiotic effects, as both date polyphenol extract and digested whole fruit extract selectively and significantly increase bifidobacteria (Eid et al., 2014), which is important in enhancing colon health (Gibson & Roberfroid, 1995). The PAC sourced from cranberries is reported for exerting prebiotic activity by increasing abundance of Akkermansia *muciniphila* (Anhê et al., 2015), which lends to a decrease in intestinal permeability. Akkermansia *muciniphila* is a gut bacterium that resides in the mucus layer of the intestinal epithelium, and which represents 3-5% of the microbial composition, rendering it the dominant bacterium that colonizes mucosal environment in healthy humans (Derrien, Vaughan, Plugge, & Vos, 2004). Akkermansia's abundance inversely correlates with body weight (Everard et al., 2011; Santacruz et al., 2010), and it has also been suggested that Akkermansia counts are crucial in affecting gut barrier function, metabolic inflammation, body composition and fat storage (Everard et al., 2013). Recently, Anhê and colleagues (2015) reported that in an 8-week treatment of HF-high sucrose diet, cranberry extract supplementation demonstrated beneficial effects on metabolic phenotypes, a well as an

increase in the relative abundance of Akkermansia, which was associated with the prevention of obesogenic-diet induced rises in gut permeability, as indicated by circulating lipopolysaccharide (LPS) levels. They are the first group to report a fruit extract exerting major prebiotic effects in the intestinal microbiota of a rodent model of diet-induced obesity (Anhê et al., 2015). Furthermore, research on the gut microbiota-muscle axis suggests the microbiota as a therapeutic target for muscle wasting as a result of the development of systemic inflammation (Bindels & Delzenne, 2013). In line with this position, Anhê and colleagues (2015) also found that cranberry extract administration completely suppressed NFκB activation in the intestines, and since NFκB is a central regulator of intestinal inflammation, this causes a decrease in TNFα and COX2 expression in the intestines as well. A general downregulation of inflammation could lead to decreases in insulin resistance, upregulation of glucose transport, and less peripheral tissue damage. Bleau, Karelis, St-Pierre, and Lamontagne (2015) reviewed how HF-diet-induced changes in the gut microbiota lend themselves to an increase in the inflammatory tone of the mucosal lining, as well as a disruption of the epithelial barrier allowing an increase in the transit of LPS into systemic circulation. Moreover, skeletal muscle cells can express toll-like receptors (TLR), and some TLRs maintain natural ligands for LPS, such as TLR-4 (Davis, Gabler, Walker-Daniels, & Spurlock, 2008). When TLRs are activated, pro-inflammatory myokines are released from cells and macrophages, and contribute to an inflammatory environment. Taken together, this all suggests that cranberry extract administration is a crucial component in a potential mechanism that would abolish any metabolic endotoxemia that developed as a result of gut leakage, and protect the physiologic environment that supports muscle cell quality and optimal functioning in the presence of a HF diet. However, the effects of PAC

supplementation on skeletal muscle quality have not directly been explored. It is important to establish the relationship between PAC supplementation and skeletal muscle quality to further understand the potential mechanisms of action, as well as contribute to the future establishment of consumption recommendations.

PAC, HF diets and skeletal muscle contractility

Skeletal muscle quality can be indicated by contractility, which includes measurements of force generation, resistance to fatigue, and recovery from fatigue. Our group recently showed that a short-term (2-week) HF diet was sufficient to cause alterations in skeletal muscle contractility among rats, indicated by a reduction in SOL muscle specific force (Andrich, Ou, et al., 2018). Harmful alterations were also observed in lipid metabolism, mitochondrial function, insulin sensitivity and oxidative stress within skeletal muscle tissue (Andrich et al., 2019; Andrich, Ou, et al., 2018; Lupien-Meilleur et al., 2019). Polyphenols Res and Uro have previously and extensively been shown to exert beneficial effects on skeletal muscle function, including but not limited to local anti-inflammatory and anti-oxidant activity, muscle soreness, force recovery, resistance to fatigue, and overall exercise tolerance (Sung, Byrne, et al., 2017; Trombold et al., 2010, 2011). There are some reported beneficial effects for PAC on tissue repair, altering muscle fiber types and energy metabolism (Mizunoya et al., 2017; Myburgh et al., 2012; Pajuelo et al., 2011), but no similar reports to those of Res and Uro on skeletal muscle contractility despite similar reports among PAC, Res and Uro of important prebiotic activity in the gut.

The gut microbiota-muscle axis is supported by Yan et al., (2016) who showed that the gut microbiota influences skeletal muscle function and phenotype. Additionally, polyphenols interact with the gut microbiota, and the gut microbiota has in turn been shown to be necessary to transform polyphenols and render their positive impact on skeletal muscle (Espín et al., 2007; King et al., 2006). The reported prebiotic effects for PAC are proposed to counter the detrimental effects of HF diets on skeletal muscle quality, facilitated by harmful alterations in the gut (Anhê et al., 2015; Bleau et al., 2015). Therefore, it can be hypothesized that PAC supplementation may prove protective against the early and harmful alterations in skeletal muscle contractility previously observed to be caused by a HF diet (Andrich, Ou, et al., 2018). In support of the existence gut-microbiota muscle axis, the present study intends to highlight the early impact of PAC as a supplementation to a HF diet on a number of contractile functions in glycolytic and oxidative skeletal muscle tissue.

Research question

With the ultimate aim of establishing the impact of cranberry-derived polyphenol extract supplementation on skeletal muscle function in rats, it is our goal to determine whether two weeks of HF diet supplemented with PAC, is sufficient to observe effects on skeletal muscle quality, as indicated by contractility. It is hypothesized that PAC polyphenol supplementation will prevent the early alteration in skeletal muscle contractility that we have previously reported in response to a HF diet among young rats.

CHAPTER 2: Methodology

Models

Animal model justification

The typical nature of not only a nutritional study, but a nutritional study involving the consumption of a diet rich in fat, and the examination of the resulting effects at the level of the skeletal muscle, represents a number of feasibility challenges for human participants. Among serious ethical considerations, individual differences, adherence, accuracy and reliability contribute to low feasibility for a study as such. Therefore, animal models have been used in lieu of human models for more than 60 years (Peckham, Entenman, & Carroll, 1962) with rats and mice being the most common species (Speakman, Hambly, Mitchell, & Król, 2007). Rat breeds used in metabolic studies include Sprague-Dawley and Wistar (Marques et al., 2016), Fisher 344 (Levy, Clore, & Stevens, 2004), Lewis (Schmiedt et al., 2011) and Long-Evans (Woods, Seeley, Rushing, D'Alessio, & Tso, 2003). Zucker Diabetic rats (which lack the leptin receptor and arguably maintain an obese genotype; Kurtz, Morris, & Pershadsingh, 1989) and Otsuka Long-Evans Tokushima (Kawano, Hirashima, Mori, & Natori, 1994; Schroeder et al., 2009) are predisposed to the development of obesity and obesity-related phenotypes.

Popular mice models include C57/BL6, 129X1, DBA/2 and FVB/N breeds as they are susceptible to the development of HF diet-induced obesity, glucose intolerance and insulin resistance (Montgomery et al., 2013). *Ob/ob* mouse models have a lack of leptin expression and develop obesity and mild insulin resistance, and *db/db* mice have a leptin-receptor deficiency and develop both obesity and diabetes (Giesbertz et al., 2015). Both are popular models for obesity and T2D studies. Among other species altogether, Syrian Golden Hamsters exhibit more robust disturbances in lipid metabolism than rats (Ebara et al., 1994), and are an ideal model for dyslipidemia as they develop similar apolipoprotein levels as humans (Ahn et al., 1994).

Sprague-Dawley and Wistar are arguably the most commonly used types of rats for obesity- and metabolic syndrome-related research. When submitted to the same rich-in-fat diet, both breeds displayed a higher body weight, a higher mass of adipose tissue, increased sizes of mesenteric adipocytes, hyperglycemia as indicated by oral glucose tolerance tests, and elevated blood levels of adiponectin and leptin compared to regular chow fed rats (Marques et al., 2016). Both types of rats displayed similar modulations of the gut microbiota, however, most of these metabolic effects were observed either in a more pronounced manner, or earlier among the Wistar rats (Marques et al., 2016). This renders Wistar rats ideal models for research on the short-term metabolism of excess dietary fats.

The sex, and the age of the animal at the beginning of the protocol are important factors for the physiological adaptations influenced by the diet. Rats reach puberty towards their fifth week of life (Sengupta, 2013). Among females, sexual maturity represents 4- to 5-day estrus cycles, where food intake and body weight fluctuate depending on the phase of the cycle (ter Haar, 1972; Westwood, 2008). By the adult stage of life, which is around 8 weeks, the body weight of male rats increases more rapidly than female rats, and remains more elevated for the duration of adult life (Kwekel, Desai, Moland, Branham, & Fuscoe, 2010). As such, the phases of estrus cycles would have to be considered if the protocol consisted of a cohort of female rats, and it would be no more accurate to mix male and female rats for comparison of groups due to the effects of estrus cycles among females. Therefore, the use of male rats is encouraged for diet-related research (Giles, Jackman, & MacLean, 2016). When considering the age of the model, young rats appear to be more susceptible to the effects of excess dietary fats than adult or older rats (Erdos et al., 2011). Following an 8-week protocol, a number of metabolic syndrome

symptoms were observed among rats that were 3 weeks old at the start of the protocol, yet not among rats that began the protocol at adult age (Cheng, Ton, Phang, Tan, & Abdul Kadir, 2017). Moreover, our group has previously used young male Wistar rats submitted to 14 days of HF diet in order to measure effects on muscle contractility, This model allowed us to observe impairments in some skeletal muscle contractile function, lipid metabolism and antioxidant defense. (Andrich, Melbouci, et al., 2018; Andrich, Ou, et al., 2018; Lupien-Meilleur et al., 2019). The use of young male rats is therefore ideal and effective for research on short-term, excess dietary fat consumption, and for examining effects at the muscular level, as alterations in metabolism occur rapidly and markedly.

