Neuromechanical Measurement of the Effect of Carbohydrate Mouth Rinse on Human Performance in Strength and Elite Cycling Endurance

by

Matthew Jensen
BSc (Honours), University of Western Ontario, 2002

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University of Victoria

Supervisory Committee

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Abstract

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The overarching goal of this dissertation is to refine methods employed for assessing neuromuscular changes and associated power/force outputs during various perturbations of fatigue, direct or perceived, induced by either exercise or nutritional interventions, with associated performance outcomes.

To address this goal, we collected physiological and biomechanical data from subjects across a set of experiments designed to induce different levels of fatigue by the implementation of various exercise and nutritional interventions to cause various levels of fatigue in an ecologically valid manner. The data sets were collected during a single joint task and during cycling trials. During these experimental trials, we collected measures of kinetics (force and cycling power) as well as muscle activation (EMG) and physiological measures (heart rate, rating of perceived exertion, blood lactate, blood glucose, ventilation, oxygen uptake and carbon dioxide production) to investigate the overall performance, as well as potential mechanisms for improved performance related to the exercise and nutritional interventions.

In order to substantially enhance the collection of cycling kinetics and kinematics, we have developed an innovative sensor that improved the measurement resolution (temporal and spatial) of a commercial research grade power meter. Using these improved measures alongside advanced muscle activity analysis, we could ameliorate an experimental framework that could be used to investigate changes in fatigue and coordination pattern associated with exercise and nutritional interventions.

Investigation of the effects of a CHO mouth rinse vs. placebo on force and muscle activity during a very short (<3 min) neuromuscular demanding fatiguing trial demonstrated a consistent change in EMG median frequency related to increased fatigue in both experimental conditions, providing little evidence of change in neuromuscular strategy associated with CHO mouth rinse.

Further investigation explored the effects of a CHO mouth rinse vs. placebo using fundamental physiological measures of neuromuscular activation and overall performance measures during an ecologically valid late endurance cycling time trial. Our results demonstrated that while there was no overall effect noticed for time to completion, there was a significant decrease in performance in the time to complete various components of the time trial during the placebo trial only. Muscle activity of the lower leg (MG and SOL) demonstrated a modification in frequency only evident during the placebo condition.

Application of principal component analysis to power output and the EMG intensity profiles of the muscles of the lower leg during the pedal cycle revealed a more detailed understanding of the effect of CHO mouth rinse on performance during cycling. The average power output profile in WASH showed an earlier onset in the pedal cycle, greater duration and higher amplitude versus PLA during the TT. Additionally, only the PLA condition showed a significant increase in muscle activation throughout the time trial, which could be evidence of fatigue. This dissertation shows for the first time that CHO mouth rinse may have a substantial effect on the maintenance of power while mitigating the impact of neuromuscular fatigue, in late endurance performance, further strengthen our assertion that CHO may, in fact, minimize the changes in performance that are associated with fatigue during late endurance fatiguing events.

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Dedication

This work is dedicated to my family. You have made me stronger, better and more fulfilled than I could have ever imagined. I love you to the moon and back.

1 Introduction

The use of the macronutrient carbohydrate (CHO) to improve or maintain performance during athletic events has been the focus of a large body of research in the field of nutritional interventions in sport and exercise (Jeukendrup, 2004; Stellingwerff & Cox, 2014). Furthermore, a developing area of research has recently been meta-analyzed and even shows that a simple CHO mouth rinse appears to enhance performance during different exercise events (Peart, 2017), yet its effect and a potential mechanism for change in performance has yet to be fully elucidated. For example, CHO mouth rinse has been suggested to stimulate receptors in the oral cavity that activate areas of the brain associated with reward and pacing (Chambers, Bridge, & Jones, 2009). Explicitly, the areas of the anterior cingulate cortex, dorsolateral prefrontal cortex and ventral striatum, which mediate the behavioural and autonomic responses to rewarding stimuli, have been shown to be activated following CHO mouth rinse use (Chambers et al., 2009). The associated musculoskeletal neural activation following the use of CHO mouth rinse has been hypothesized to underpin the improvements in overall performance in athletic events where CHO mouth rinse has been studied, with most studies being endurance activity protocols of ~60 min (Peart, 2017; Stellingwerff & Cox, 2014).

This Chapter will introduce CHO mouth rinse, its potential underlying central/neural mechanisms and review the rationale and history of investigations into the effect of CHO mouth rinse on performance. Also, this Chapter will discuss the set of biomechanical tools and techniques that can be used to investigate changes in neuromuscular performance associated with this intervention.

1.1 Carbohydrate mouth rinse and fatigue

CHO supplementation during exercise contributes to performance in two ways 1) direct contribution of CHO energy via exogenous CHO oxidation (Jeukendrup, 2004; T. Thomas, Erdman, & Burke, 2016) and/or 2) mental/cognitive stimulation of the central nervous system (CNS) likely due to a stimulation of the pleasure and reward centers of the brain by CHO exposure to the oral cavity (Chambers et al., 2009). CHO feeding during prolonged strenuous exercise has been shown to postpone development of fatigue in trained individuals (Coyle et al., 1983). This delay in fatigue is because CHO is essential for muscle contraction and CHO depletion is linked to neuromuscular fatigue during exercise (Costill & Hargreaves, 1992). During prolonged exercise (>2 h), with or without CHO supplementation, CHO depletion will occur, with more muscle glycogen depletion occurring in favour of preserving hepatic glucose levels (McConell, Fabris, Proietto, & Hargreaves, 1994a); although liver glycogen depletion will also occur to some extent during exercise (Jeukendrup et al., 1999). As CHO supplementation research commonly employs an overnight fast, it is important to note that liver glycogen stores are also depleted after a single over-night sleep or short-term 8h fast (Maughan, Fallah, & Coyle, 2010). The amount of muscle versus liver glycogen depletion is complex and depends on the exercise intensity and exogenous CHO intake rates. Accordingly, Ataide-Silva et al. (2016) demonstrated the largest performance benefits of CHO mouth rinse specifically when subjects were both muscle and liver glycogen depleted, compared to fed or just liver glycogen depleted (via an overnight fast). As both glycogen stores start to deplete during prolonged exercise, the positive effect of CHO intake significantly increases performance in a progressively linear manner ($r = 0.356$; $P = 0.004$; (Stellingwerff & Cox, 2014)) and the potential performance improvement of a CHO mouth rinse may have added benefit (Stellingwerff & Cox, 2014). During performance situations with ever-increasing levels of glycogen depletion, there is evidence that muscle activation and power changes reflect a fatigued state more so than during non-depleted conditions (Ataide-Silva et al., 2016; Lane, Bird, Burke, & Hawley, 2012). This potential evidence of muscle fatigue and the concomitant decrease in power output is thought to be an internal neural protective strategy that results from reduced CHO and energy availability (Ataide-Silva et al., 2016). Fatigue has been shown to alter neuromuscular (NM) strategies during maximal performance; this includes the differential activation of muscles to limit force production to maintain fuel resources and limit tissue damage (St Clair Gibson, Lambert, & Noakes, 2001). For example, maximal voluntary knee extensions and leg cycling sprints result in a considerable amount of NM fatigue related to alterations in quadriceps activation, limiting performance (Billaut, 2011). Taken together, it is suggested that CHO mouth rinse may improve performance by modifying the perception and motor response to fatigue, which may be augmented with low endogenous CHO availability (liver and muscle glycogen), and thus allow athletes to work at higher power outputs compared to placebo conditions.

Importantly, most of the CHO mouth rinse strength/power activity based studies and endurance activity based studies did not utilize subjects in a fatigued state, where a decrease in performance may be due to central and/or peripheral mechanism(s) related to energy depletion (Allen, Lamb, & Westerblad, 2008). This is important, as central mechanisms of performance enhancement may be more readily evident in a fatigued state. Previously, Luden et al. (2016) reported that CHO mouth rinse used during a ~3 min TT, performed after 3 hours of exercise with no CHO ingestion, resulted in a 3.8% improvement in their time trial performance. Although it is essential to establish a potential improvement during late endurance exercise in a state where nutritional depletion and neuromuscular fatigue is prevalent, the level of nutritional depletion utilized by Luden et al. (2016) is not representative of real-world conditions, as some ingestion of

CHO is recommended to optimize performance during prolonged exercise situations (T. Thomas et al., 2016).

As noted above, it has been shown that CHO stimulates receptors in the mouth that activate areas of the brain associated with motor control and reward (Chambers et al., 2009). Accordingly, it has been suggested that CHO mouth rinse specifically, may be more effective when used in a post-absorptive overnight fasted state (when the oral receptors have a greater sensitivity to CHO) (Table 1-1) (Ataide-Silva et al., 2016; Lane et al., 2012; Trommelen et al., 2015). Fasting not only results in reduced liver glycogen content but is thought to sensitize CHO receptors and therefore result in a greater central drive to improve performance in response to CHO (Lane et al., 2012). Additionally, the effect of a CHO mouth rinse on performance has been reported to be greater where muscle and liver glycogen in diminished (Ataide-Silva et al., 2016; Kasper et al., 2016). Therefore, the majority of previous studies have incorporated an overnight fasting protocol designed to create a clean non-nutrition impacted study design, but also a CHO reduced state in subjects in an attempt to maximize the potential observed benefit of a CHO mouth rinse (Table 1-1). However, this study design lack full ecological validity, as endurance athletes do not skip breakfast before competitive situations.

1.2 Carbohydrate mouth rinse possible neural mechanisms

Neural imaging (fMRI) investigations into the effect of CHO mouth rinse on performance have shown that CHO in the oral cavity stimulates the pleasure and reward centres of the brain (Chambers et al., 2009). Additionally, Gant et al. (2010) found an immediate increase in maximum voluntary force following CHO ingestion and a larger change in motor evoked potentials (MEPs) in fatigued vs non-fatigued conditions. A significant finding of Gant et al. (2010) is that due to the time course of the effect, the immediate increase in voluntary force associated with CHO mouth rinsing could not have been associated with CHO metabolism in the stomach or intestines due to any inadvertent CHO consumption, but must be linked to the stimulation of oral receptors by CHO in the mouth. As stated previously, one possible mechanism for this short-lasting improvement is through the enhanced neural drive to motor units from a supraspinal source (Gandevia, 2001). Following a volitional contraction, the level of intensity for a subsequent maximal effort may be centrally inhibited based on afferent input to limit damage to muscle tissue and ward off energy depletion (St Clair Gibson & Noakes, 2004). This afferent input could result in the down-regulation of motor efferent commands resulting in decreased motor output (St Clair Gibson et al., 2001). Thus the CHO mouth rinse may act to attenuate centrally mediated inhibition of motor output possibly through afferent signals associated with CHO availability in the mouth (Gant et al., 2010). This finding is consistent with studies evaluating direct cortical measures during CHO use, demonstrating activation of anterior cingulate cortex and striatum immediately following CHO mouth rinse (Chambers et al., 2009). The authors attributed this activation to reward recognition and Gant et al. (2010) support an enhancement in corticospinal activation. This could suggest that while there are short-term benefits to a CHO mouth rinse combating centrally mediated fatigue, there may be a detriment to repeated maximal bouts or prolonged sustained endurance where accurate pace judgement is required.

1.3 Neuromuscular fatigue

Muscle fatigue, in the context of physical activity, can be defined as ‘any exercise-induced decrease in maximal voluntary force or power produced by a muscle or muscle group’ (Bigland-Ritchie, Jones, Hosking, & Edwards, 1978). Muscle fatigue can be divided further into peripheral or central fatigue. Peripheral fatigue refers to a loss of force caused by processes occurring at or distal to the neuromuscular junction (Gandevia, 2001), or in simpler terms fatigue within the muscle itself. Central fatigue represents the failure of the nervous system to drive the muscle maximally and is defined as a progressive exercise-induced reduction in voluntary activation or neural drive to the muscle (Gandevia, 2001). Supraspinal fatigue, specifically, is defined as an exercise-induced decline in force caused by the suboptimal output from the motor cortex (Taylor, Todd, & Gandevia, 2006).