The influence of a given diet on metabolism, especially among rodents is directly related to caloric density. In the Atwater system, one gram of lipids contains 9 calories, whereas one gram of each carbohydrates and proteins contains 4 calories. The prevailing belief in the literature is that rats will consume the same amount of food daily regardless of the type of diet (Ramirez & Friedman, 1990). Accordingly, rats submitted to a HF diet will consume a greater number of calories every day.

It is important to note that anti-obesogenic effects (Halton & Hu, 2004) have been demonstrated for proteins, even in the presence of excess lipids (Pichon, Huneau, Fromentin, & Tomé, 2006). Therefore, considerations must be made in order to maintain similar percentages of calories from proteins in the experimental and control diets. As a result, the increased portion of lipids in the HF diet is related to a decrease in portion of carbohydrates. Companies such as Research Diets and Teklad (Envigo) offer HF diets that are specially formulated to contain sufficient quantities of micronutrients and proteins.

Accordingly, the HF diet recipe suggested by Research Diets, which sources fats from lard and soybean oil, represents the ideal diet for our model. A preference for the taste of linoleic acid, which is dominant in lard (± 11% linoleic acid) and contained in soybean oil as well, as been demonstrated among rats (Mizushige, Inoue, & Fushiki, 2007). This could explain why a lard-based diet showed increased obesogenic effects compared to a hydrogenated vegetable oil-based (± 4% linoleic acid) diet, despite both having the same caloric density and quantity of saturated fats (Kubant et al., 2015). Further, an excess consumption of linoleic acid has been shown to contribute to adipogenic effects by being converted into arachidonic acid. Arachidonic acid then acts on endocannabinoids (which are important for regulating appetite) 2-arachidonylglycerol (2-AG), 1-AG and anandamide to cause hyperactivity and ultimately increase adiposity (Alvheim et al., 2012). A lard and soybean oil-based diet has also been shown to have greater adiposity than a coconut oil-based diet even though the lard and soybean oil only contain 40% and 14% saturated fat respectively, while the coconut oil contains 90% saturated fat (Buettner, Schölmerich, & Bollheimer, 2007; Deol et al., 2015). One explanation lies with the precise lipid species contained in the triglycerides from each source of fat (Takeuchi, Noguchi, Sekine, Kobayashi, & Aoyama, 2006). Coconut oil is mainly composed of lauric acid (12:0), while lard is composed of palmitic (16:0) and stearic acid (18:0); which are evidently longer-chain acids. Longer-chain acids are oxidized to lesser extents, and favored for storage (DeLany, Windhauser, Champagne, & Bray, 2000).

Recent research has shown that depending on the desired outcomes, the protocol for adipogenic diet administration can be effective in as little as two weeks, as well as across the animal's whole life (Iwasa, Matsuzaki, Yano, & Irahara, 2018). Among rats, significant weight gain is typically observed around 4 weeks of adipogenic diet consumption (Ramirez & Friedman,

1990; Suk & Shin, 2015). However, others report that adiposity increases more rapidly than body weight among rats (Schmiedt et al., 2011; Woods et al., 2003) substantiating important metabolic changes that precede significant weight gain.

PAC polyphenol justification

PAC has been recently and extensively studied for its prebiotic effects on the relative abundance of Akkermansia within the gut, as well as for improving features of metabolic syndrome that are associated with increased proportions of Akkermansia (Anhê et al., 2016, 2015). In 2015, Anhê and colleagues administered 200mg/Kg cranberry extract by gavage to 10-week old C57BL/6J mice fed a high-fat-high-sucrose (65% lipid, 15% protein, 20% carbohydrates) diet for 8 weeks. Compared to chow-fed and water-gavaged mice, the cranberry extract contributed to important metabolic and hepatic benefits. This included reduced diet-induced weight gain and visceral obesity, reduced liver weight and triglycerol accumulation, blunted hepatic and intestinal oxidative stress and inflammation, increased insulin tolerance, decreased intestinal triglyceride content, and an increased proportion of Akkermansia (Anhê et al., 2015).

It has been previously and extensively shown that Res and Uro exert beneficial effects on skeletal muscle function, while the metabolic benefits of PAC have only recently begun to find establishment. Whether or not PAC polyphenols exert beneficial effects on the skeletal muscle contractility has yet to be explored.

Animal model

The protocol for this project was approved by the Comité Institutionnel de Protection des Animaux (CIPA) of UQAM (protocol reference number: 0619-974-0620). For this project, young male Wistar rats (Charles River, St-Constant, QC), approximately 4 weeks old, weighing between 100-125g were used. The rats underwent an acclimatization period of 4 to 7 days in the UQAM animal facility before being randomized into one of 3 groups: control; submitted to a powdered, regular chow (RC) diet, high fat; submitted to a high fat (HF) diet, and high fat plus PAC supplementation; submitted to the same HF diet, but supplemented with cranberry extract polyphenol proanthocyanidin (HF + PAC). Access to food was ad libitum for a period of 14 days during which, the animals were submitted to a 12-hour light and dark cycle beginning at 6:00. The rats were weighed at the same hour every second day in order to accurately assess changes in body mass. At the end of the 14-day protocol, the rats were fasted for a period of 2-4 hours, anesthetized with isoflurane gas (induction: 5%, maintenance: 3%) at 1 L/min of oxygen for the harvesting of tissues and blood, and then sacrificed. All procedures for this project were carried out with strict adherence to the National Institute of Health's guidelines for the care and use of laboratory animals, and all efforts were made in order to minimize discomfort to the animal

Diets

The caloric content for this diet was calculated based on the modified Atwater system, where proteins and carbohydrates contain 3.5 kcal/g, and fats contain 8.5 kcal/g. The powdered regular chow diet (Charles River Rodent Diet n° 5075, Cargill Animal Nutrition, MN, USA) had a caloric load of 2.89 kcal/g, with the macronutrient composition consisting of 55.2%

carbohydrates (65.6% kcal), 18% protein (21.4% kcal) and 4.5% fats (13% kcal). The HF and HF + PAC diets were prepared from pure ingredients according to a commercially available recipe (D12492, Research Diets Inc., New Brunswick, NJ, USA) with alternative Mineral and Vitamin mixes (CA.170915, CA.40060 respectively, Envigo Teklad Diets, Envigo, USA). The HF diets consisted of 5.24kcal/g, with macronutrient compositions consisting of 26.3% carbohydrate (20% kcal), 26.2% protein (20% kcal) and 34.9% fat (60% kcal). The HF +PAC diet followed the same formula (Table 4), with the addition of sufficient purified PAC to represent 0.47mg/0.4kg rat/day. This value was compared to a generous mass of 400g for a rat, resulting in the recommendation of 0.233mg PAC/day.

The carbohydrate sources in the HF diets included maltodextrin and sucrose (64.5% and 35.5% respectively), the protein sources included Casein and L-Cystine (98.5% and 1.5% respectively), and the fat sources included lard and Soybean oil (90.7% and 9.3% respectively). The diets also contained cellulose (64.6 g/Kg), calcium carbonate (7.1 g/Kg), dicalcium phosphate (16.8 g/Kg), potassium citrate (21.3 g/Kg) and choline bitartrate (2.6 g/Kg) as well as mineral (35 g/Kg) and vitamin (10 g/Kg) mixes (Table 4).

Table 4: Ingredient composition in g/KG of the HF and PAC diets. 6Kg was made for each diet

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

Metabolic chambers

The rats were housed individually in Oxymax CLAMS (Columbus Instruments, OH, USA) metabolic chambers for the duration of the 14 days. The temperature of the chambers was maintained at 22 ± 2 ºC. The O_2 and CO_2 sensors where calibrated every day for the first quarter of the protocol, after which point, we experienced recurring issues with the air pump, namely, that air was not accurately being pumped through the analyzers. After numerous unsuccessful attempts at solving the issue, it was determined that the pump itself was broken. It was therefore

turned off in order to minimize unnecessary noise pollution for the rats. Therefore, we were unable to collect sufficient nor reliable gas exchange data. As a result, we are unable to assess O_2 and CO_2 concentration calculations by calorimetry, the respiratory exchange ratios, nor the energetic expenditure, values which the equipment and software normally support. In each chamber, the food rests in a device on an individual scale (PL1502E, Mettler Toledo, Switzerland), designed in such a way to minimize food lost to random displacement by the rat, as well as foraging. The food was replenished, the rats were weighed and manipulated, and the cages were cleaned every second day.

Muscle contractility

The muscle contractility system (1205A, Aurora Scientific, Aurora, ON, Canada) was calibrated before every experiment with no weight, and 5g, 10g, and 20g weights. While the rat remained anesthetized, the extensor digitorum longus (EDL) and soleus (SOL) muscles were harvested from the right leg. Sutures were tied with Seraflex surgical silk (size 3.0; Serag-Wiessner, Naila, Germany) to each tendon, while carefully preserving muscle tissue integrity. The muscle was then mounted vertically between two parallel electrodes in a temperature-controlled, 32 °C Krebs solution bath. The silk thread attached to one end of the muscle was tied to a fixed arm at the bottom of the bath, while the silk thread at the other extremity was tied to the arm of a servo-motor (Figure 1). The optimal length of contraction (Lo) was determined by gradually adjusting the length of the muscle while administering 5Hz stimulations at 15 volts. Once obtained, this optimal length is recorded (both in mm and grams of force on the servo-motor) and used for the duration of the contractility procedures. A “pre” 120Hz stimulation was

then delivered to the muscle in order to further tighten the knots and assure their hold on the muscle. This stimulation, as well as each of the following stimulations were administered on the device's standard 80-volt setting. The muscle was then rested for a 15-minute period. The protocol then proceeded with a force-frequency interval, which included 600ms-long stimulations at 5Hz, 10Hz, 30Hz, 50Hz, 75Hz, 90Hz, 100Hz and 120Hz separated by 2-minute rests. After the force frequency protocol, the muscle was rested for 3 minutes, then underwent a fatigue protocol. This consisted of 50Hz stimulations to the EDL and 75Hz stimulations to the SOL every 2 seconds for a 3-minute period. The amount of time to observe a 50% reduction from the initial force value was identified as the muscle's fatigue resistance. The muscle was then rested for 3 minutes before undergoing a recovery protocol. The recovery protocol used the same frequencies as the fatigue, but stimulations were delivered every 90s for a 30-minute period. The recovery protocol indicates the percentage of the maximum strength (recorded during the fatigue protocol) the muscle was able to recover. All variables; maximum force, time to maximum (the time between the onset of contraction and the maximum force produced), and half relaxation time (the time required for a 50% reduction of the force following the peak of contraction) were obtained and determined using the DMC software (Aurora Scientific, Aurora, ON, Canada). Once all the simulations were complete, the muscles were removed from the apparatus, carefully dried, cleaned and weighed. The specific force (N/cm^2) was calculated for each muscle by dividing the product of the muscular force (g) and acceleration due to gravity ($9.81 m/s^2$) by the estimated cross-sectional area (CSA) of the muscle. The muscle CSA is estimated by dividing the mass of the muscle by the product of the density ($1.06 mg/mm^{-3}$) and the optimal length (L_o).

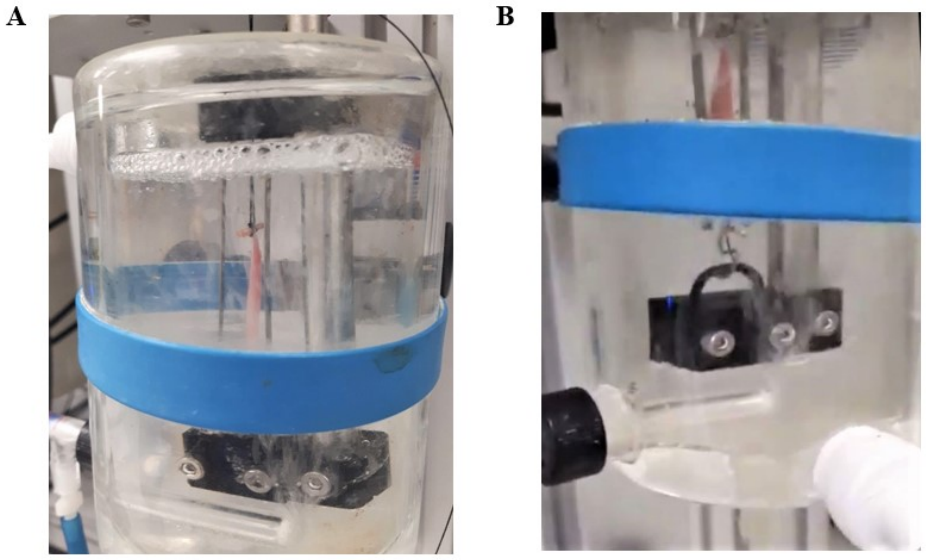

Figure 1: *EDL muscle mounted in contractility apparatus showing A; the upper tendon attaching above to the servomotor, B; the lower tendon fixed to the apparatus*

Fat deposits

Following the extraction of both the EDL and SOL muscles, blood was drawn from the heart, and the animal was terminated by cutting the heart away from the rest of the body. Fat stores were then collected from four sites: mesenteric, epididymal, extraperitoneal and subcutaneous, and weighed. The mesenteric fat pad consisted of the adipose tissue surrounding the GI tract, from the gastroesophageal sphincter to the end of the rectum. Special care was taken to distinguish and remove pancreatic cells. The epididymal fat pad included the adipose tissue surrounding the ureters and bladder. The extraperitoneal fat pad included the adipose deposits posterior to each kidney, and along the lumbar muscles. Finally, the SC fat was gathered from a rectangular region of skin extending from the median line of the abdomen to the spine, and the first right rib to the right hip.

Statistical analysis

Sample sizes were calculated based on our previous studies (Andrich, Melbouci, et al., 2018; Andrich, Ou, et al., 2018), and using power set at 0.8 (80%) and significance set at $p < 0.05$. All values are presented as means ± SD. One-way analysis of variance (ANOVA) statistical tests, with the diet as the main effect, were used to compare values between the 3 groups (RC, HF and PAC). Eta squared values indicate the portion of the variance accounted for by the diet groups. A value of 0.01 indicates a small effect size, 0.06 is medium, and +0.14 is large. A mixed linear regression model was used to analyze the curves for the EDL recovery and SOL fatigue protocols, while a quadratics test was used to analyse the curves for the EDL fatigue and SOL recovery protocols. Statistical analyses were performed using SPSS 24.0 (IBM Corporation, Armonk, NY, USA) and SAS Studio 3.5 (SAS Institute, Cary, NC, USA).

CHAPTER 3: Manuscript

Cranberry-derived polyphenol supplementation to mitigate the effects of a high-fat diet on skeletal muscle contractility in rats

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Abstract

Background: Polyphenol intake is gaining focus for its vast beneficial effects on organs throughout the body. Proanthocyanidin (PAC) is a cranberry-derived polyphenol shown to contribute to improved metabolic functions. Our group has previously shown that a short-term high-fat (HF) diets influences skeletal muscle quality as indicated by altered contractility. Other polyphenols such as Urolithins and Resveratrol were previously shown to improve skeletal muscle functions. The effects of dietary PAC on skeletal muscle function have yet to be explored.

Objective: The goal of this research project is to determine whether dietary PAC supplementation can prevent the impairments of a short-term HF diet on skeletal muscle contractility in young male rats.

Methods: Thirty young male Wistar rats (100-125g; *n*=10/group) were randomly submitted to a regular chow (RC) diet, a HF diet, or a HF + PAC-supplemented diet (PAC; 0.233mg/day) for 14 days, and with weight and food intake measured every two days. After two weeks, specific force, fatigue resistance and recovery from fatigue were measured in the *extensor digitorum longus* (EDL, glycolytic) and the soleus (SOL, oxidative) muscles using an *ex vivo* muscle contractility system. Visceral and subcutaneous (SC) fat stores were collected and weighed before the rat was terminated.

Results: Body weight was similar for all diet groups at the beginning of the experimental period, and statistically higher after 14 days in the HF group compared to the RC group ($P = 0.005$), but not compared to the PAC group. The RC and PAC groups were not significantly different in terms of body weight at the end of the 14 days. Animals treated with HF and PAC diets

displayed significantly higher ($P < 0.001$) caloric intake per day than those treated with RCD. The subcutaneous (SC) fat pad, and the sum of the visceral fat deposits were both significantly heavier (SC $P = 0.002$ and 0.02 respectively; Visceral $P < 0.001$) among the HF and PAC groups compared to the RC group, but not statistically different between the HF and PAC groups. No difference was noted for specific force and muscle fatigue. Significant differences ($P < 0.001$) were detected between the HF and PAC groups for recovery from fatigue in the EDL muscle only.

Conclusion: PAC supplementation influenced force recovery in the EDL muscle, while not affecting specific force or fatigue resistance. PAC supplementation also did not benefit weight gain, or body composition in comparison to the HF group.

Résumé

Contexte: Les polyphénols sont largement étudiés pour leurs vastes effets bénéfiques sur les systèmes de l'ensemble de l'organisme. La proanthocyanidin (PAC) est un polyphénol dérivé de la canneberge pouvant contribuer à l'amélioration des fonctions métaboliques. Notre groupe a déjà démontré qu'un régime alimentaire à court terme riche en lipides (HF) influence la qualité des muscles squelettiques, ce qui se traduit par une altération de la contractilité musculaire. D'autres polyphénols tels que les Urolithines et le Resveratrol ont déjà démontré qu'ils amélioraient les fonctions des muscles squelettiques. Les effets de PAC sur les fonctions des muscles squelettiques doivent encore être étudiés.

Objectif: Le but de ce projet de recherche est de déterminer si une supplémentation alimentaire en PAC peut protéger contre les effets d'un régime HF à court terme sur la contractilité des muscles squelettiques chez les jeunes rats mâles.

Méthodes: Trente jeunes rats Wistar mâles (100-125 g; *n*=10/groupe) ont été soumis aléatoirement à un régime de moulée (RC), à un régime HF, ou à un régime HF + PAC (PAC; 0.233mg/jour) durant 14 jours, avec le poids et la consommation alimentaire mesurés tous les deux jours. Après deux semaines, la force spécifique, la résistance à la fatigue et la récupération de la fatigue ont été mesurées dans les muscles *extensor digitorum longus* (EDL, glycolytique) et soléaire (SOL, oxydatif) avec un système de contractilité musculaire *ex vivo*. Les réserves de lipides viscérales et sous-cutanées (SC) ont été prélevées et pesées avant le sacrifice de chaque rat.

Résultats: Le poids corporel était similaire pour tous les groupes au début de la période expérimentale, mais statistiquement plus élevé après 14 jours dans le groupe HF par rapport au

groupe RC ($P = 0,005$), mais pas par rapport au groupe PAC. Les groupes RC et PAC n'étaient pas significativement différents en termes de poids corporel à la fin des 14 jours. Les animaux traités avec les régimes HF et PAC présentaient un apport calorique quotidien significativement plus élevé ($P < 0,001$) que ceux traités avec le RC. Des différences significatives ($P < 0,001$) ont été observées entre les groupes HF et PAC pour la récupération de la fatigue dans le muscle EDL uniquement. Aucune différence n'a été observée entre les groupes en ce qui concerne la force spécifique ou la résistance à la fatigue dans l'un ou l'autre des muscles. Le dépôt adipeux sous-cutané (SC), et la somme des dépôts lipidiques viscéraux était significativement plus lourd (SC $P = 0,002$ et 0,02 respectivement; viscéraux $P < 0,001$) dans les groupes HF et PAC que dans le groupe RC, mais pas statistiquement différente entre les groupes HF et PAC.

Conclusion: La supplémentation en PAC a influencé la récupération de la force dans le muscle EDL, tout en n'affectant pas la force spécifique ou la résistance à la fatigue. La supplémentation en PAC n'a pas non plus influencé la prise de poids ou la composition corporelle par rapport au groupe HF.

Introduction

Important advances in nutritional sciences and practical dietary recommendations have occurred over recent years. However, it is evident that much work remains to improve human diets as indicated by the ever-growing population of chronic diseases aggravated by poor or malnutrition, and skeletal muscle impairments. Individual differences, including lifestyle, age and gut microbial properties ought to be considered when exploring dietary approaches in order for the body to function optimally.