The limiting factors of muscle fatigue have been debated since the model was introduced in the late 1880s by Angelo Mosso. Is performance limited by intrinsic properties of the muscles themselves (peripheral), or by the central nervous system (central) (Gandevia, 2008)? It is well known now that both contribute to overall fatigue, but the exact extent of each mechanism is still not well known and could depend on exercise intensity, duration, physical or mental fatigue state and fitness level of the subject (Enoka & Duchateau, 2008). The main issue surrounding this debate is the complexity of muscle fatigue. Muscle fatigue can refer to, in part, an individual's perception (St Clair Gibson et al., 2003) or decline in mental function (Lorist, Kernell, Meijman, & Zijdewind, 2002), and it can describe the gradual decrease in the force capacity of muscle and measured as a reduction in muscle force (Allen, 2001), and it can be inferred by a change in electromyographic activity (Kallenberg, Schulte, Disselhorst-Klug, & Hermens, 2007). During submaximal contractions, the body will aim to maintain force by altering physiological processes generating force as fatigue develops. This change will be noted in the muscle by two main neuromuscular strategies as 1) recruitment of additional motor units and, 2) adjustment of motor unit firing rates. These processes allow for measurement of the fatigue state of an individual to be measured through changes in performance variables (e.g. force output) together with measurements of specific changes in muscle activation through electromyography (EMG) (De Luca, 1997). The information that is regularly extracted from EMG is amplitude, timing, and frequency, and fatiguing exercise is most likely to alter all of these features.

1.4 Use of surface electromyography in the study of neuromuscular fatigue

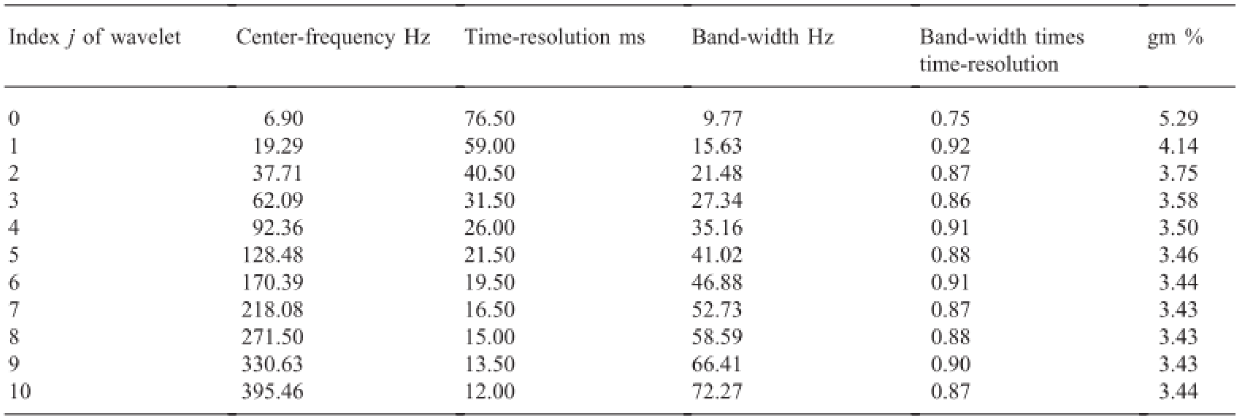
The use of EMG in the study of neuromuscular fatigue has been well established in static muscle contraction (Gerdle, Larsson, & Karlsson, 2000; Masuda, Masuda, Sadoyama, Mitsuharu, & Katsuta, 1999). Increasingly, investigators are exploring measurement of muscle fatigue during dynamic muscle contraction as this represents more realistic, functionally and performance relevant information (Hug & Dorel, 2009). EMG spectral parameters (mean and median frequency of EMG power spectrum) and EMG amplitude (mean and root mean square) are accepted measurements of muscle fatigue within static contractions.

1.4.1 Median frequency and amplitude of electromyography

A decrease in median frequency (MDF) of the EMG signal suggests a decrease in motor unit (MU) firing rate, MU recruitment and/or a decrease in conduction velocity (Billaut, 2011) and can be used as an index of fatigue (De Luca, 1997; Nagata, Arsenault, & Gagnon, 1990). Additionally, EMG root mean square (RMS) amplitude has been positively correlated with muscle force as greater MU recruitment, and higher firing rate contributes to an increase in the cumulative EMG amplitude (Karlsson & Gerdle, 2001; Larsson, Karlsson, Eriksson, & Gerdle, 2003). The amplitude of the EMG signal, however, does not indicate the high or low-frequency components of the signal. It is important to note that following fatigue, in conjunction with lower force production, EMG RMS may increase, while MDF may decrease (Gandevia, Allen, Butler, & Taylor, 1996). Therefore considering RMS alongside shifts in EMG frequency content is necessary to adequately account for the factors that alter these variables (De Luca, 1997).

1.4.2 Wavelet analysis of electromyography

EMG signals carry more information than what is usually resolved in RMS analysis, which includes the frequency of the signal that contains information pertaining to the pattern of muscle fibre activation. The frequency content of an EMG signal can be analyzed using the Fourier transform (e.g. in the calculation of MDF). However, this requires recording of the EMG signal over a substantial time period and can cause the timing of the muscle activity to be lost (Merletti & Lo Conte, 1997). Advanced approaches to EMG decomposition have been developed to allow the amplitude, timing and frequency content of an EMG signal to be resolved all at the same time (Figure 1-1) using non-linear scaled wavelets of specified resolution (Von Tscharner, 2000). Use of wavelet analysis allows analysis of both time and frequency content of the EMG signal facilitating evaluation of frequency changes in the context of muscle excitation duration (Von Tscharner, 2002; Wakeling, Pascual, & Nigg, 2002; Wakeling, Pascual, Nigg, & von Tscharner, 2001). Changes noted in the EMG signal in both frequency and time, correspond to modifications of muscle fibre recruitment strategies within the muscle (Wakeling, Uehli, & Rozitis, 2006). For example, a shift towards higher frequency content in EMG signal, without a change in intensity has been attributed to recruitment of fast twitch muscle fibres (Wakeling et al., 2006). Wavelet analysis has the advantage of being event and intensity oriented, meaning there is more detail with respect to the functional aspects of muscle activation compared to classical EMG analysis. During repetitive task activity such as cycling, wavelet-based analysis allows EMG signal to be resolved into time and frequency components for each pedal cycle. This provides sufficient detail that allows subtle changes in muscle activity pattern to be detected and has been used to show that the onset of higher frequency components occur at different points of the pedal cycle (Blake, Champoux, & Wakeling, 2012; Von Tscharner, 2002; Wakeling, Blake, & Chan, 2010).



Index *j* of wavelet	Center-frequency Hz	Time-resolution ms	Band-width Hz	Band-width times time-resolution	gm %
0	6.90	76.50	9.77	0.75	5.29
1	19.29	59.00	15.63	0.92	4.14
2	37.71	40.50	21.48	0.87	3.75
3	62.09	31.50	27.34	0.86	3.58
4	92.36	26.00	35.16	0.91	3.50
5	128.48	21.50	41.02	0.88	3.46
6	170.39	19.50	46.88	0.91	3.44
7	218.08	16.50	52.73	0.87	3.43
8	271.50	15.00	58.59	0.88	3.43
9	330.63	13.50	66.41	0.90	3.43
10	395.46	12.00	72.27	0.87	3.44

Figure 1-1: Wavelet parameters used for EMG Analysis. Adapted from "Intensity analysis in time-frequency space of surface myoelectric signals by wavelets of specified resolution," V. Von Tscharner, 2000, *Journal of Electromyography and Kinesiology*, *10*(6), p. 436. Copyright 2000 by Elsevier Science Ltd.

1.5 Carbohydrate mouth rinse effect on performance

Carbohydrate (CHO) is the primary substrate for high-intensity exercise (Hawley & Leckey, 2015), and CHO intake has been demonstrated to have a positive effect on short duration high-intensity (~3min), short duration high-intensity endurance (~1hr; 2.6%) and high-intensity long endurance (>2hr; 6.2%) performance (Stellingwerff & Cox, 2014). The impact of CHO supplementation on metabolism, and subsequent performance is multi-factorial and depends on; length and intensity of exercise, CHO intake rate, subject training status and type of exercise (Jeukendrup, 2004, 2010). Glycogen stores can become depleted during high-intensity/prolonged duration exercise (~75 to 90 min) and the current consensus statement recommendations are for the consumption of ~30-60 g CHO·h^{-1} (of ~4-8% CHO solution) during an endurance event (1-2.5 h) or up to 90 g CHO·h^{-1} during ultra-endurance events (>4 h) (T. Thomas et al., 2016). However, 30-50% of endurance athletes experience some level of gastrointestinal (GI) issues during endurance exercise resulting in significant challenges in meeting these levels of CHO intake (de Oliveira, Burini, & Jeukendrup, 2014). Furthermore, GI problems have been shown to be more severe and more likely to occur in athletes with a history of GI problems (Pfeiffer et al., 2012), with CHO intake itself (de Oliveira & Burini, 2014) and as exercise time increases (Peters et al., 1993). Therefore, CHO intake supplementation to support prolonged and intense endurance exercise will induce an ergogenic effect, but also may lead to GI upset causing an ergolytic effect. A potential solution to GI upset due to CHO ingestion is the use of CHO mouth rinse during exercise. Studies have shown significant improvement (13 of 21 studies) in high-intensity endurance exercise performance (~1 h) by rinsing the mouth with a CHO solution, without oral CHO consumption, compared to rinsing the mouth with a non-CHO solution (non-caloric; Table 1-1).

Prior to the initial CHO mouth rinsing study in 2004 (Carter, Jeukendrup, & Jones, 2004), it had been consistently shown that performance is improved when CHO is ingested during shorter endurance events (<1 h), when in fact only a small percentage of the ingested CHO would actually have been absorbed, transported and oxidized for ATP production by the muscle (Jeukendrup, Brouns, Wagenmakers, & Saris, 1997). This suggests that there is potential to obtain improvement or maintenance of performance during short duration high-intensity and endurance events through simple CHO exposure in the mouth, which may mitigate the complications of gastrointestinal distress associated with CHO ingestion. The following sections will highlight the 3 main exercise paradigms where CHO mouth rinse has been used as an ergogenic aid to improve performance, which are presented in Tables 1-1, 1-2 and 1-3.

1.5.1 Effect during glycogen non-limiting, short duration exercise (~60 min)

The performance effects of CHO mouth rinse have primarily been studied over short duration high-intensity cycling and running protocols lasting approximately 60 min, where muscle glycogen is not limiting to performance, with an average improvement in performance of 4.4% (90% CI [2.20, 6.64])(Table 1-1). It is important to note the average performance improvement when not including any time to exhaustion (TTE) protocols is 1.72% (90% CI [0.93, 2.64]). There have been improvements as high as 30.3% (Fraga et al., 2017) and on average an improvement of 16.3% (90% CI [7.18, 25.33]) when using CHO mouth rinse during TTE protocols, however TTE is known to be a highly variable test (CV>10%), especially in recreationally trained subjects, resulting in large effect sizes and percent change outcomes (Currell & Jeukendrup, 2008). However the fact 18 of 21 studies have found on average 1.72% improvement is important because the smallest worthwhile change in elite athletes to effect results has been suggested to be ~0.4% (Hopkins, 2004). Of the 21 studies included in Table 1-1, only 3 studies found an adverse effect on performance when using CHO mouth rinse; however, none of them was a significant decrement.

Table 1-1: Summary of all Carbohydrate mouth rinse studies during high-intensity short duration cycling and running, in varying fasted states.