The gut microbial properties are a particularly interesting link for nutritional and health science research, as the gut interacts directly with the available dietary components, influences whole-body metabolism, and has recently been linked to a number of widespread health conditions (Adolph et al., 2018; Bindels & Delzenne, 2013; Forkosh & Ilan, 2019) including systemic inflammation (Fang et al., 2018) and diabetes mellitus (T2D; Delzenne & Cani, 2011), which maintain important links to skeletal muscle quality and function. Skeletal muscle quality can be indicated by contractility, which includes measurements of force generation, resistance to fatigue, and recovery from fatigue. Our group recently showed that a short-term (2-week) high fat (HF) diet was sufficient to cause alterations in skeletal muscle contractility among rats, indicated by a reduction in SOL muscle specific force (Andrich, Ou, et al., 2018). Harmful alterations were also observed in lipid metabolism, mitochondrial function, insulin sensitivity and oxidative stress within skeletal muscle tissue (Andrich et al., 2019; Andrich, Ou, et al., 2018; Lupien-Meilleur et al., 2019).

A growing area of interest in the area of nutritional sciences and gut (patho)physiology is the importance of prebiotic nutrients. Prebiotic nutrients include fermentable fibers and the

micronutrient polyphenols. Polyphenols are a group of biologically active compounds found in what are commonly classified as “functional foods”. This notion is grounded in the idea that biological activity extends beyond basic nutrition, which is represented by energetic nutrients. Polyphenols pass through the digestive tract in order to enter the body to exert their unique effects, such as the modulation of pro-inflammatory gene expressions (Carmela Santangelo, Rosaria Varì & Roberta Di Benedetto, 2007), induction of muscle hypertrophy (Rodriguez et al., 2017), decreasing hepatic and intestinal oxidative stress and inflammation, and improving insulin sensitivity (Anhê et al., 2015), as well as radical scavenging activity, lending to important antioxidant effects (Gülçin, 2006). However, the specific effects of each type of polyphenol have yet to be fully elucidated.

Promising data indicate that specific nutraceuticals modulate the gut microbiota to ultimately potentiate skeletal muscle function. This has given rise to the Gut Microbiota-Skeletal Muscle axis (Bindels & Delzenne, 2013; Yan et al., 2016): a platform on which to explore dietary substances’ implications as they extend beyond basic nutrition.

Resveratrol (Res), a polyphenol commonly found in grapes has been extensively researched for its effects on whole body metabolism (Sung, Byrne, et al., 2017), exercise performance (Dolinsky et al., 2012), muscle deconditioning (Mortreux et al., 2019) and gut microbiota composition (Etxeberria et al., 2015; Sung, Kim, et al., 2017). Res consumption has been shown to benefit skeletal muscle physiology, as well as cause an alteration in the gut microbiota, which in turn plays a crucial role in mediating beneficial effects on skeletal muscle exercise tolerance and glucose homeostasis (Sung, Kim, et al., 2017). Urolithin (Uro), derived from gut-mediated transformations of ellagitannins upon degradation of ingested pomegranate

has been shown to exert beneficial effects on tissue recovery, and acute ergogenic effects on muscle strength and soreness in the elbow flexors (Trombold et al., 2010, 2011). Pomegranate supplementation has also been associated with the preservation of muscle fiber integrity and muscle function in situations where inflammation and catabolism would be favored in humans (Bellone et al., 2018), as well as an improvement in muscle cell quality across the stages of life among mice (Ryu, 2016).

Supplementation with proanthocyanidins (PAC), a subclass of polyphenols with important concentrations in cranberries, apples and date fruit is associated with an alteration in energy metabolism by increasing the expression of genes such as PPARγ and PGC1α (Pajuelo et al., 2011), which contributes to increased skeletal muscle oxidative capacity. PAC is also associated with improved processes of muscle repair, and changing muscle fiber types (Mizunoya et al., 2017; Myburgh et al., 2012). Finally, PAC is reported for exerting potent prebiotic activity crucial for the exertion of benefits at the level of skeletal muscle (Anhê et al., 2015; Eid et al., 2014). Despite the improvements in metabolic functions previously observed for PAC, its effects on skeletal muscle contractile properties have never been investigated.

The gut microbiota-muscle axis is supported by Yan et al., (2016) who showed that the gut microbiota influences skeletal muscle function and phenotype. Polyphenols interact with the gut microbiota, and the gut microbiota has in turn been shown to be necessary to transform polyphenols and render their positive impact on skeletal muscle (Espín et al., 2007; King et al., 2006). The reported prebiotic effects for PAC are proposed to counter the detrimental effects of HF diets on skeletal muscle quality, facilitated by harmful alterations in the gut (Anhê et al., 2015; Bleau et al., 2015). Therefore, it can be hypothesized that PAC supplementation may

prevent the prompt impairments in skeletal muscle contractility previously observed to be induced by a HF diet (Andrich, Ou, et al., 2018). The present study intends to highlight the early impact of PAC as a supplementation to a HF diet on a number of contractile functions in glycolytic and oxidative skeletal muscle tissue.

Methods

Animal model

The protocol for this project was approved by the Comité Institutionnel de Protection des Animaux (CIPA) of UQAM (protocol reference number: 0619-974-0620). For this project, young male Wistar rats (Charles River, St-Constant, QC), approximately 4 weeks old, weighing between 100-125g were used. The rats underwent an acclimatization period of 4 to 7 days in the UQAM animal facility before being randomized into one of 3 groups: control; submitted to a powdered, regular chow diet (RC), high fat; submitted to a high fat diet (HF), and high fat plus proanthocyanidin supplementation; submitted to the same HF diet, but supplemented with cranberry extract proanthocyanidin (PAC). Animals were given unrestricted access to food for a period of 14 days during which, the animals were submitted to a 12-hour light and dark cycle beginning at 6:00. The rats were weighed at the same hour every second day in order to accurately assess changes in body mass. At the end of the 14-day protocol, the rats were fasted for a period of 2-4 hours, anesthetized with isoflurane gas (induction: 5%, maintenance: 3%) at 1 L/min of oxygen for the harvesting of tissues and blood, and then sacrificed. All procedures for this project were carried out with strict adherence to the National Institute of Health's guidelines

for the care and use of laboratory animals, and all efforts were made in order to minimize discomfort to the animal

Diets

The caloric content for this diet was calculated based on the modified Atwater system, where proteins and carbohydrates contain 3.5 kcal/g, and fats contain 8.5 kcal/g. The powdered regular chow diet (Charles River Rodent Diet n° 5075, Cargill Animal Nutrition, MN, USA) had a caloric load of 2.89 kcal/g, with the macronutrient composition consisting of 55.2% carbohydrates (65.6% kcal), 18% protein (21.4% kcal) and 4.5% fats (13% kcal). The HF and HF + PAC diets were prepared from pure ingredients according to a commercially available recipe (D12492, Research Diets Inc., New Brunswick, NJ, USA) with alternative Mineral and Vitamin mixes (CA.170915, CA.40060 respectively, Envigo Teklad Diets, Envigo, USA). The HF diets consisted of 5.24kcal/g, with macronutrient compositions consisting of 26.3% carbohydrate (20% kcal), 26.2% protein (20% kcal) and 34.9% fat (60% kcal). The HF +PAC diet followed the same formula (Table 5), with the addition of sufficient purified PAC to represent 0.47mg/0.4kg rat/day. This value was compared to a generous mass of 400g for a rat, resulting in the recommendation of 0.233mg PAC/day.

The carbohydrate sources in the HF diets included maltodextrin and sucrose (64.5% and 35.5% respectively), the protein sources included Casein and L-Cystine (98.5% and 1.5% respectively), and the fat sources included lard and Soybean oil (90.7% and 9.3% respectively). The diets also contained cellulose (64.6 g/Kg), calcium carbonate (7.1 g/Kg), dicalcium phosphate (16.8 g/Kg), potassium citrate (21.3 g/Kg) and choline bitartrate (2.6 g/Kg) as well as mineral (35 g/Kg) and vitamin (10 g/Kg) mixes (Table 5).

Table 5: Ingredient composition in g/KG of the HF and PAC diets. 6Kg was made for each diet

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

Metabolic chambers

The rats were housed individually in Oxymax CLAMS (Columbus Instruments, OH, USA) metabolic chambers for the duration of the 14 days. The temperature of the chambers was maintained at 22 ± 2 ºC. In each chamber, the food rests in a device on an individual scale (PL1502E, Mettler Toledo, Switzerland), designed in such a way to minimize food lost to random displacement by the rat, as well as foraging. The food was replenished, the rats were weighed and manipulated, and the cages were cleaned every second day.

Muscle contractility

The muscle contractility system (1205A, Aurora Scientific, Aurora, ON, Canada) was calibrated before every experiment with no weight, and 5g, 10g, and 20g weights. While the rat remained anesthetized, the extensor digitorum longus (EDL) and soleus (SOL) muscles were harvested from the right leg. Sutures were tied with Seraflex surgical silk (size 3.0; Serag-Wiessner, Naila, Germany) to each tendon, while carefully preserving muscle tissue integrity. The muscle was then mounted vertically between two parallel electrodes in a temperature-controlled, 32 °C Krebs solution bath. The silk thread attached to one end of the muscle was tied to a fixed arm at the bottom of the bath, while the silk thread at the other extremity was tied to the arm of a servo-motor (Figure 2). The optimal length of contraction (Lo) was determined by gradually adjusting the length of the muscle while administering 5Hz stimulations at 15 volts. Once obtained, this optimal length is recorded (both in mm and grams of force on the servo-motor) and used for the duration of the contractility procedures. A “pre” 120Hz stimulation was then delivered to the muscle in order to further tighten the knots and assure their hold on the muscle. This stimulation, as well as each of the following stimulations were administered on the device’s standard 80-volt setting. The muscle was then rested for a 15-minute period. The protocol then proceeded with a force-frequency interval, which included 600ms-long stimulations at 5Hz, 10Hz, 30Hz, 50Hz, 75Hz, 90Hz, 100Hz and 120Hz separated by 2-minute rests. After the force frequency protocol, the muscle was rested for 3 minutes, then underwent a fatigue protocol. This consisted of 50Hz stimulations to the EDL and 75Hz stimulations to the SOL every 2 seconds for a 3-minute period. The amount of time to observe a 50% reduction from the initial force value was identified as the muscle’s fatigue resistance. The muscle was then rested for 3 minutes before undergoing a recovery protocol. The recovery protocol used the

same frequencies as the fatigue, but stimulations were delivered every 90s for a 30-minute period. The recovery protocol indicates the percentage of the maximum strength (recorded during the fatigue protocol) the muscle was able to recover. All variables; maximum force, time to maximum (the time between the onset of contraction and the maximum force produced), and half relaxation time (the time required for a 50% reduction of the force following the peak of contraction) were obtained and determined using the DMC software (Aurora Scientific, Aurora, ON, Canada). Once all the simulations were complete, the muscles were removed from the apparatus, carefully dried, cleaned and weighed. The specific force (N/cm^2) was calculated for each muscle by dividing the product of the muscular force (g) and acceleration due to gravity ($9.81m/s^2$) by the estimated cross-sectional area (CSA) of the muscle. The muscle CSA is estimated by dividing the mass of the muscle by the product of the density ($1.06mg/mm^{-3}$) and the optimal length (L_o).

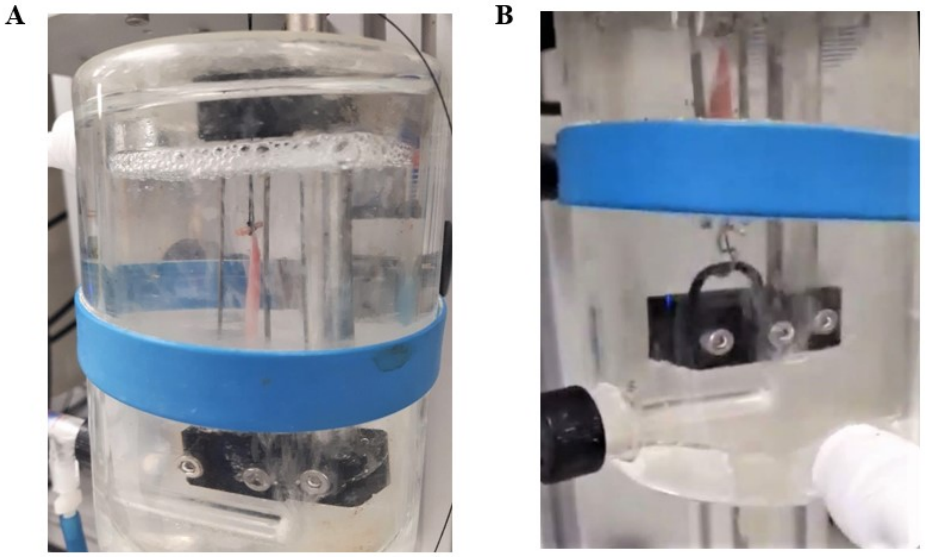

Figure 2*: EDL muscle mounted in contractility apparatus showing A; the upper tendon attaching above to the servomotor, B; the lower tendon fixed to the apparatus*

Fat deposits

Following the extraction of both the EDL and SOL muscles, blood was drawn from the heart, and the animal was terminated by cutting the heart away from the rest of the body. Fat stores were then collected from four sites: mesenteric, epididymal, extraperitoneal and subcutaneous, and weighed. The mesenteric fat pad consisted of the adipose tissue surrounding the GI tract, from the gastroesophageal sphincter to the end of the rectum. Special care was taken to distinguish and remove pancreatic cells. The epididymal fat pad included the adipose tissue surrounding the ureters and bladder. The extraperitoneal fat pad included the adipose deposits posterior to each kidney, and along the lumbar muscles. Finally, the SC fat was gathered from a rectangular region of skin extending from the median line of the abdomen to the spine, and the first right rib to the right hip.

Statistical analysis

Sample sizes were calculated based on our previous studies (Andrich, Melbouci, et al., 2018; Andrich, Ou, et al., 2018), and using power set at 0.8 (80%) and significance set at $P < 0.05$. All values are presented as means ± SD. One-way analysis of variance (ANOVA) statistical tests, with the diet as the main effect, were used to compare values between the 3 groups (RC, HF and PAC). Eta squared values indicate the effect size for the diet groups. A mixed linear regression model was used to analyze the curves for the EDL recovery and SOL fatigue protocols, while a quadratics test was used to analyse the curves for the EDL fatigue and SOL recovery protocols. Statistical analyses were performed using SPSS 24.0 (IBM Corporation, Armonk, NY, USA) and SAS Studio 3.5 (SAS Institute, Cary, NC, USA).

Results

Biometric parameters and food intake

Body weight was similar for all diet groups at the beginning of the experimental period (Table 6). Body weight was statistically higher after 14 days in the HF group compared to the RC group ($P = 0.005$), but not compared to the PAC group. The RC and PAC groups were not significantly different in terms of body weight at the end of the 14 days (Table 6). When considering caloric intake per day, animals treated with HF and PAC diets displayed significantly higher ($P < 0.001$) values than those treated with RC. However, there was no difference in energy intake per day between the HF and PAC groups (Table 6). The sum of the three visceral fat deposits (mesenteric, epididymal and extraperitoneal) were significantly heavier ($P < 0.001$) among the HF and PAC groups compared to the RC group, but not statistically different between the HF and PAC groups. The subcutaneous (SC) fat pad was also significantly heavier ($P = 0.002$ and 0.02 respectively) among the HF and PAC groups compared to the RC group, but not statistically different between the HF and PAC groups. The HF and PAC diets resulted in similar total sums of fat deposits, which were significantly heavier ($P < 0.001$) than those from the RC diet (Table 6).

The mean weight every two days for the HF and PAC groups is shown as a difference in weight from the RC group in Figure 3. Towards the end of the experimental protocol, there is a trend for the HF group to increase weight each day by nearly double the amount that the PAC group increased relative to the RC group.

Table 6: Biometric parameters and average food intake of RC, HF and PAC-fed rats

	RC	HF	PAC
Initial body weight (g)	172.95 ± 7.76	174.63 ± 6.07	173.52 ± 6.17
Final body weight (g)	303.65 ± 14.91	328.56 ± 16.00**	316.70 ± 16.90
Body weight gain (g)	130.70 ± 12.13	153.93 ± 12.95**	143.18 ± 16.73
Food intake (g/day)	27.80 ± 2.03	21.60 ± 2.22***	21.20 ± 1.65***
Food intake (kcal/day)	80.34 ± 5.86	113.17 ± 11.62***	111.07 ± 8.62***
Visceral fat (g)	8.50 ± 0.87	12.27 ± 1.87***	11.50 ± 1.24***
Subcutaneous fat (g)	3.40 ± 0.73	5.10 ± 1.21*	4.69 ± 0.89*

RC, regular chow; HF, high fat diet; PAC, proanthocyanidins-supplemented high-fat diet; *, significant difference with RC;
*: $P < 0.05$
**: $P < 0.01$
***: $P < 0.001$

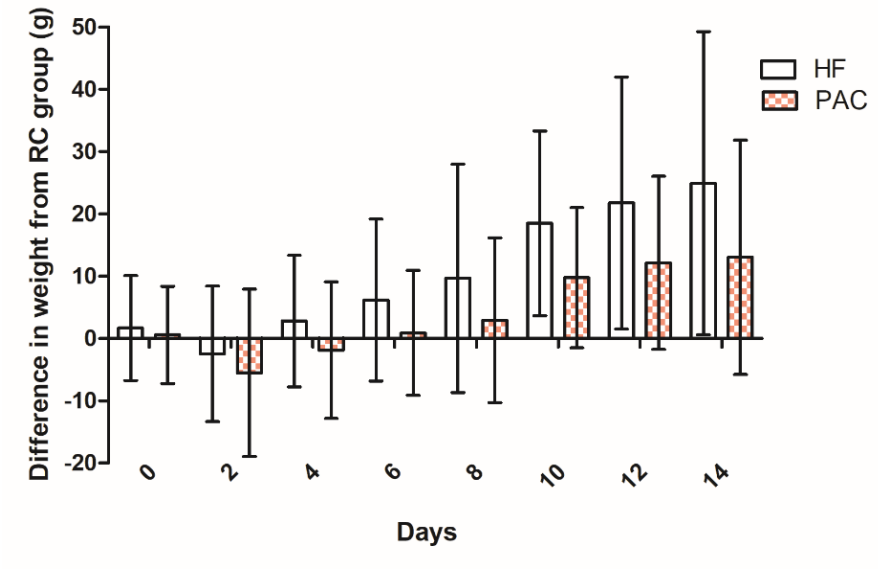

Figure 3: *Difference in weight of the HF and PAC groups compared to the RC group (represented by the x-axis). Results are presented as means ± SD for n= 10; HF, high-fat diet; PAC, proanthocyanidins-supplemented high-fat diet*

Skeletal Muscle Parameters and Contractility

Table 7: Skeletal muscle parameters

	RC	HF	PAC
EDL Length (mm)	3.23 ± 0.20	3.19 ± 0.16	3.22 ± 0.34
SOL Length (mm)	2.82 ± 0.16	2.82 ± 0.18	2.75 ± 0.30
EDL weight (mg)	138.30 ± 10.04	139.84 ± 12.50	138.66 ± 14.68
SOL weight (mg)	130.89 ± 14.39	141.48 ± 13.04	136.81 ± 13.77
EDL CSA (mm^2)	4.05 ± 0.30	4.14 ± 0.32	4.10 ± 0.54
SOL CSA (mm^2)	4.39 ± 0.54	4.75 ± 0.49	4.74 ± 0.60

RC, regular chow; HF, high fat diet; PAC, proanthocyanidins-supplemented high-fat diet

The length, weight and CSA of both the EDL and SOL muscles were similar in all groups (Table 7). Eta squared values indicated very small group effects for the length, weight and CSA of the EDL muscle, as well as the length of the SOL muscle. Medium to large effect sizes were recorded for the weight and CSA of the SOL muscle (η^2 = 0.099 and 0.094 respectively). Contractility tests showed similar specific force productions among all groups for both the EDL and SOL muscles (Figure 4A and 4B). Concerning further contractility tests, the focus will remain on differences between the HF and PAC groups. Both the HF diet, and the HF diet supplemented with PAC did not evoke any difference in the time to maximum contraction (Figure 4C and 4D), nor in the half-relaxation time (Figure 4E and 4F) in either muscle, however eta squared values for group on half-relaxation time were 0.122 in the EDL, and 0.083 in the SOL, indicating medium effect sizes.