Study	N	Subjects	Exercise	CHO Solution	Rinse Duration	Fasted State	Perf. Effect vs. Placebo	% Diff vs Placebo	$p \leq 0.05$
Carter et al. (2004)	9	ET, males (7) females (2)	~1-h cycling TT (914 kJ)	25ml of 6% GLU or non-caloric placebo (P) mouth rinse	5 s x 8	4hr Fast	59.57min (GLU) vs. 61.36min (P)	3.0%	Yes
Whitham and McKinney (2007)	7	RT males	15 min running warm-up at 65% $\dot{V}O_{2max}$ followed by 45 min running TT	~25ml of 6% GLU or 3% unsweetened lemon juice(P) mouth rinse	5 s x 10	4hr Fast	9333m (GLU) vs. 9309m (P)	0.3%	No
Rollo et al. (2008)	10	ET males	10min warm-up at 60% $\dot{V}O_{2max}$ followed by 30min treadmill running TT performance test	25ml of 6% GLU or non-caloric placebo (P) mouth rinse	5 s x 10	Overnight	6584m (GLU) vs. 6469m (P)	1.8%	Yes
Beelen et al. (2009)	14	ET males	Total cycling work done for ~1h TT at self-selected PO	25ml of 6% GLU or non-caloric placebo (P) mouth rinse	5 s x 8	2hr Fast stand. Breakfast	68.14min (GLU) vs. 67.52min (P)	-0.9%	No
Chambers et al. (2009)	8	ET males	~ 1-h cycling TT (914 kJ)	25ml of 6% GLU or non-caloric placebo (P) mouth rinse	5 s x 5	Overnight	60.4min (GLU) vs. 61.6min (P)	2.0%	Yes
Rollo et al. (2010)	10	ET males	1 hr treadmill running	25ml of 6% GLU or non-caloric placebo (P) mouth rinse	5 s x 4	Overnight	14298m (GLU) vs. 14086m (P)	1.5%	Yes
Pottier et al. (2010)	12	ET males	~1 hr cycling TT (975kJ)	25ml of 6% GLU or non-caloric placebo (P) mouth rinse	5 s x 8	3hr Fast	61.7min (GLU) vs. 64.1min (P)	3.8%	Yes

ET = Endurance Trained; RA = Recreationally Active; RT = Recreationally Trained; W_{max} = Maximum Workload; MIE = Moderate Intensity Exercise; HIE = High-intensity Exercise; CHOI = Carbohydrate Ingestion; CHOR = CHO mouth rinse; GLU = Glucose; P = non caloric Placebo

Table 1-1: (continued)

Study	N	Subjects	Exercise	CHO Solution	Rinse Duration	Fasted State	Perf. Effect vs. Placebo	% Diff vs Placebo	$p \leq 0.05$
Rollo et al. (2011)	10	ET males	1 hr treadmill running	25ml of 6.4% GLU or non-caloric placebo (P) mouth rinse	5s x 4	Overnight and Fed	14283m (CHOR) 14515m (CHOI) 14190 (P)	0.7% Wash 2.3% Ingest	No: Wash Yes: Ingest
Fares and Kayser (2011)	13	RT males	60% W_{max} until exhaustion Cycling (~55min)	25ml of 6% GLU or non-caloric placebo (P) mouth rinse	5-10 s x varying	3hr (FED) Overnight(FAST)	56.6 min (FED) vs. 54.7 min (P); 53.9 min (FAST) vs. 48.3 min (P)	FED: 3.4% FAST: 11.0%	Yes
Lane et al. (2012)	12	ET males	~1-h cycling TT at self-selected PO	20ml of 10% MALT or non-caloric placebo (P) mouth rinse	10 s x 8	Fed and Overnight Fasted	286 W (FED-MALT) vs. 281 W (FED-P) vs. 282 W (FST-MALT) vs. 273 W (FST-P)	FED: 1.8% FAST:3.6%	Yes
Gam et al. (2013)	10	ET males	~65 min cycling TT (1000 kJ)	25ml of 6% GLU or non-caloric placebo (P) mouth rinse	5 s x 8	4hr Fast	65.7min (GLU) vs. 67.6min (P)	2.9%	Yes
Sinclair et al. (2014)	11	RT males	30 min cycling TT	25ml of 6% GLU or non-caloric placebo (P) mouth rinse.	5 s x 5 10 s x 5	4hr Fast	155.6W (10 s) 152.4W (5 s) 145.7W (P)	10 s: 6.6% 5 s: 4.6%	Yes
Jeffers et al. (2015)	9	Male cyclists	45min at 70% Wmax followed by 15min TT (11min break)	25ml of 6.4% CHO non-caloric placebo (P) mouth rinse.	5 s x 9	4hr Fast	248W CHO 248W P	0%	No
Ispoglou et al. (2015)	7	Trained Male Cyclists	~1hr cycling TT	4,6,8% CHO or non-caloric placebo (P) mouth rinse	5 s x 8	3hr Fast	62min (P) 62.8min (4%) 63.4min (6%) 63min (8%)	4%: -1.3% 6%: -2.2% 8%: -1.6%	No
Trommelen et al. (2015)	14	Trained Male Cyclists	~1-h Cycling TT	6.4% Sucrose (S) or non-caloric placebo (P) mouth rinse	5 s x 8	Overnight(FAST) 2hr (FED)	68.6 min(FAST-P) 69.6 min (FAST-S) 67.6 min(FED-P) 69.0 (FED-S)	FAST: -1.5% FED: -2.1%	No

ET = Endurance Trained; RA = Recreationally Active; RT = Recreationally Trained; W_{max} = Maximum Workload; MIE = Moderate Intensity Exercise; HIE = High-intensity Exercise; CHOI = Carbohydrate Ingestion; CHOR = CHO mouth rinse; GLU = Glucose; P = Placebo

Table 1-1: (continued)

Study	**N**	**Subjects**	**Exercise**	**CHO Solution**	**Rinse Duration**	**Fasted State**	**Perf. Effect vs. Placebo**	**% Diff vs Placebo**	$p \le 0.05$
Devenney et al. (2016)	12	RA males	~1 h Cycling TT	6% or 16% CHO (MD) solution or non-caloric placebo (P) mouth rinse	5 s x 8	2-3 hrs	58.8min (6%) 57.9min (16%) 62.3min (P)	6% = 5.8% 16% = 7.3%	Yes CHO vs P, No 6% vs 16%
Bastos-Silva et al. (2016)	13	Physically Active Males	Test to exhaustion at MIE (80%) or HIE (110%) on cycle ergometer	6.4% CHO or non-caloric placebo (P) mouth rinse.	HIE: 10 s x 1 MIE: 10 s x 5	2hr Fast	MIE: CHO (76.6 min) P (65.4min). HIE: CHO (177.2s) P (163s)	MIE: 15.8% HIE: 8.4%	No HIE Yes MIE
Ataide-Silva et al. (2016)	8	Physically Active Males	30 min Constant load followed by 20km cycling TT	6.4% CHO or non-caloric placebo (P) mouth rinse.	10 s x 8	Overnight (FAST)	FAST 41.82 min P 43 min	2.8%	No
						2hr (FED)	FED 40.92 min P 40.7 min	0.5%	No
Kulaksiz et al. (2016)	9	RA males	20km cycling TT	3%, 6% or 12% CHO vs non-caloric placebo (P) mouth rinse	5 s x 7	Overnight	40.1 min (3%) 40.1 min (6%) 39.3 min (12%) 40.2 min (P)	3%: 0.3% 6%: 0.3% 12%: -2.3%	No
Fraga et al (2017)	6	ET males	Run to exhaustion at 85% $\dot{V}O_{2max}$	8% Rinse, 6% ingestion vs non-caloric placebo (P) mouth rinse	10 s x varying	Overnight	43.7 min (CHOR) 43.0 min (CHOI) 32.2 min (P)	MR: 30.3% Ingest: 28.8%	Yes
James et al. (2017)	11	Competitive male cyclists	~1-h Cycling TT (844 kJ)	7%, 14% CHO vs non-caloric placebo (P) mouth rinse	5 s x 8	Overnight	57.3 (7%), 57.4(14%) 59.5 (P)	7%: 3.8% 14%: 3.6%	Yes

ET = Endurance Trained; RA = Recreationally Active; RT = Recreationally Trained; W_{max} = Maximum Workload; MIE = Moderate Intensity Exercise; HIE = High-intensity Exercise; CHOI = Carbohydrate Ingestion; CHOR = CHO mouth rinse; GLU = Glucose; P = Placebo

1.5.2 Effect during glycogen limiting conditions

Depending on the subjects fitness exercise intensity, substrate availability and duration it is generally thought that glycogen can become limiting to performance in ~75 to 90min of sustained intense exercise (Impey et al., 2018). Thus, CHO ingestion is required for long duration events (>90 min) to attenuate glycogen depletion and is generally accepted that it will improve prolonged endurance capacity (Coyle et al., 1983; Jeukendrup, 2010; Romijn et al., 1993). However, GI symptoms impacting performance are more likely to intensify once CHO ingestion rates increase and time of event increases (Peters et al., 1993; Pfeiffer et al., 2012). The use of CHO mouth rinse during late endurance exercise is in its infancy, with just a single study; however, early findings suggest CHO mouth rinse may be beneficial (Luden et al., 2016), which has potential importance for athletes affected by GI upset following CHO ingestion. CHO mouth rinse has the potential to limit GI distress, but also increase performance during long-duration exercise/events (>90 min). It is interesting to note that the effect of CHO mouth rinse has been evident when glycogen depletion is more prevalent as it is in a late endurance performance or when CHO availability is limited prior to exercise (Table 1-2). For example, Ataide-Silva et al. (2016) showed that there was only evidence of beneficial effect of CHO mouth rinse during fasted and depleted state, with no performance gains when in a fed state. This suggests that CHO mouth rinse may have an effect on the fatigue state of performance related to CHO depletion. This could indicate that the effect of CHO mouth rinse is magnified under conditions of CHO depletion. To date, only 4 studies have looked at the impact of CHO mouth rinse on performance during a glycogen reduced state, compared to the ~21 studies performed during short duration high-intensity exercise. Of the 4 studies, 2 have found a significant impact on performance, with one of them being a TTE protocol (21.7%). While Luden et al. (2016) did not find a significant increase in performance, they did find that CHO mouth rinse would ‘likely’ enhance performance versus placebo, with a 3.8% improvement in a 2-km time trial while in a reduced glycogen state. Future research needs to be performed in this area before a CHO mouth rinse strategy should be recommended for the later stages of a race, and research is conducted with greater ecological validity that mimics typical CHO ingestion during a race.

Table 1-2: Summary of carbohydrate mouth rinse studies that utilized a glycogen reducing exercise protocol.

Study	N	Subjects	Depletion Protocol	Exercise	CHO Solution	Rinse Duration	Fasted State	Perf. Effect vs. Placebo	% Diff	$p \leq 0.05$
Ali et al. (2016)	9	RT male cyclists/ triathletes	30 min cycling @ 70% PP/ 3 x 50s print/ 45 min @ 70% PP	~1-h Cycling TT	15% CHOR, 7.5% CHOI vs PLAR/I. CHO intake rate = 65.4 ± 6.6 (g/hr)	8s x 8	Overnight	CHOR 68.4 min PLAR 68.3 min	-0.15%	No
								CHOI 65.3 min PLAI 68.7 min	5.07%	Yes
Kasper et al. (2016)	8	RA males	Exhaustive running evening prior to test 45 min at 65% $\dot{V}O_{2max}$ day of test	1 min HIT running/1 min walking	10% CHO or non-caloric placebo mouth rinse.	10s @ 4 min interval	Overnight	CHOR 52 min PLAR 36 min	36.4%	Yes
Luden et al. (2016)	8	Trained male cyclists	120min 55% W_{max}, 30km TT, 15 min rest (MVCs), 10min 35-55% W_{max}	2km TT	6.4% CHO or non-caloric placebo mouth rinse.	5s x 3	Standardized Breakfast 2 hr prior	CHOR 192.4 s PLAR 200.1 s	3.92%	No
Ataide-Silva et al. (2016)	8	Physically Active Males	90 min @ 70% PPO, 6x1min @ 125% PPO	30 min Constant load followed by 20km cycling TT	6.4% CHO or non-caloric placebo mouth rinse.	10s x 4	Depleted (DEP)	DEP 46.3 min PLA 48 min	7.08%	Yes

ET = Endurance Trained; RA = Recreationally Active; RT = Recreationally Trained; CHOI / PLAI = Carbohydrate Ingestion / Placebo Ingestion; CHOR / PLAR = Carbohydrate Rinse / Placebo Rinse; HIT = High-intensity; PPO = Peak Power Output

1.5.3 Effect during maximal strength, power and sprint exercise

In recent years, the number of studies examining the use of CHO mouth rinse as an ergogenic aid during maximal strength/power/sprint type exercise has increased (Table 1-3). However, the results of these studies have produced mixed results, both supporting (11.8% average improvement, n=7) and refuting (-0.6% average decrease, n=8) the positive effect of CHO mouth rinse. This is unlike the 18 out of 21 studies predominately reporting positive outcomes when CHO mouth rinse is used during high-intensity cycling/running studies. Recent work by Decimoni et al. (2018) who looked at the effect of CHO mouth rinse on resistive training hypothesized that the effect is only observed when higher exercise volumes/longer duration are utilized. This may be due to the fact the duration of the exposure to fatiguing stimuli may need to accumulate for the CHO mouth rinse to activate the dopaminergic pathways of the ventral striatum that affects the reward/motor functions of the basal ganglia, which may counteract the effects of fatigue (Chambers et al., 2009). However this hypothesis does not explain why during cycling sprint (<45 s) performance protocols using CHO mouth rinse, a greater increase in initial power output has been observed, however with a greater decrement in power over time when compared to a non-CHO mouth rinse (Beaven, Maulder, Pooley, Kilduff, & Cook, 2013; Dorling & Earnest, 2013). This short-term benefit of CHO mouth rinse would not induce the extended fatigue stimuli needed to activate the dopaminergic pathway; therefore, other central mechanisms/factors must be activated by CHO to induce short-term performance benefits. This short-term benefit of CHO mouth rinse seems evident for repeated efforts; however, to date, no studies have investigated the effect of CHO mouth rinse during short duration maximum strength/power following an acute fatiguing protocol and repeated bouts. Measurement of the use of CHO mouth rinse in this context may be of value in athletic events that require maximum repetitive exertions such as during jumping or sprinting events. The current research has yet to provide any evidence that CHO mouth rinse is detrimental to performance during maximal strength/power/sprint type exercise, since only 2 studies showed a decrease in performance, however not significant. Another 3 studies found a 0% (null) improvement, which doesn’t support CHO mouth rinse as a performance enhancement but also doesn’t portray it as being detrimental. The type of test protocols utilized for the studies presented in Table 1-3 cover a wide range, and the type of subjects are equally varying. This might also explain the mixed results when using CHO mouth rinse to increase maximal power.

Table 1-3: Summary of carbohydrate mouth rinse studies during maximal strength/power/sprint type exercises in varying fasted states.

Study	N	Subjects	Exercise	CHO Solution	Fasted State	Rinse Duration	Perf. Effect vs. placebo	% Diff	*$p \leq 0.05$*
Chong et al. (2011)	14	Male Cyclists	30-s maximal cycling sprint	6.4% Malt, 7.1% GLU or non-caloric placebo (P) mouth rinse	Overnight	5 s	Peak Power P: 1203W GLU: 1189W MALT: 1191W	-1.4%	No
Painelli et al. (2011)	12	RT Strength Males	Maximum strength testing (1RM) and 6 sets until failure at 70% of 1RM	25ml of 6% GLU or non-caloric placebo (P) mouth rinse	8hr Fast	10-15 s varying	101kg (GLU) vs. 101kg (P)	0%	No
Beaven et al. (2013)	12	RT Males	5 x 6 s sprints with 24 sec recovery on a cycle ergometer	25ml of 6% GLU or non-caloric placebo (P) mouth rinse	2hr Fast	5 s x 6	Sprint 1: GLU +39W vs. P	N/A	Yes
Bortolotti et al. (2013)	9	Under 15 soccer players	Repeated Sprints, 6x40m	6% 100ml	NA	10 s x 1	No difference	0%	No
Dorling and Earnest (2013)	8	RA Males	Repeated sprint ability tests (LIST)	25ml 6.4% MALT or water (P)	Fasted (Time NA)	5 s x 27	0.8% smallest worthwhile effect using 90% CI	0%	No
Chong et al. (2014)	12	Competitive Male Cyclists	45s Cycling Sprint	10% GLU, 9% MALT or non-caloric placebo (P) mouth rinse	Overnight	5 s x 11	10%: 1188W 9%: 1042W P: 1036W	10%: 16.7% 9%: 0.6%	Yes 10%GLU
Phillips et al. (2014)	12	RA Males	30s cycling ergometer sprints @ 0.075 g/kg	8 x 5s rinses with 25ml of 6% CHO or PLA)	2hr Fast	5 s x 8	Peak Power Output 13.51 W/kg (CHO) vs 13.2 W/kg (PLA)	2.3%	Yes
Rollo et al. (2015)	11	Male soccer players	Loughborough Intermittent Shuttle Running Test (LIST)	25ml of 10% GLU or non-caloric placebo (P) mouth rinse	3hr Fast	10 s x 11	0.2s set as smallest worthwhile change. Chance of beneficial, negligible or detrimental was 86%, 10% and 4% respectively	0.8% 90% CI	No

GLU = Glucose; MALT = Maltodextrin; ET = Endurance Trained; RA = Recreationally Active; RT = Recreationally Trained; CMJ = Countermovement jump; IMTP = Isometric mid-thigh pull; BP = Bench Press

Table 1-3: (continued)

Study	N	Subjects	Exercise	CHO Solution	Fasted State	Rinse Duration	Perf. Effect vs. placebo	% Diff	$p \leq 0.05$
Bastos-Silva et al. (2017)	12	RT Strength Male	Training Load Volume (TLV), Leg Press, Bench Press	6.4% GLU or non-caloric placebo (P) mouth rinse	2hr Fast	10 s	CHO increased Rep and TLV only during BP vs Control	17% BP 13% TLV	Yes
Bazzucchi et al. (2017)	18	Young Men	3 x MVC pre, 5x30 isokinetic fatiguing contraction, 1 MVC post	6.4% GLU, MALT, non-caloric placebo and no rinse control	Overnight	10 s	Total Work GLU: 4316J MALT: 4249J P: 3853J	GLU: 11.3% MALT: 9.8%	Yes
Clarke et al. (2017)	12	Healthy Males	CMJ height, IMTP PF, 10m sprint, BP and back squats	6% CHO solution, non-caloric placebo mouth rinse or no rinse	Overnight	10 s before each exercise	Improved CMJ height, 10m sprint, BP and squats		CMJ, 10m, BP, Squat = Y IMTP = N
Dolan et al. (2017)	10	College Male Athletes	Yo-Yo Intermittent Recovery Test	6% CHO solution or non-caloric placebo (P) mouth rinse	Overnight	10 s	CHO: Level 37 P: Level 35	NA	No
Dunkin and Phillips (2017)	12	RT Males	BP 1rep max (RM), followed by repetitions till failure (@40% 1RM)	25mL 18% CHO or non-caloric placebo (P) mouth rinse	2hr	10 s x 2	NA	NA	No
Krings et al. (2017)	14	Healthy Males	5 x 15s maximal cycling sprint	50mL 10% CHO or non-caloric placebo (P) mouth rinse	NA	10 s x 6	10%: 646W P: 656W	-1.5%	No
Decimoni et al. (2018)	15	RT Women	3 sets resistance exercise bouts with 10 repetitions	6% MALT or non-caloric placebo (P) mouth rinse	Overnight	10 s	Total Workload MALT: 7589 P:6678	12%	Yes

GLU = Glucose; MALT = Maltodextrin; ET = Endurance Trained; RA = Recreationally Active; RT = Recreationally Trained; CMJ = Countermovement jump; IMTP = Isometric mid-thigh pull; BP = Bench Press

1.5.4 Carbohydrate mouth rinse dose response

The CHO concentration level of the mouth rinse required to elicit a positive effect on performance has been investigated (Ispoglou et al., 2015; James et al., 2017; Kulaksız et al., 2016; Wright & Davison, 2013). James et al. (2017) showed that competitive male cyclists using a mouth rinse with CHO concentration levels of 7% and 14% did better than placebo mouth rinse, but that any increase in concentration levels did not have any further effect on cycling time trial performance. In contrast, Ispoglou et al. (2015) showed no cycling TT performance differences with CHO mouth rinse concentrations of 4, 6 and 8% versus a non-caloric placebo condition (0%). However, it is important to note that Ispoglou et al. (2015) employed only a 3 hr pre-activity fast, whereas the testing protocol of James et al. (2017) included participants that were overnight fasted. It is interesting to note that the majority of CHO mouth rinse studies have used a CHO concentration level of 6.4% for their rinse solutions. This might be due to the fact that this is the CHO concentration commonly used in sports drinks consumed by athletes (Gatorade, Lucozade). Given the conflicting studies, whether there is a CHO% dose-response remains to be further examined. However, there also seems to be a dose-response relationship with total mouth exposure, as suggested by Sinclair et al. (2014) that looked at the effect of CHO mouth rinse duration of either 5 s or 10 s during a 30 min cycling TT. Their findings would suggest that the longer 10 s CHO mouth rinse (6.8%) versus 5 s CHO mouth rinse (4.6%) has a greater effect on cycling TT performance, which may be attributed to the prolonged exposure to the oral receptors.

1.6 Kinetic and kinematic data consolidation to measure changes in neuromuscular performance

1.6.1 The requirement of a high-resolution cycling power measurement

The metric most commonly measured and linked to performance outcomes during cycling is power. As such, there are many commercial power meters used to compare athletic interventions during scientific, competitive and recreational use. However, most commercial power meters only provide low-resolution readings (≤10Hz, with most power meters at 2Hz) of power which may lack critical detail during transitions, sprint starts, progressive fatigue or when considering bilateral differences under different cycling conditions (Bini & Hume, 2014).

The SRM PowerMeter (Figure 1-2) is one of the most commonly used power meters and has been used previously as the gold standard for validation of many power meters (Abbiss, Quod, Levin, Martin, & Laursen, 2009; Bertucci, Duc, Villerius, Pernin, & Grappe, 2005; Bini, Hume, & Cerviri, 2011; Czajkowski, Bouillod, Dauriannes, Soto-Romero, & Grappe, 2016; Gardner et al., 2004). A major limitation of the SRM system is that it only provides power measurement at 2Hz and provides an estimation of angular velocity once per pedal revolution. This sampling frequency improves to 200Hz only when using the SRM ergometer and a specially designed torque analysis box. However, this does not directly improve the measurement of power, as the angular velocity is not collected at high resolution. As a guiding principle for signal measurement, the sampling resolution for a signal of interest should be greater than twice the highest frequency component of the signal (Robertson, Caldwell, Hamill, Kamen, & Whittlesey, 2014). During cycling, the highest frequency component needs to be determined when considering that the torque and angular velocity of a cycle crank change continuously throughout a pedal stroke. To our knowledge, this determination of minimum sampling rate for torque and angular velocity in cycling has not been established. For example in human movement (e.g., walking, running, jumping), measurement sampling rates of 160Hz to 500Hz are required to adequately sample kinematic and kinetic data respectively (Hori et al., 2009; Winter, 2009). Furthermore, an average of the power per cycle by power meters that assumes a constant angular velocity throughout the entire cycle revolution may provide inaccurate results during rapid acceleration (i.e., BMX/track cycling sprint start). This can also limit the ability to produce a more detailed comparison of power by crank angle, as well as compare differences between leading and trailing limbs. In detailed cycling measurement, it is customary to collect sufficient samples to display power based on the angular position of the crank arm with degree precision. This enables a more accurate and detailed representation of the cycling kinetics, and more detailed metrics can be calculated given a sufficient sampling rate (Bertucci, Taiar, Toshev, & Letellier, 2008). For example, using low-resolution classical parameters of cycling criteria Bertucci et al. (2008) was not able to determine differences between regional level and elite level athletes. However, with improved resolution, a new set of biomechanical parameters were determined, and they could be used to determine differences in pedalling characteristics between the groups and provide more accurate feedback. This greater number of metrics may also be used to evaluate differences in cycling interventions related to technique, training, equipment, injury status and/ or nutritional interventions. However, many of the studies that have utilized highly detailed analysis of power were done using only a cycle-averaged angular velocity, which may lead to inaccurate values of power (Bertucci et al., 2008; Carpes, Rossato, Faria, & Bolli Mota, 2007). Therefore, a more accurate measurement of angular velocity is necessary to address these high-performance measurement needs in cycling (Figure 1-3.

Figure 1-2: SRM PowerMeter Science Road. Retrieved February 2, 2018, from http://www.srm.de/products/srm-powermeter/science-road/. Copyright 2015 SRM GMBH.

Along with higher resolution power and angular velocity readings, the ability to have accurate position during the pedal stroke allows surface EMG signal to be synced/time aligned to pedal position (Figure 1-4). This is critical to identify any changes in activation/performance/patterning in individual and multiple muscles during cycling (Blake & Wakeling, 2015).

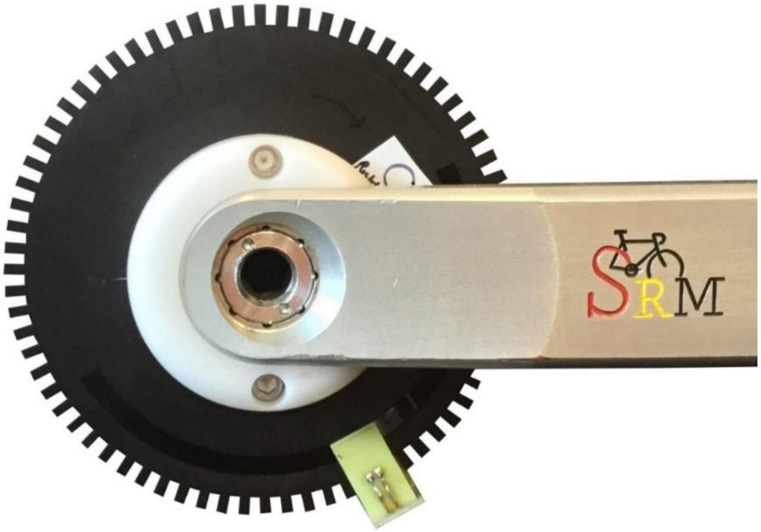


Figure 1-3: Custom SRM add-on device
used to measure torque and angular velocity every 5 degrees during a pedal cycle.