Neither diet induced any difference in resistance to fatigue, as indicated by the time to reach 50% of maximal force during the fatigue protocol in the EDL muscle (Figure 5A), and the time to reach 60% of maximal force in the SOL muscle (Figure 5B). There was however, a

medium effect size (η^2 = 0.074) for resistance to fatigue in the SOL muscle only. There was no difference in the total decrease in force from beginning to end of the fatigue protocol in either muscle for either diet group (Figure 5C and 5D), and there was no difference in the slopes that represent the decrease in force across the fatigue protocol between the HF and PAC groups for each muscle (Figure 5E and 5F).

There was no significant difference in the force at the beginning of the recuperation protocol between the HF and PAC groups for the EDL nor the SOL muscles (Figure 6A and 6B), but in the EDL, the HF group maintained a significantly lower (P = 0.02) percent force at the beginning of the recovery protocol than the RC group (Figure 6A). Nonetheless, large effect sizes (η^2 = 0.252 EDL; and η^2 = 0.166 SOL) were recorded for the force at the beginning of the recuperation protocol in both muscles. In the SOL muscle, there was no difference in the recovery from fatigue between the HF and PAC groups, but they were both significantly lower (P < 0.01) than the slope of the recovery curve of the control (RC) group (Figure 6D). In the EDL muscle, there was a significant (P < 0.01) group effect for the recuperation, where PAC supplementation significantly (P < 0.01) increased force recovery when compared to the results obtained in the HF-diet group (Figure 6C). This shows that among the PAC-supplemented rats, more force was recovered on average, every 90 seconds between stimulations in the EDL muscle. However, the total force recovered at the end of the recuperation protocol was not significantly different between the PAC and HF groups for either muscle (Figure 6E and 6F) despite also maintaining medium to large effect sizes (η^2 = 0.190 EDL; and η^2 = 0.096 SOL).

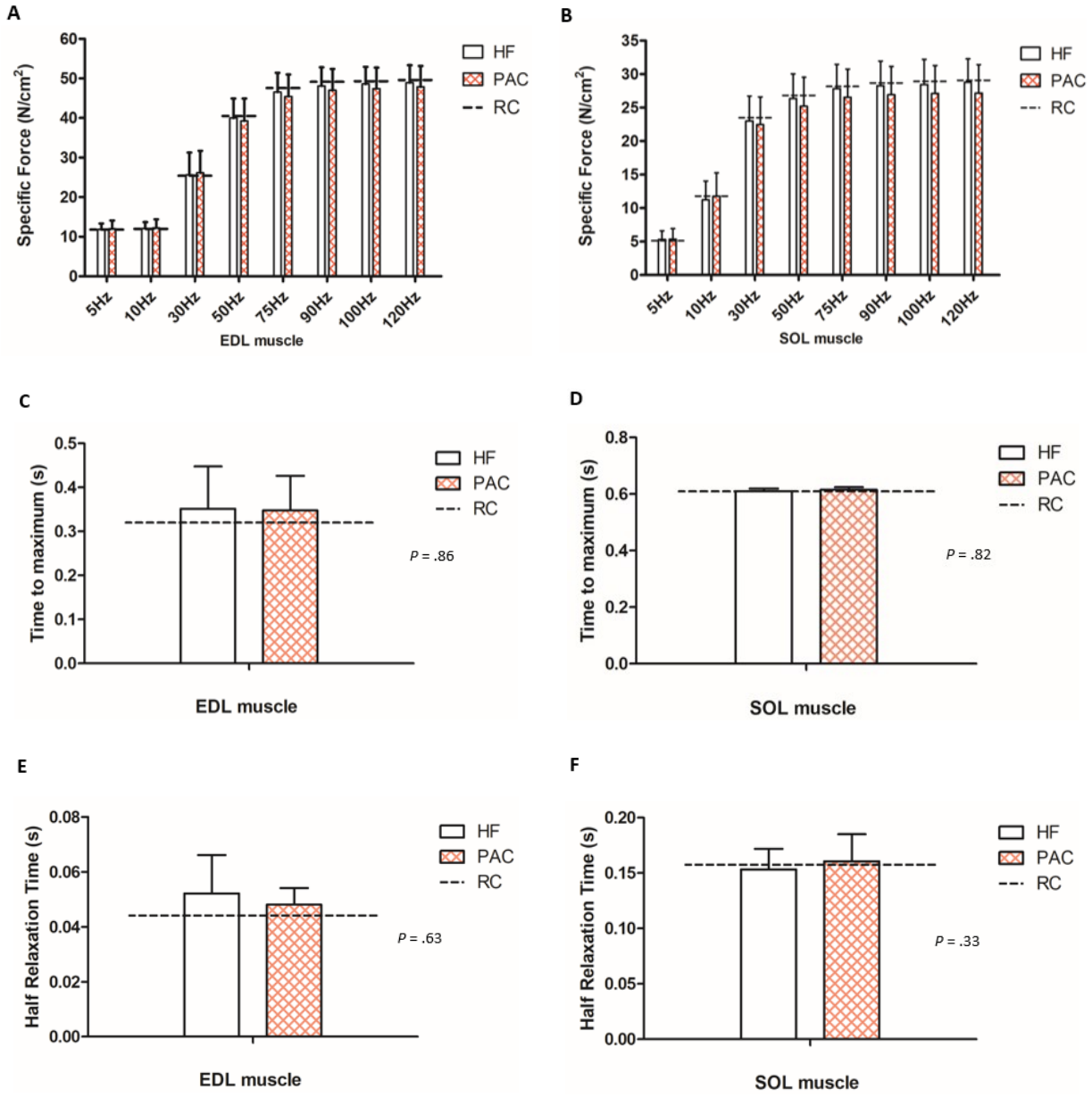

Figure 4: *Skeletal muscle specific force of EDL (A) and SOL (B) muscles of young rats submitted to HF or PAC (or RC) diets for 14 days. Time to maximum contraction in the EDL (C) and SOL (D) muscles, and half-relaxation time in the EDL (E) and SOL (F) muscles are shown. ANOVA statistical tests were used to compare values from the three diets groups, P-values displayed for the difference between HF and PAC groups obtained from Tukey's post-hoc tests. Results presented as means ± SD for n = 10. No significant difference between groups at P < 0.05*

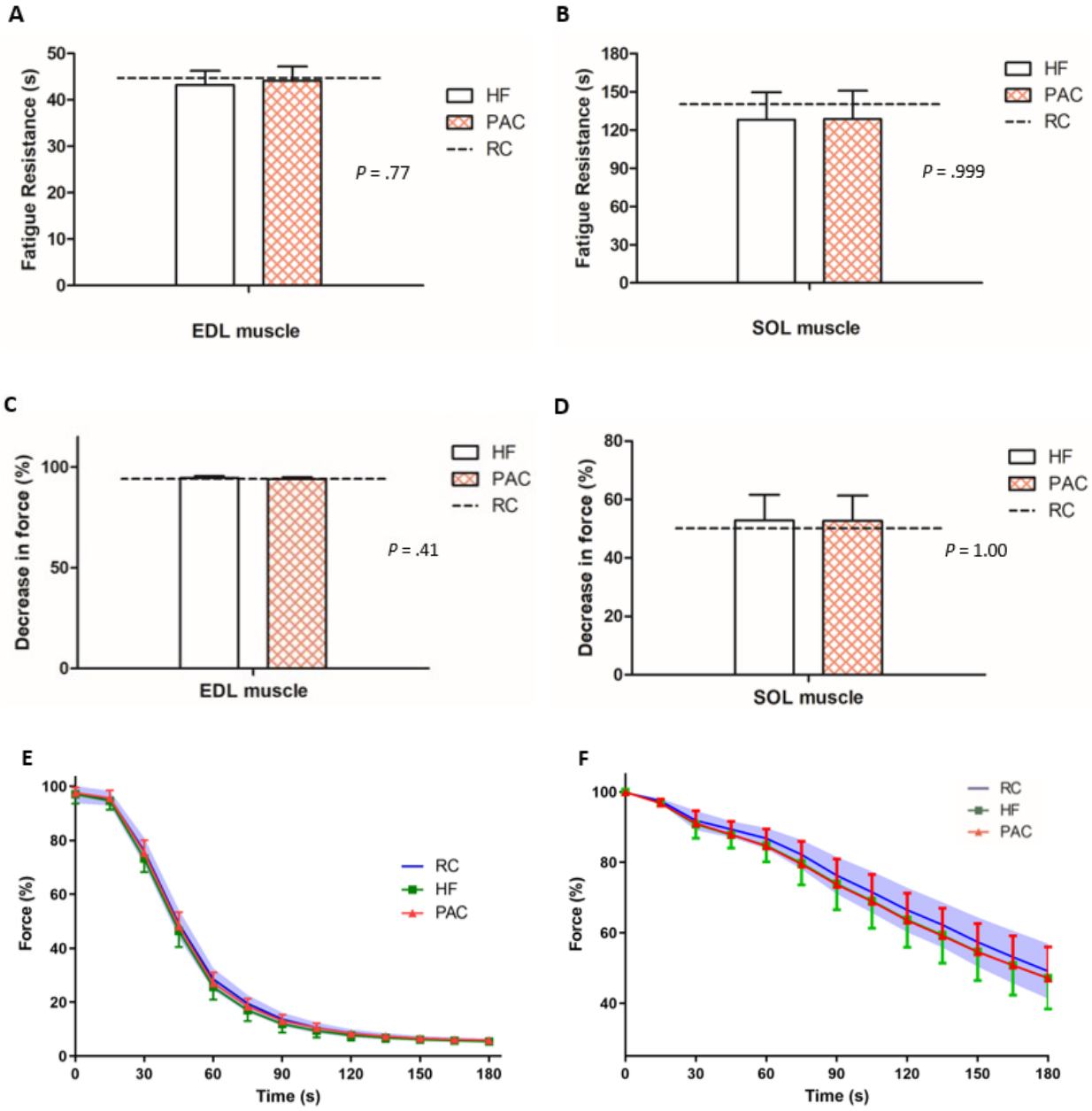

Figure 5: *Skeletal muscle fatigue resistance of EDL (A) and SOL (B) muscles of young rats submitted to HF or PAC diets for 14 days. RC group represented as horizontal dashed line to represent the mean of typically developing rats. Total decrease in force as a result of the fatigue protocol in the EDL (C) and SOL (D) muscles, fatigue curves in the EDL (E) and SOL (F) muscles. ANOVA statistical tests were used to compare values from the three diets groups, P-values displayed for the difference between HF and PAC groups obtained from Tukey's post-hoc tests, as well as quadratic and mixed linear regression models for the curves. Results presented as means ± SD for n = 8-10.*

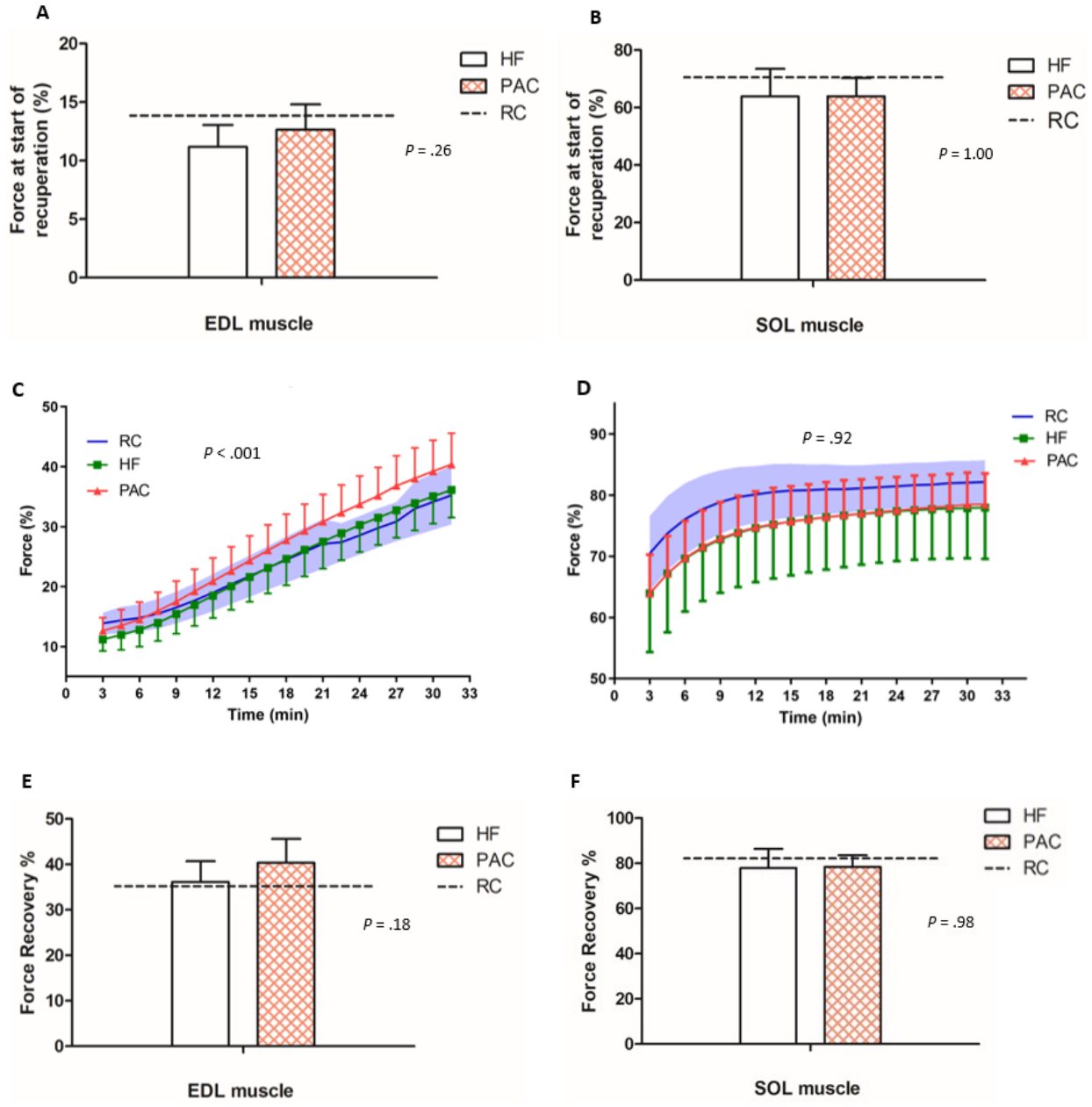

Figure 6: *Percent of force at the beginning of the recovery protocol for the EDL (A) and SOL (B) muscles of young rats submitted to HF or PAC diets for 14 days. RC group represented as horizontal dashed line to represent the mean of typically developing rats. Recovery curves in the EDL (C) and SOL (D) muscles, and total force recovered as a result of the recovery protocol in the EDL (E)and SOL (F) muscles are shown. ANOVA statistical tests were used to compare values from the three diets groups, P-values displayed for the difference between HF and PAC groups obtained from Tukey's post-hoc tests, as well as quadratic and mixed linear regression models for the curves. Results presented as means ± SD for n = 8-10.*

Discussion

Skeletal muscle contractility remains a key indicator of quality of skeletal muscle function. The present study intended to highlight the early impact of PAC as a supplementation to HF diets on a number of contractile functions in glycolytic and oxidative skeletal muscle tissue. This is the first study to evaluate the effects of PAC supplementation against the effects of a HF diet on skeletal muscle force, fatigue resistance and recovery. It had been previously shown that the gut microbiota influences skeletal muscle function and phenotype (Yan et al., 2016). The polyphenol PAC has been shown to improve metabolic functions by altering the gut microbiota (Anhê et al., 2015), which has been reviewed for a potential to improve skeletal muscle quality (Bleau et al., 2015). Our group previously showed that a short-term (2-week) HF diet caused harmful alterations at the level of the skeletal muscle (Andrich, Ou, et al., 2018). Therefore, given the alterations caused by a HF diet, the prebiotic impact of PAC on the gut microbiota, and the important gut microbiota-skeletal muscle axis, we aimed to identify whether PAC supplementation would alter the early adaptations to a short-term HF diet that have been previously observed for skeletal muscle contractility (Andrich, Ou, et al., 2018).

The present study demonstrates that PAC supplementation influences the recovery of force in the EDL (glycolytic) muscle of young rats submitted to 14 days of HF diets by way of significantly improving the slope of the recovery across the recuperation protocol. PAC supplementation did not benefit weight gain, or body composition compared to the HF group. The improved recovery of force in the EDL muscle is rendered especially interesting for a number of reasons. First, there was no significant difference between the PAC-supplementation and HF diet groups in force generation (Figure 4), in the extent of force reduction as a result of

the fatigue protocol (Figure 5C, E), nor in the resistance to fatigue (Figure 5A) in the EDL muscle. Second, there was no difference in the percent of force of the EDL muscle between HF and PAC groups at the beginning of the recuperation protocol (Figure 6A). Further, the percent of force recovered as a result of the recuperation protocol (at the end) was also similar in the HF and PAC groups (Figure 6E). This implies that the rate at which EDL contractile functions in the PAC group recovered was significantly increased over time despite no detection of a significant effect on percent force recovery amplitude at the beginning or end of our experiments.

Similar effects were observed by (Howatson et al., 2010), where isometric strength recovered significantly faster following strenuous exercise as a result of treatment with tart-cherry juice, among recreational marathon runners who consumed tart cherry juice 5 days prior to the marathon, the day of, and 48 hours after. Pomegranate-derived polyphenols have also been suggested to exert beneficial effects on muscle tissue recovery, indicated by an attenuation of strength reduction as early as 2 hours post-eccentric exercise among resistance trained men supplemented with pomegranate juice for 15 days (Trombold et al., 2011). An increase in membrane stability among myocytes mediated by polyphenols is one of the mechanisms suggested for the observation of improved tissue recovery (Trombold et al., 2011), and has not yet been explored for PAC. Reduced muscle damage during exercise recovery was additionally observed as a result of 500 mg green tea extract supplementation per day for 15 days among untrained men who performed exercises to induce delayed onset muscle soreness (da Silva, Machado, Souza, Mello-Carpes, & Carpes, 2018). This study examined plasma creatine kinase (CK) activity as an indicator of muscle damage, and observed lower CK activity in the green tea group, indicating that fewer fibers were damaged during exercise. Oxidative damage and

antioxidant status were unaltered by supplementation (da Silva et al., 2018). Unlike the damage inflicted on the muscle as a result of strenuous marathon running, and eccentric exercises, our design aimed to explore whether PAC administration could protect contractile properties against alterations caused by HF, low-quality diets.

Interestingly, the beneficial effects of PAC were observed in the EDL muscle, but not in the SOL muscle. The HF diet proved to be particularly harmful for force recovery compared to the control groups for both muscles, but the PAC-supplemented group only exhibited beneficial effects in the EDL muscle, which is glycolytic. Perhaps short-term PAC-supplementation drives protectives effects against HF diets in glycolytic tissue more readily than in oxidative. Alternatively, alterations caused by PAC against the effects of a HF diet in oxidative fibers may simply remain less detectable after only 2 weeks, especially considering the number of large effect sizes recorded among contractile properties in the SOL muscle.

In an exploration of recovery from repeated contraction in isolated mouse EDL muscles, Blackwood, Hanya and Katz (2018) substantiated that phosphorylase plays an important role in glycogen restoration during recovery. Glycogen phosphorylase is a key enzyme in the mobilization and use of muscle glycogen reserves, impeding the accumulation of glycogen and contributing to accelerated fatigue during a subsequent bout (Blackwood et al., 2018). The glycosidic derivative of Res has been identified as a potent glycogen phosphorylase inhibitor (Mavrokefalos, Myrianthopoulos, Chajistamatiou, Chrysina, & Mikros, 2015). Interestingly, resveratrol was recently shown to influence the protection and regulation of muscle glycogen in exercised and non-exercised (30 minute swimming procedure) rat models (Bicer, Baltaci,

Mogulkoc, Baltaci, & Avunduk, 2019). A recent finding for the effects of PAC treatment that might be related was improved insulin sensitivity in HF-fed mice (Anhê et al., 2015).