1.6.2 Advanced measurement of muscle efficiency and patterning with EMG wavelet analysis

Wavelet analysis of EMG frequency content together with kinetic measurement of power output during continuous cycling has demonstrated that the power output from the limb is impacted by coordination of the muscles of the leg more so than the maximum power output from any one muscle itself (Blake & Wakeling, 2015; Wakeling et al., 2010). Additionally, muscles across the leg demonstrate systematic phase shifts of muscle excitation noted with shifts in EMG frequency relative to the pedal cycle and dependent on cadence and power output during prolonged cycling trials (Blake & Wakeling, 2015). This suggests the importance of measurement of multiple muscles of the leg that contribute to force production during cycling as changes in neuromuscular performance of multiple muscles may contribute to overall power output. Furthermore, this suggests that during interventions aimed at maintaining optimal performance during prolonged cycling, it is important to understand how the intervention may influence detailed measurements of neuromuscular fatigue and associated muscle excitation. The ability to collect higher resolution power output together with recent work by Blake et al. (2015) to develop advanced EMG analysis techniques to improve the understanding of how individual muscle excitations and coordination between muscles change in response to cycling demands, presents a great potential to investigate detailed changes in muscle activation and power output during nutritional interventions.

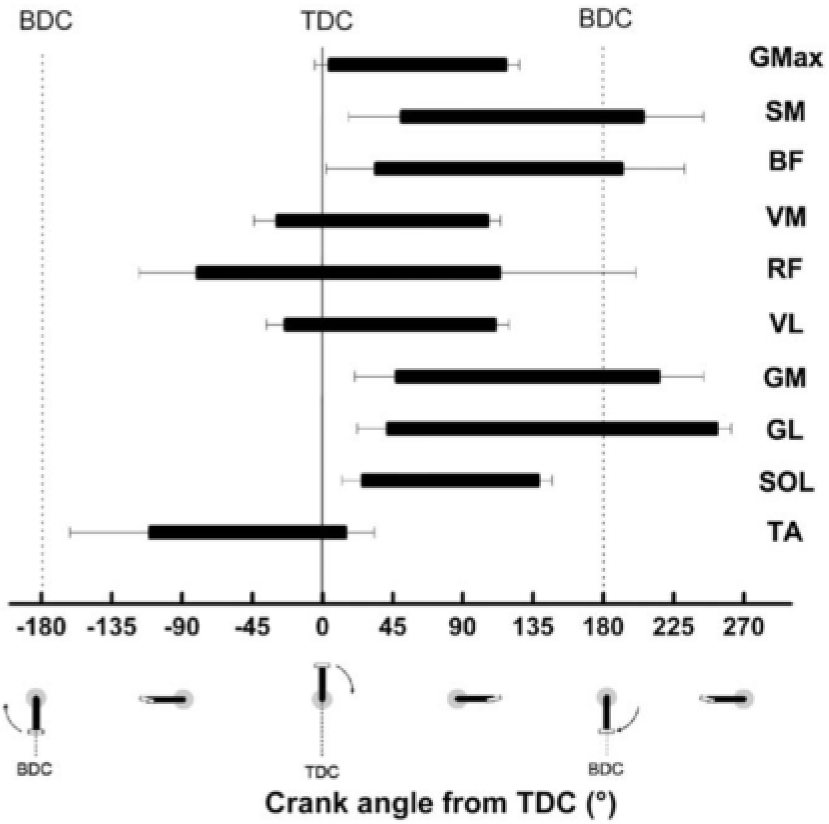


Figure 1-4: Mean onset, offset and duration of EMG activation during a pedal cycle. Adapted from "Intra-session repeatability of lower limb muscles activation pattern during pedalling," by S. Dorel, A. Couturier, and F. Hug, 2008, *Journal of Electromyography and Kinesiology*, *18*(5), p. 862. Copyright 2007 Elsevier Ltd.

1.7 Outline and specific aims of this dissertation

The primary goal of these projects is to refine methods for assessing neuromuscular changes and associated power/force outputs during various perturbations of direct, or perceived, fatigue induced by either exercise or nutritional interventions, with associated performance outcomes.

In order to address this goal, we collected physiological and biomechanical data from subjects across a set of experiments designed to induce different levels of fatigue by the implementation of various exercise and nutritional interventions to cause various levels of fatigue in an ecologically valid manner. The data sets were collected during a single joint task (Chapter 2) and during cycling trials (Chapter 3 and 4). During these experimental trials, we collected measures of kinetics (force and power) as well as muscle activation (EMG) and physiological measures (heart rate, rating of perceived exertion, blood lactate, blood glucose, ventilation, oxygen uptake and carbon dioxide production) to investigate the overall performance, as well as potential mechanisms for improved performance related to the exercise and nutritional interventions.

In order to substantially enhance the collection of cycling kinetics and kinematics, we have developed an innovative sensor that improved the measurement resolution (temporal and spatial) of a commercial research grade power meter (Figure 1-3). Using these improved measures alongside advanced muscle activity analysis (Blake et al., 2012; Blake & Wakeling, 2012, 2015; Von Tscharner, 2000; Wakeling et al., 2010), we were able to ameliorate an experimental framework that could be used to investigate changes in fatigue and coordination pattern associated with exercise and nutritional interventions (Chapter 4).

The studies, outlined in this document, are the first to evaluate these exercise and nutritional interventions with combined biomechanical and physiological measures. Further, the experimental framework can form the basis for mechanistic investigations of a different technique, training, equipment, injury status and/ or nutritional interventions in sports performance.

To date, the biomechanical investigations of the effect of CHO mouth rinse have been limited by a lack of ecological validity in study design. This limits the ability to fully understand the potential impact of this intervention on performance enhancement in high-performance sport. To the best of our knowledge, this is the first in a series of investigations to integrate biomechanical, physiological and performance outcomes during CHO mouth rinse in both simple and ecologically valid cycling tasks, of which we will now further elaborate on the series of studies.

The aim of Chapter 2 was to investigate the effects of a CHO mouth rinse vs. placebo (artificial sweetener) on force and muscle activity during a very short (<3 min), fatiguing, neuromuscular demanding trial. We hypothesized that a single CHO mouth rinse post-fatigue would provide enhanced force performance during a maximal isometric knee extension contraction in an acute fatigued state and that changes would be positively correlated with modification to muscle activation. Our results demonstrated a consistent change in EMG median frequency related to increased fatigue in both experimental conditions in all three muscles tested, providing little evidence of change in neuromuscular strategy associated with CHO mouth rinse. Therefore, we sought to develop an experimental protocol that would facilitate a more detailed investigation of neuromuscular activation.

In Chapter 3, we aimed to compare the effects of a CHO mouth rinse vs. placebo (artificial sweetener) using fundamental physiological measures of neuromuscular activation and overall performance during an ecologically valid late endurance cycling time trial that included a standardized pre-trial meal plan and a fatiguing protocol (2 hr of steady cycling at ~60% $\dot{V}O_{2max}$ followed by an ~ 30 min time trial) to replicate the late stages of a cycling race. We hypothesized that CHO mouth rinse will improve power output and TT performance compared to placebo. During this investigation, we found that while there was no overall effect noticed for time to completion, there was a significant change in the time to complete various components of the time trial, only noticed in the CHO mouth rinse trial. Additionally, muscle activity of the lower leg (MG and SOL) demonstrated a modification in frequency only evident during the placebo condition. Taken together this would suggest that the CHO mouth rinse may act to mitigate the effect of fatigue during late endurance performance. However, using the simple measures of median frequency and overall intensity it was difficult to ascertain if this change in muscular activation is truly a modification in neuromuscular strategy.

Based on this limitation in the potential to investigate mechanisms of changes in neuromuscular performance, the aim of Chapter 4 was to use a more complex neural and biomechanical analysis technique to examine the potential changes in coordination and neuromuscular performance associated with various levels of fatigue. This involved combining high-resolution cycling power alongside wavelet analysis of EMG and principal component analysis (PCA) to determine the kinetic, kinematic and neural components that map onto using a CHO mouth rinse or placebo conditions. This is the first study to use advanced neuromechanical analysis to examine the effect of CHO mouth rinse in a late endurance cycling trial. The results of this investigation show noticeable differences between WASH and PLA for both power output and muscle activation. The average power output profile in WASH showed an earlier onset in the pedal cycle, greater duration and higher amplitude versus PLA during the TT. Additionally, only the PLA condition showed a significant increase in muscle activation throughout the time trial, which could be evidence of fatigue. This shows for the first time that CHO mouth rinse may have a substantial effect on the maintenance of power while mitigating the impact of neuromuscular fatigue, in late endurance performance, further strengthen our assertion that CHO may, in fact, minimize the changes in performance that are associated with fatigue during late endurance fatiguing events.

Portions of this dissertation have been published, or are in the process of being published elsewhere. Portions of Chapter 2 have been published in the *International Journal of Sport Nutrition and Exercise Metabolism* (Jensen, Stellingwerff, & Klimstra, 2015). Portions of Chapter

3 have been published in *Journal of Medicine and Science in Sport and Exercise* (Jensen, Klimstra, Sporer, & Stellingwerff, 2018). The contents of Chapter 4 are being prepared to be submitted for publication.

2 Carbohydrate mouth rinse counters fatigue related strength reduction[1]

2.1 Abstract

The purpose was to determine the effect of carbohydrate (CHO) mouth rinse on maximal voluntary contraction (MVC) and neuromuscular output in a fatigued state. It was hypothesized that CHO mouth rinse would potentiate torque output in a fatigued state. In a double-blind, cross-over design, 12 competitive male athletes (9 rowers, 1 cyclist, 1 runner and 1 volleyball player) initially performed 3 x 5 s MVC isometric knee extensions followed by a 50% MVC contraction until volitional exhaustion, with quadriceps muscle activity measured via electromyography (EMG). Immediately after, either an 8% CHO maltodextrin (WASH) or non-caloric artificial sweetener (PLA) was mouth rinsed for 10 s, prior to 3 x 5 s final MVCs. Fatigue caused a significant decline in post-fatigue MVC trial 1 for 3 second average torque ($p = 0.03$) and peak torque ($p = 0.02$) for PLA. This fatigue related decline in torque was not noticed for WASH, with a 2.5% and 3.5% less attenuation in peak and average torque, respectively in post-fatigue MVC1 compared to PLA. The effect size for MVC trial 1 between WASH/PLA was seen to be small positive (ES=0.22; 55% likelihood of positive). Overall, for EMG RMS, there were no significant differences between PLA and WASH amongst all muscles. EMG median frequency showed comparable results between conditions with significant reductions due to fatigue. Taken together, this evidence suggests that the attenuation of torque post-fatigue was less for CHO mouth rinse than a placebo. Even though the gains were marginal, these discoveries may play an important role in sports performance, as small performance effects can have significant outcomes in real-world competitions.

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[1] Jensen, M. P., Stellingwerff, T., & Klimstra, M. (2015). Carbohydrate Mouth Rinse Counters Fatigue Related Strength Reduction. *International Journal of Sport Nutrition and Exercise Metabolism, 25*(3), 252–261.

2.2 Introduction

Carbohydrate (CHO) consumption can improve performance during long-duration endurance exercise (>90mins), with the primary mechanisms likely related to maintenance of high rates of CHO oxidation and hypoglycemia prevention (Jeukendrup, 2004). While these mechanisms support energy/metabolic benefits of CHO during prolonged exercise, there have also been positive effects of CHO on shorter duration (<1 h), high-intensity exercise. Interestingly, acute and short-term effects appear unrelated to muscle glycogen concentrations (Jeukendrup, 2004; Romijn et al., 1993) or through increased availability of plasma glucose (Carter, Jeukendrup, Mann, & Jones, 2004). Therefore, the acute performance enhancement from CHO intake during high-intensity exercise (<1 h) is probably based on alternate mechanisms.

It is theorized that CHO ingestion may impact short duration high-intensity exercise performance (<1 h) through central/cognitive effects (Chambers et al., 2009). Recent studies have shown improvement for high-intensity endurance exercise (~1 h) by rinsing the mouth with a CHO solution, without oral CHO consumption (Carter, Jeukendrup, & Jones, 2004; Chong et al., 2011; Gam et al., 2013; Rollo & Williams, 2011). The results support a central mechanism as CHO mouth rinse has been shown to increase activation of pleasure and reward centres of the brain (Chambers et al., 2009) and improve performance related to the duration of oral cavity CHO exposure(Sinclair et al., 2014).

The performance effects of CHO mouth rinse have primarily been studied over short duration cycling (Carter, Jeukendrup, & Jones, 2004; Gam et al., 2013; Sinclair et al., 2014), and running (Rollo et al., 2010, 2011; Whitham & McKinney, 2007), with only a few studies examining CHO mouth rinse on maximal strength/power type exercise, finding contradictory performance outcomes (Beaven et al., 2013; Chong et al., 2011; Painelli et al., 2011). However, all of these strength/power based studies did not utilize subjects in a pre-fatigued state, where a decrease in performance may be due to central and/or peripheral mechanism(s) related to energy availability (Allen et al., 2008). Thus, central mechanisms of performance enhancement may be more readily evident in a fatigued state. This is supported by Gant et al. (2010) who found an immediate increase in motor output due to a CHO mouth rinse following a fatiguing contraction, suggesting central modifications in muscular fatigue and power output.

The fatigue state of an individual can be directly measured through performance modifications as well as indirectly by changes in muscle activity through electromyography (De Luca, 1997). Based on the idea that CHO mouth rinse may benefit endurance performance in part through central mechanisms, it can be hypothesized that EMG may be useful to quantify changes in muscle activation associated with a CHO mouth rinse. Therefore, the aim of this study was to investigate the effect of a CHO mouth rinse on 1) muscular force production and 2) neuromuscular output (EMG), during a maximal isometric knee extensor contraction in an acute fatigued state. This is the first study of its kind to combine measures of neuromuscular and force performance to evaluate the effect of CHO mouth rinse on performance. We hypothesized that a single CHO mouth rinse post-fatigue would provide enhanced force performance during a maximal isometric knee extension contraction in an acute fatigued state and that changes would be positively correlated with modification to muscle activation.

2.3 Methods

2.3.1 Participants

Twelve competitive male athlete participants (26.7 ± 6.7 years, 78.2 ± 5.5 kg and 184.9 ± 6.1 cm, for age, body mass (BM) and height (mean ±SD), respectfully) were recruited and all gave written consent under Human Ethics at the University of Victoria. The study participants were regularly active (training 3-4 times a week at the time of the study) and had previous athletic experience from varsity to national level (9 rowers, 1 cyclist, 1 runner and 1 volleyball player).

2.3.2 Participant setup

Upon laboratory arrival, each participant was prepared for EMG electrode placement by shaving hair and cleaning the skin with 70% alcohol. EMG electrodes (Delsys Trigno, USA) were then placed on anatomical landmarked sites over the *vastus lateralis* (VL), *rectus femoris* (RF) and *vastus medialis* (VM) (Barbero, Merletti, & Rainoldi, 2012). The distance from the anterior superior iliac spine (ASIS) to the lateral and medial border of the patella was used as reference lines for identical placement of the EMG electrodes on the participants' perceived dominant leg for each trial. The anatomical landmark (AL) for the VL was the distal portion of the muscle belly and oriented 20 degrees (°) with respect to the reference line on the lateral side of the patella. The electrode was placed 165mm from the AL. The AL for the VL was the distal portion of the muscle belly and oriented 50° with respect to the reference line on the medial side of the patella. The electrode was placed 95 mm from the AL. The AL for the RF was a line between the ASIS and the superior part of the patella. The electrode was placed at 50% of the distance of the AL, as previously described (Barbero et al., 2012). All measurements were recorded so that on subsequent trials all electrodes could be positioned in the same place. EMG and force were sampled at 2 kHz with a 16-bit A/D converter (USB-1616F; Measurement Computing, Norton, MA).

Prior to experimental trials, all participants completed a standardized sub-maximal cycling warm-up for 15 minutes (70-80 rpm) on a cycle ergometer at varying intensities normalized to the participants' body weight (2, 3 and 4% body weight) (Monark Ergomedic 828E; Sweden). Following the warm-up, subjects were seated in a Cybex-6000 with the axis of rotation of the leg-extension dynamometer aligned to the knee joint line. The backrest and seat angles were adjusted so that the hip was at 80° of flexion, with a strap placed across the pelvis and upper body to minimize extraneous movements. The dominant leg to be tested was secured and fixed to the dynamometer lever arm by a pad secured 1 cm above the lateral malleolus. Contra-lateral knee extension was restrained at the distal tibia, and all contractions were performed at 60° angle of knee flexion (0°=full extension). During experimental trials, participants folded their arms across their chest and received visual force feedback on a computer monitor. Knee extension force was measured through the use of a tension/compression load cell (Omegadyne, LC101-500; USA) attached between the Cybex-6000 lever arm and base of the Cybex-6000. Torque (Nm) was then calculated as the product of the measured force and the lever arm length for each individual subject.

2.3.3 Experimental protocol

Each participant completed two experimental trials in double-blind, randomized crossover fashion while maintaining regular training for the duration of the study and to abstain from any intense exercise, caffeine and alcohol in the previous 24 h before each test. The trials were separated by 4.6 ± 2.2 days, and subjects were overnight fasted and conducted each trial at the same time (between 7-10 am) to minimize the effects of diurnal variation. Participants recorded their food and liquid consumption for 24-hrs prior to the first trial, which they replicated for the subsequent trial.

The experimental trial consisted of maximal and submaximal isometric knee extension tasks while knee extensor muscle activity and force were collected (Figure 2-1). Before each trial, the participants were familiarized to isometric knee extension tasks by performing three slow ramp isometric knee extensions at a submaximal level. Participants then performed three isometric maximal voluntary contraction (MVC) knee extensions lasting 5 s separated by 60 s rest to get baseline measurements, with the highest achieved torque used to calculate the target torque for the fatiguing endurance task (Figure 2-1).

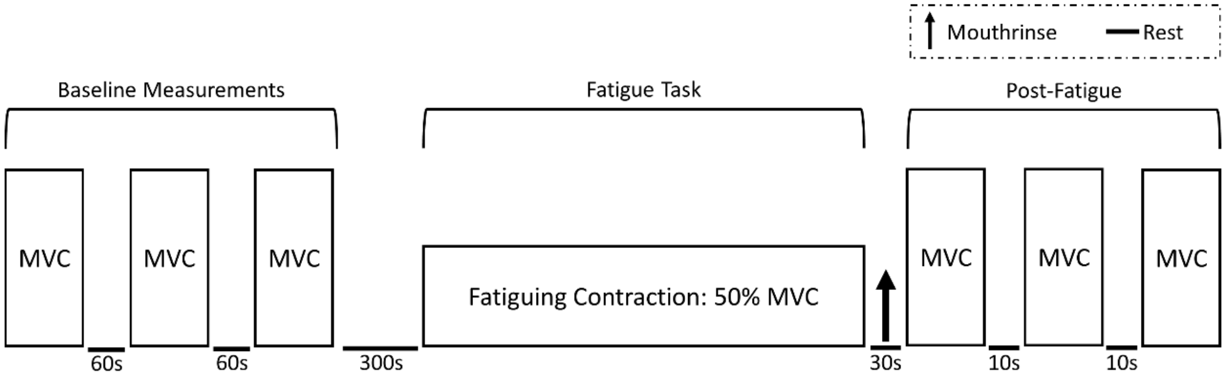

Figure 2-1: Schematic diagram of the experimental protocol. Mouth rinse administered following fatigue task and before post-fatigue maximal voluntary contraction (MVC).

Following 5 minutes of rest, participants then performed a fatiguing contraction at 50% of MVC (with visual torque feedback) until volitional exhaustion, defined as the point where the torque dropped more than 10% from the target level for more than 5 seconds. Immediately post-fatigue, participants rinsed their mouth with one of two randomized solutions (WASH vs. PLA) for 10 s and then expelled all of the solution into a beaker, over a 30 s rest period. The last exercise set consisted of 3 x 5 s MVCs with 10 s rest between each contraction (Figure 2-1). Participants were given constant and consistent verbal encouragement for all MVCs and during the fatiguing contraction.

The CHO mouth rinse consisted of 8% maltodextrin. Both the WASH and PLA solutions were supplemented with 0.2% artificial sweetener, sucralose (MIO, Kraft Food, USA) to make the solutions indistinguishable. The mouth rinse solutions were coded by a non-affiliated researcher to ensure double-blinding. Drinks were taste-matched and comprised the same quantity of energy-free ingredients (artificial-sweetener, colourings, and flavouring).

2.3.4 Data analysis

Of the first three baseline measurements, the greatest peak torque was used to compare to all three post-fatigue trials. Average torque was calculated using the last 3 s of each MVC, whereas peak torque was defined as the maximal torque reached at any point during the 5 s maximal contraction. The total area under the load-time curve was calculated and compared between days to ensure that a common level of cumulative effort was required by each subject to reach volitional exhaustion in the fatiguing contraction on separate days. Raw EMG signal was filtered using a second-order zero-phase Butterworth band-pass filter using low and high cut-off frequencies of 10 Hz and 500 Hz respectively. EMG root mean square (RMS) and median frequency (MDF) were calculated using a 300ms interval centred around absolute maximal torque for all MVCs (Farfán, Politti, & Felice, 2010). Baseline MDF and RMS was extracted from the baseline MVC that had the greatest peak torque. RMS and MDF maximal values were determined using a 300ms moving window for the entire raw signal for each baseline trial. The average maximum windowed RMS/MDF from the three baseline MVCs was used to normalize all RMS/MDF values. For each window, a discrete Fast Fourier Transform (FFT Hanning) was used to calculate MDF (Merletti & Lo Conte, 1997).

2.3.5 Statistics

Repeated measure ANOVAs were used to determine the effect of mouth rinse (WASH vs. PLA) and TIME (PRE, $\text{POST}_{1,2,3}$), on Peak torque, 3 s Average torque, RMS, and MDF. Whenever a significant *F*-value was obtained for main effect or interactions, a Tukey post-hoc test was performed. A priori planned orthogonal contrasts were made between pre-fatigue baseline value and first trial post-fatigue, as well as comparing post-fatigue trials 2 and 3 (Tabachnick & Fidell, 2013). This was done to examine the acute effect of WASH on isometric force production post-fatigue as well as the effect over repeated bouts. To determine whether the differences between PLA and WASH trials were meaningful, magnitude-based inferences were calculated for torque. Cohen's effect size (ES) calculations were made between baseline and all 3 post-fatigue trials and interpreted in relation to the likelihood of exceeding the smallest worthwhile change (ES <0.2 trivial, >0.2 small, >0.6 moderate, >1.2 large). Magnitudes of the standardized effects were interpreted using the threshold of ±0.20, which was deemed trivial. The effect of the intervention was expressed as 90% confidence intervals, and the probabilities of the intervention being effective was expressed as either positive, trivial or negative (Hopkins, Marshall, Batterham, & Hanin, 2009).

To test for differences in the cumulative effort during the fatiguing contraction between days, a paired t-test was used. Intraclass correlation coefficient (ICC) was used to express relative reliability of the EMG signal measures. The reliability between trials was calculated using all baseline EMG raw data and was determined as the estimator of test-retest reproducibility. ICC reproducibility was characterized using the following criteria: good (0.8-1.0), fair (0.6-0.79) and poor (<0.6) (Sleivert & Wenger, 1994). All data were analyzed using SPSS (Version 20, Chicago IL). Data are reported as means ± SE, with significance set at $p<0.05$.

2.4 Results

2.4.1 Torque

Repeated measures ANOVA demonstrated that there was significant main effect for TIME for peak and average torque with collapsed conditions (WASH/PLA) showing that grouped fatigue trials 1, 2, 3 were significantly lower than pre-fatigue values. There was no significant difference in measures of peak torque or average torque between pre-fatigue WASH and PLA conditions (peak torque WASH: 364.0 ± 12.6 vs. PLA: 351.5 ± 10.9 Nm, $p = 0.24$; and average torque WASH:

344.2 ± 11.2 vs. PLA: 334.1 ± 10.2 Nm, $p = 0.27$; Figure 2-2A & 2-3A). Planned contrasts for WASH showed that post-fatigue MVC1 for peak and average torque did not have a significant decrease ($p > 0.05$) from pre-fatigue baseline (Figure 2-2A & 2-3A). Planned contrasts for PLA showed a significant decrease from baseline in peak torque for post-fatigue trial 1 ($p = 0.02$) and average torque for trial 1 ($p = 0.03$). The peak torque for all post-fatigue MVCs for WASH had a smaller decrease from baseline compared to PLA (MVC1: WASH: -31.7 ± 9.9 vs. PLA: -38.7 ± 9.8 Nm; MVC2 WASH: -30.3 ± 9.5 vs. PLA: -32.7 ± 8.3 Nm; and MVC3: WASH: -31.6 ± 7.4 vs. PLA: -33.1 ± 7.7 Nm). Effect size for peak torque MVC1, MVC2, MVC3 between WASH/PLA was seen to be small positive (ES = 0.22; 55%), trivial (ES = 0.10; 76%), and trivial (ES = 0.05; 71%), respectively. For average torque, WASH had a smaller decrease from baseline compared to PLA for all three post-fatigue trials (MVC1: WASH: -25.9 ± 10.1 vs. PLA: -36.0 ± 11.5 Nm; MVC2: WASH: -27.2 ± 8.8 vs. PLA: -32.5 ± 8.5 Nm; and MVC3: WASH: -28.2 ± 7.3 vs. PLA: -30.6 ± 8.2 Nm). Effect size for average torque MVC1, MVC2, MVC3 between WASH/PLA was seen to be small positive (ES = 0.32; 64%), trivial (ES = 0.16; 50%), and trivial (ES = 0.07; 55%), respectively. There was no significant difference between PLA and WASH cumulative effort during the fatiguing contraction on separate days ($p > 0.05$).