On another hand, autophagy, a cellular process that clears damaged organelles and aggregated proteins, is an essential process for skeletal muscle recovery (Call & Nichenko, 2020). Ryu (2016) reported that both autophagy and mitophagy gene expressions were greater in young worms supplemented with UroA, and that the ability to stimulate autophagy and mitophagy is conserved in mammalian muscle and intestinal tissue. Although these observations were for UroA and not PAC, this shows that these genes are potential targets for polyphenols, which would lend to similar observations for force recovery.

Many of the known effects of polyphenol activity on skeletal muscle tissue are specific to other polyphenols, however, given that similar observations to ours on force recovery in type II fibers are reported, a potential remains for the mechanism of action of PAC to maintain similarities. Nevertheless, the precise mechanism linking PAC consumption to recovery of force in the EDL muscle is beyond the scope of the present study. Our results only indicate that PAC supplementation to a short-term HF diet influences the contractile property of force recovery in 14 days.

The present study also shows that PAC administration prevents weight gain by maintaining no significant difference with the final weight of the RC group (Table 3). As expected, the final weight of the HF group was significantly different from the RC group. However, a gradient effect is observed when the final weight of the PAC group is compared to both other groups, as it is not significantly different from the final weight of the RC or the HF groups. Anhê and colleagues (2015) observed similar effects for their RC and HF-high-sucrose-

fed rats supplemented with cranberry extract, but their third group, HF-high-sucrose without supplementation gained significantly more weight than both groups. An important difference however, is that their study spanned 8 weeks, whereas ours only spanned two. The daily food intake in g/day, the daily energy intake in kcal/day, and the mass of fat pads maintained the same trend in the HF and PAC groups relative to the RC group (Table 3). In other words, both the HF and PAC groups took in significantly less grams of food than the RC group each day, but significantly more energy, and developed similar fat deposits while only the HF group developed a significantly different final mass than the RC group.

We analyzed resistance to fatigue and found no difference in the EDL or SOL muscles of HF and PAC-fed rats, but when exploring resistance to fatigue, we had to analyse the SOL muscle against a 40% reduction in force, compared to a 50% reduction in force of the EDL muscle, because a number of SOL samples did not reach a 50% decrease in force during the fatigue protocol. Other groups have observed a delay in the onset of fatigue, in situ, as a result of polyphenol ((-)-epicathechin from cocoa) treatment and treadmill running for 15 days in 1-year-old male mice muscle (Nogueira et al., 2011). However, our subset of samples of SOL muscles that did not observe a 50% decrease in force consists of samples from all three diet groups. Out of $n = 10$ in each group, 5 RC, 5 HF and 4 PAC muscles did not decrease to 50%. This observation lies with a potential flaw in our fatigue protocol as opposed to an effect of diet. When a 50% reduction was changed to a 40% reduction for the SOL muscle, the groups were evenly represented with only 1 sample from each not decreasing to 60% of maximal force within the fatigue protocol. Altogether, this suggests that for the SOL muscle, the fatigue protocol ought

to either be extended to 3.5 or 4 minutes in duration, or delivered with higher frequency (Hz) stimulation.

A strength of the current study was the rigor and consistency surrounding muscle tissue extraction. This is supported by the small standard deviations recorded for the specific forces, which also indicate the that protocol was well followed. Despite these measures for consistency, only two muscles in the whole rat were tested, and even though the SOL and EDL samples represent slow-twitch oxidative and fast-twitch glycolytic fibers, the findings of this study do not necessarily lend themselves to conclusions for all the muscles in the body. Further, in vitro muscle testing compared to in vivo, allows the potential for parameters such as bath temperature, extraction and frequency to affect the results. Furthermore, our study intended to build on our previous work, which compared the effects of a HF diet to a RC diet on contractile properties (Andrich, Ou, et al., 2018), and was modeled on the Anhê et al., (2015) study, which consisted of a RC, a HF-diet and a HF + cranberry extract supplemented group. Moreover, our model is of valuable for isolating the effects of PAC on contractile properties, and represents a good model for exploring the specific mechanisms associated with PAC and recovery in the future.

Another noteworthy feature is the concentration of PAC used in our study. It was adapted for a rodent model from an approximation of 35mg/day for a 60Kg human, which is an entirely reasonable amount that one can consume from food sources. The fat source in our diet is quite homogeneous however, which differs from the reality of a human diet. Finally, these results are evidently limited in their impact due to the lack of potential extrapolation from rodents to a human model. Nonetheless, this study leads to a number of important future recommendations. The first, is exploring the longer-duration effects of PAC on skeletal muscle function. This

includes functional aspects such as repair, satellite cell recruitment and hypertrophy, as well as fiber type-switching, metabolism and anti-inflammatory and anti-oxidant activity. A two-week, short term protocol is crucial in exploring the early adaptations to the treatment, especially among a youth model, as we have done, but a longer-duration model, such as 6 or 8 weeks, or numerous months allows the opportunity to explore more chronic adaptations such as those observed by Anhê et al., (2015) and Ryu, (2016). A longer-duration PAC-supplementation is especially supported by the medium to large group effect sizes observed for contractile properties whose difference did not reach statistical significance. Additionally, exploring the effects of PAC on skeletal muscle submitted to endurance, resistance or high intensity interval training will likely highlight important actions and effects as in the case with other polyphenols, such as flavanols (Nogueira et al., 2011), Uro (Trombold et al., 2011) and Res (Sung, Byrne, et al., 2017). The importance of combining polyphenols in future studies must also not be overlooked. As shown in Table 2, the majority of food sources contain a number of different phenolic compounds, with some containing polyphenols from differing classes as well. It is possible that polyphenols may act synergistically when ingested with others, which would enhance their beneficial effects. This is highly representative of a real human diet, and was recently observed for pomegranate with date fruit (Rosenblat et al., 2015), and Res with quercetin (Etxeberria et al., 2015). It was also briefly suggested by Nieman et al., (2017).

Although we did not measure gut bacteria, Wistar rats have been proven to be a particularly susceptible model to negative changes in the gut microbiota as a result a HF diet (Marques et al., 2016), and PAC supplementation has been proposed to be linked to prebiotic effects in the gut (Anhê et al., 2015), which lend themselves to improved skeletal muscle health

status relative to fatty model muscles (Yan et al., 2016). Our findings support the existence of the gut microbiota-skeletal muscle axis. However, in light of the existence of various gut-organ axes (Adolph et al., 2018; Fang et al., 2018; Forkosh & Ilan, 2019), there is potential for confounding interactions with other organs as a result of the prebiotic effects observed for PAC supplementation that must not be overlooked. Other gut-organ axes that might influence observations at the level of the skeletal muscle include metabolic organs such as the pancreas and the liver as a result of governing important processes related to skeletal muscle function such as insulin secretion and glucose storage. Moreover, important links have been reported between gut health status and systemic inflammation, which undoubtably influences skeletal muscle quality (Bindels & Delzenne, 2013). Therefore, future studies ought to explore the effects of PAC supplementation on other organs and inflammation in relation to skeletal muscle function.

In this study, we have highlighted important influences for PAC on glycolytic tissue recovery. This could prove especially advantageous in a context where a volume of exercise is executed and followed by a short recovery period before a subsequent performance, such as the case with hockey, tennis and soccer. In the future, an analysis of animal model behaviours, lipidomic, satellite cell function, fiber typing, insulin sensitivity and inflammatory status ought to be conducted in order to understand the mechanism of action of PAC-supplementation on skeletal muscle tissue.

Ideally, in a not-so-distant future, our working knowledge on the effects and mechanisms of actions of polyphenols will resemble that of vitamins, which would allow for isolation of purified forms, and/or production of synthetic forms. Similar to consuming a daily multi-vitamin,

when the effects are fully elucidated, humans could be able to supplement their diet with a multi-polyphenol; a poly-polyphenol tablet.

CHAPTER 4: Conclusion

There is currently a strong realization that polyphenol intake influences the functioning of organ systems throughout the body. One underexplored organ is the skeletal muscle. It is known that short-term diets that are high in excess fat contribute to harmful alterations at the level of the skeletal muscle. It is expected that by consuming polyphenols, skeletal muscle function and quality will benefit. PAC is a novel polyphenol to be explored for effecting contractility (force production, fatigue resistance and force recovery). The effects of PAC on skeletal muscle contractility were assessed against a short-term HF diet. This model considered a concentration of PAC that is realistic in terms of what can be consumed from a human diet. The primary outcomes measured are predominantly physical functions and effects.

There is much that remains be to explored on the topic of PAC supplementation to HF diets and skeletal muscle function that extends beyond the scope of this project. It is necessary to elucidate the physiological mechanisms behind the action of dietary polyphenols. If this project was to be followed up, it would be interesting to explore the effects of PAC supplementation among rats on behaviour changes, and more severe muscular and vascular injuries. It would also be worthwhile to explore the effects that result from a longer experimental protocol, such as 6 or 8 weeks, or numerous months. In this regard, an analysis of a wide-range of metabolic actions would contribute to a more complete understanding of PAC supplementation as well. Finally, a fourth experimental group, submitted to a RC diet supplemented with PAC could be included in order to compare the observed effects of PAC against a control.

Any work done that helps to understand the physiology of skeletal muscle function and influences, especially force recovery carries important clinical implications. Our results highlight recovery as an aspect of contractility influenced by PAC that may represent a crucial mechanism

for the optimization of skeletal muscle health. It will be crucial to identify and propose a mechanism that could link PAC supplementation to improved force recovery despite a HF diet. Even more so, it will be crucial to substantiate this mechanism among humans. As rats were the subjects in our model, future studies should explore the safety and effectiveness of PAC among humans.

Altogether, these findings will bring valuable information necessary for the development of improved dietary recommendations. These findings will also contribute to the growing body of research on polyphenols, and skeletal muscle function, as well as the gut microbiota-skeletal muscle axis. The ability to effectively use polyphenols to both protect and enhance skeletal muscle function in individuals will provide key advances in health and performance management.

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