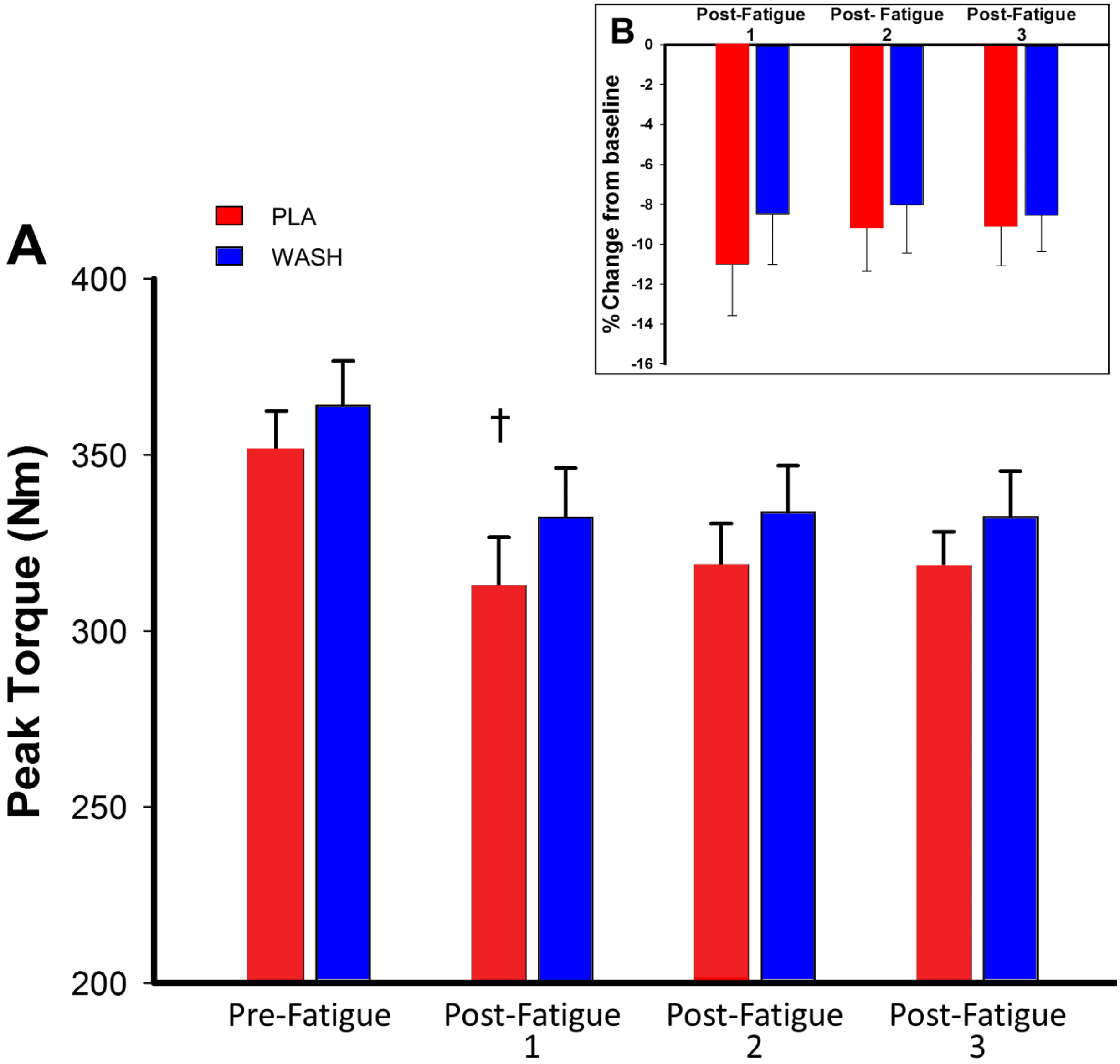

Figure 2-2: The effect of CHO mouth rinse on (A) peak torque of pre- and post-fatigue MVCs, (B) peak torque decrease (%) from baseline.† represent significantly decreased torque production in post-fatigue MVCs compared to pre-fatigue for PLA condition, *p* < 0.05. Data are presented as means ± SE.

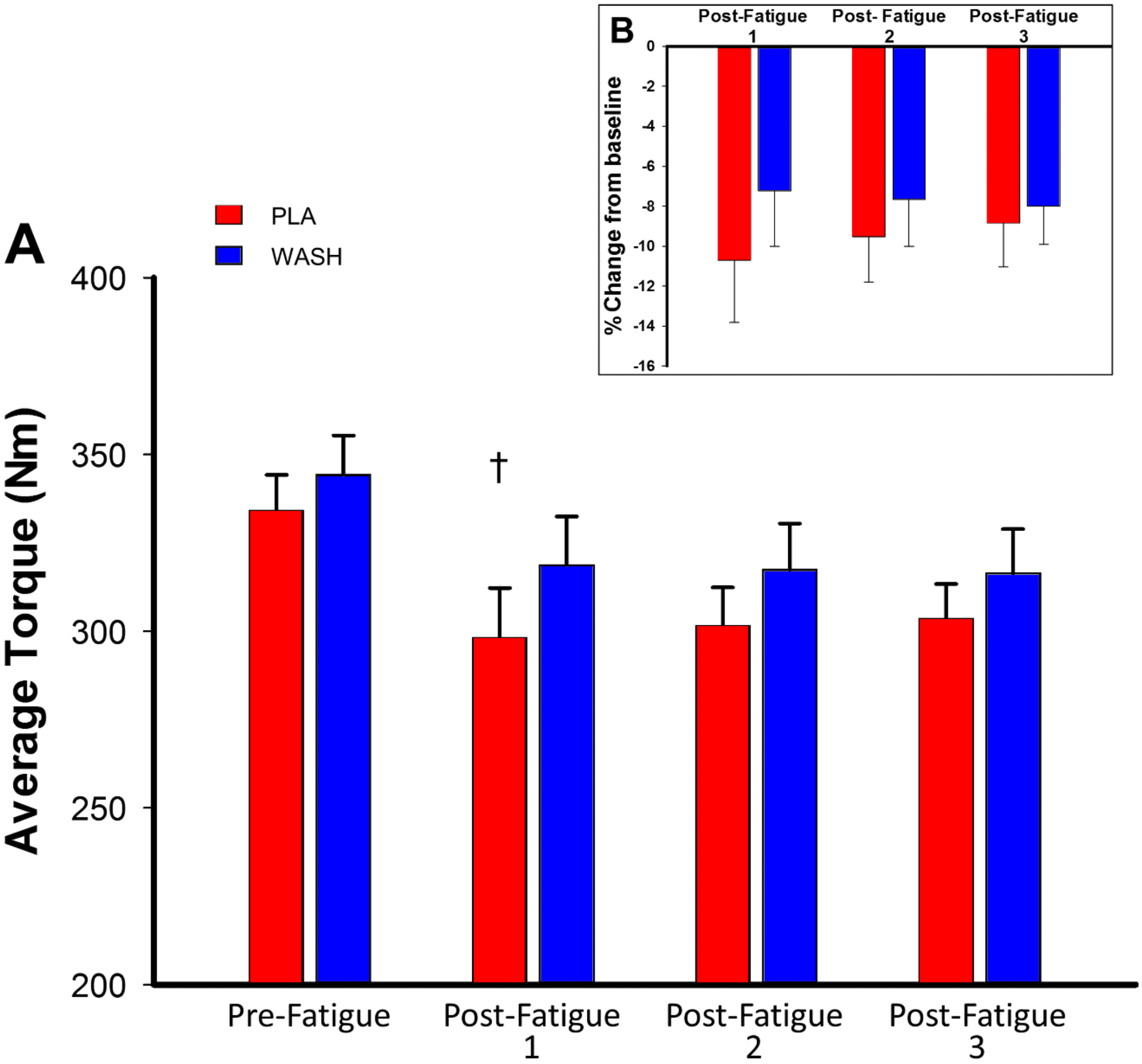

Figure 2-3: The effect of CHO mouth rinse on (A) average torque of pre- and post-fatigue MVC, (B) average torque decrease (%) from baseline.† represents significantly decreased average torque production in post-fatigue MVCs compared to pre-fatigue for PLA condition, *p* < 0.05. Data are presented as means ± SE.

2.4.2 RMS

Repeated measures ANOVA showed there was no significant main effect for TIME for VL, RF and VM, with collapsed conditions (WASH/PLA) ($p > 0.05$). There was no significant difference between trial days on absolute RMS values ($p > 0.05$) (Figure 2-4). The reliability for the absolute RMS values showed good reproducibility for the VL (ICC=0.98) VM (ICC=0.92) and RF (ICC=0.80).

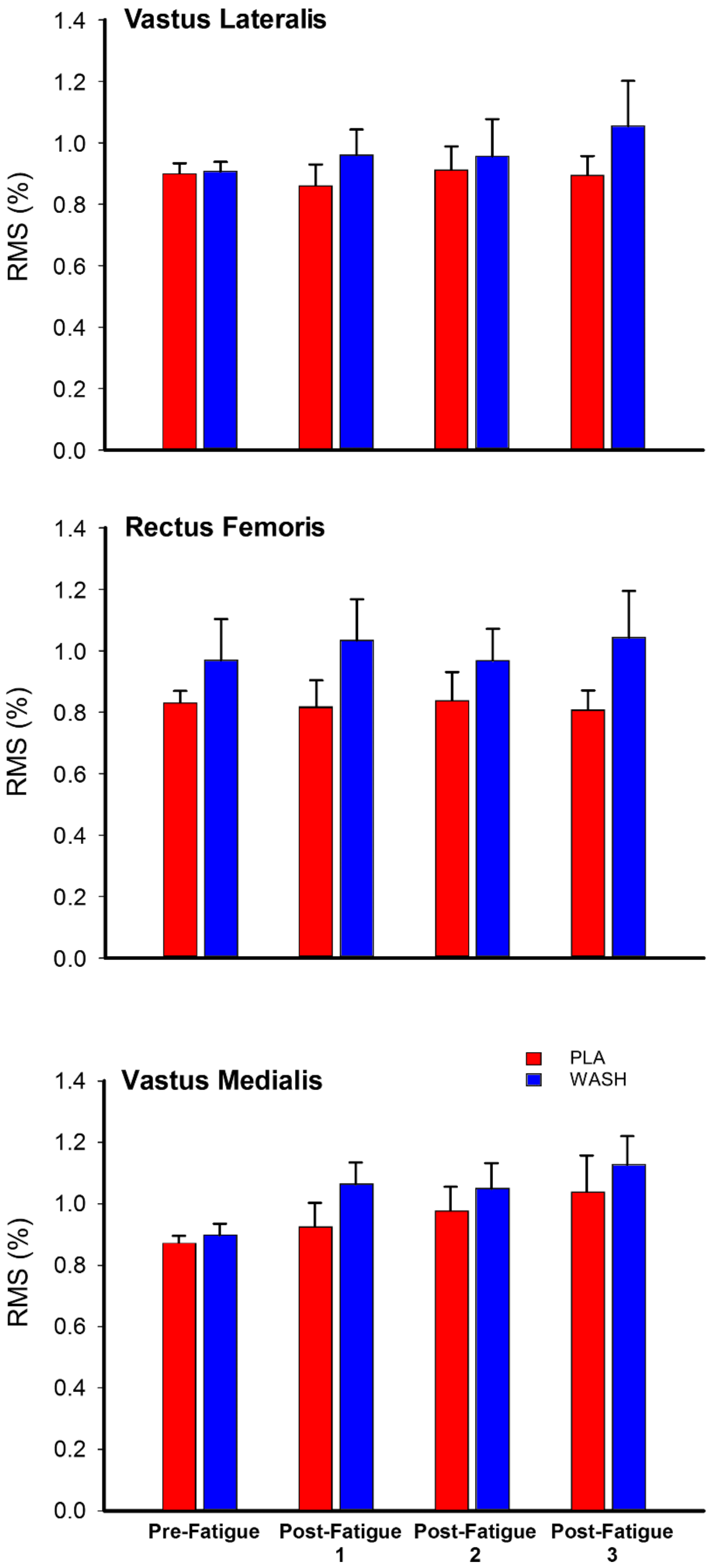

Figure 2-4: Normalized group EMG RMS Amplitude for pre- and post-fatigue MVCs. Data are presented as means ± SE.

2.4.3 MDF

Repeated measures ANOVA demonstrated that there was a significant main effect for TIME for VL, RF and VM, with collapsed conditions (WASH/PLA) showing that grouped fatigue trials 1, 2, 3 were significantly lower than grouped pre-fatigue values ($p < 0.05$). The absolute pre-fatigue values for VL, RF, and VM were WASH: 121.5 ± 9.6, 127.1 ± 7, and 127.9 ± 10 Hz, PLA: 124.4 ± 11.4, 120.8 ± 6.8 and 120.3 ± 9.3, respectively. Planned contrasts for RF showed a significant decrease from baseline, for both WASH ($p < 0.01$) and PLA ($p = 0.02$) for post-fatigue trial 1 (Figure 2-5). There was no significant difference between trial days for MDF ($p > 0.05$). The reliability for MDF showed good reproducibility for the *VL* (ICC=0.81), and *VM* (ICC=0.83) and fair reproducibility for *RF* (ICC=0.74).

None of the 12 participants were able to distinguish taste differences between WASH and PLA solutions clearly.

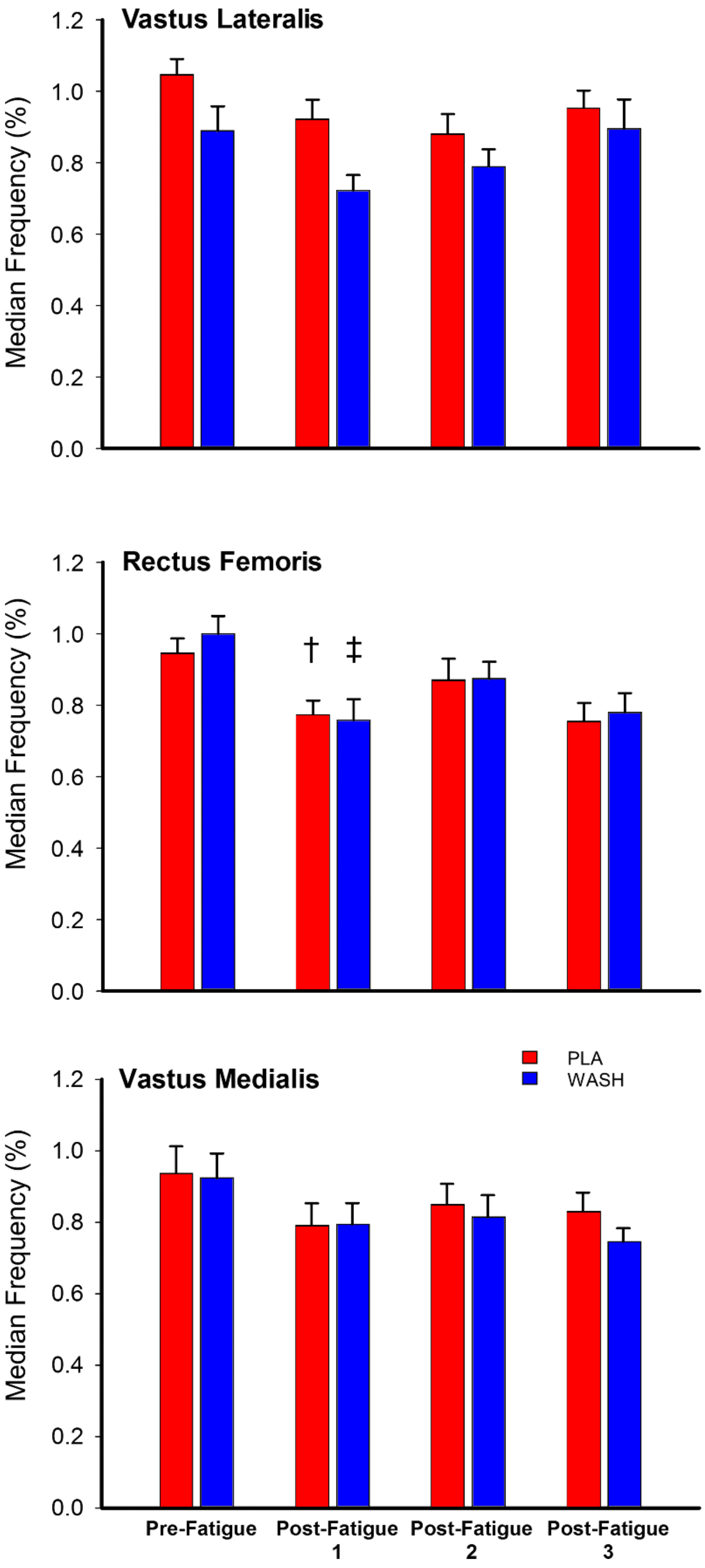

Figure 2-5: Normalized group EMG Median Frequency for pre- and post-fatigue MVCs. Data are presented as means ± SE. † (PLA) ‡ (WASH) represent significantly decreased Median Frequency in post-fatigue MVCs compared to pre-fatigue, $p < 0.05$.

2.5 Discussion

This is the first study to demonstrate that a CHO mouth rinse resulted in decreased torque attenuation as compared to a non-caloric control mouth rinse, in a fatigued state. This finding has the potential for real-world application in short duration activities at high exercise intensities, such as sprinting/jumping, cycling, and/or weight-lifting where the ability to acutely increase maximal force/strength in a fatigued state could result in an increase in performance. However, while we demonstrated a decreased torque attenuation during the WASH trial, it is unclear if this effect persists over repeated trials and the effect is small. Therefore, care must be taken when considering the use of this nutritional intervention over repeated high-intensity exercise bouts. Additionally, we found that there were no differences noticed between PLA and WASH for fatigue-related measures of surface EMG. This fails to provide evidence for an acute centrally mediated modification in motor output due to a CHO mouth rinse, yet further neural investigations are necessary.

CHO mouth rinse has been shown to improve short duration, high-intensity cycling and running protocols lasting approximately 1h, where muscle glycogen is not limiting (for review see Jeukendrup, 2013). However, prior research in the field of CHO mouth rinse and its effect on maximal strength/power has shown contradictory findings, with either no performance increase (Chong et al., 2011; Painelli et al., 2011) or only a slight performance increase (Beaven et al., 2013). However, there are significant differences between these strength/power protocols, and none have examined the effect of CHO mouth rinse following an acute pre-fatiguing protocol.

In the present experiment, we observed that there was a significant attenuation in peak and average torque for PLA, while there was no significant attenuation during WASH for the first trial post-fatigue. Thus, during the CHO mouth rinse trials, peak torque production and torque maintenance did not change, supporting a potential acute influence of CHO mouth rinse on performance. This finding is in contrast to Painelli et al. (2011) who did not observe an effect of carbohydrate mouth rinse on maximal strength or strength endurance during a 1-RM and 6-RM bench press task. However, the author's point out that their results are limited due the lack of accurate force measurement and the poor day-day reliability of their task. In this experiment, accurate torque measurement during a simple constrained knee extension task enabled a sensitive and controlled evaluation of the effect of CHO mouth rinse on torque following a fatiguing contraction. In a similar controlled experiment evaluating the effect of CHO mouth rinse on upper limb force production, Gant et al. (2010) found an immediate increase in maximum voluntary force following CHO ingestion and a larger change in motor evoked potentials (MEPs) in fatigued vs non-fatigued conditions. This indicates that CHO can immediately improve human performance by increasing corticomotor excitability. The present finding of ~3% less attenuation of peak torque post-fatigue in WASH vs. PLA (Figure 2-2B) is supported by a qualitative inference of the outcome being 55% likely to be positive, while marginal, could be relevant to the elite athlete/coach. For example, the normal variation (CV) in elite high-jump has been found to be 1.7%, and 0.3 of this is the smallest worthwhile effect (Hopkins, 1999).

An interesting observation from the peak and average torque results are that through effect size calculations (Cohen's d) it appears that the effect of WASH has a diminishing influence over repeated bouts, as only the first trial post-fatigue showed a possible positive benefit while trials 2 and 3 only had a trivial effect. This would suggest that the effect of WASH on torque reduction has a short-lasting influence compared to PLA conditions and may be ineffectual for repeated bouts. Further, other studies have shown that, with sprint performances and CHO mouth rinse, there is a greater increase in initial power output with a greater decrement in performance over time versus a non-CHO mouth rinse (Beaven et al., 2013; Dorling & Earnest, 2013). Therefore, in consideration of the present results, and the findings of Beaven et al. (2013), it appears that only the initial maximal efforts following a CHO mouth rinse may demonstrate a performance gain by reducing the attenuation in strength in a fatigued state. As stated previously, one possible mechanism for this short-lasting improvement is through the enhanced neural drive to motor units from a supraspinal source (Gandevia, 2001). Following a volitional contraction, the level of intensity for a subsequent maximal effort may be centrally inhibited based on afferent input to limit damage to muscle tissue and ward off energy depletion. This could result in the down-regulation of motor efferent commands resulting in decreased motor output (St Clair Gibson et al., 2001). Thus the CHO mouth rinse may act to attenuate centrally mediated inhibition of motor output possibly through afferent signals associated with CHO availability in the mouth (Gant et al., 2010). This is consistent with studies evaluating direct cortical measures during CHO use, demonstrating activation of anterior cingulate cortex and striatum immediately following CHO mouth rinse (Chambers et al., 2009). The authors attributed this activation to reward recognition and Gant et al. (2010) support an enhancement in corticospinal activation. This could suggest that while there are short-term benefits to a CHO mouth rinse combating centrally mediated fatigue, there may be a detriment to repeated maximal bouts. However, while this may be true of short duration high-intensity events, it is important to note that long duration events do not observably suffer from this same issue (for review see Jeukendrup, 2013).

Fatigue has been shown to alter NM strategies during maximal performance. This includes the differential activation of muscles to limit force production to maintain fuel resources and limit tissue damage (St Clair Gibson et al., 2001). For example, maximal voluntary knee extensions and leg cycling sprints result in a considerable amount of NM fatigue related to alterations in quadriceps activation, limiting performance (Billaut, 2011). When evaluating EMG a decrease in

MDF suggests a decrease in motor unit (MU) firing rate, muscle fibre recruitment and/or a decrease in conduction velocity (Billaut, 2011) and can be used as an index of fatigue (De Luca, 1997; Nagata et al., 1990). Additionally, EMG RMS amplitude has been positively correlated with muscle force as greater MU recruitment, and higher firing rate contributes to an increase in the cumulative EMG. It is important to note that following fatigue, EMG RMS may increase, while MDF may decrease in conjunction with lower force production (Gandevia et al., 1996). Therefore considering RMS alongside EMG frequency content is necessary to adequately account for the factors that alter these variables including MU firing rate, MU action potential amplitude/duration/shape and conduction velocity (De Luca, 1997). In the present study, we found that there were no differences in RMS for all quadriceps muscles across all trials compared to baseline (Figure 2-4). Additionally, there was a greater decrease in MDF in RF for WASH and PLA during MVC1 following the fatiguing contraction (Figure 2-5). Taken together these results suggest that there was significant fatigue evident in both conditions. However, the current findings do not support modification in neuromuscular strategy in either PLA and WASH conditions. A possible explanation for this finding, in light of the torque results, is that WASH may subtly alter overall motor output to enable an increase in immediate torque production. This is in agreement with Gant et al. (2010) who demonstrated that CHO in the mouth immediately increases the excitability of the corticomotor pathway. However, given that this result is only evident in RF MDF, a more detailed EMG analysis and neurophysiological methodology such as interpolated twitch or reflex techniques are required to fully support or refute this interpretation (Gant et al., 2010; Zehr, 2002)

The reproducibility of surface EMG measurements across trials was quite similar and showed good/fair reproducibility, but potential methodological problems associated with surface

EMG should not be ignored (e.g., distance to motor points, distance to lateral edge of muscle, and/or orientation of muscle fibers with respect to electrode (De Luca, 1997)). Further, use of surface EMG in the present study only enabled an indirect analysis of the underlying physiological changes associated with the CHO mouth rinse on performance. However, MVC and EMG during isometric knee extensions have been shown to have high repeatability, even without familiarization sessions (Rainoldi, Bullock-Saxton, Cavarretta, & Hogan, 2001).

The most important, novel finding of this study was that the attenuation of torque post-fatigue was less for CHO mouth rinse than a placebo. Further, this performance effect of CHO mouth rinse in a fatigued state may diminish for subsequent bouts suggesting a time-limited contribution of a centrally mediated effect. In addition, CHO mouth rinse does not result in an altered NM strategy in a fatigued state as measured through EMG activity (RMS and MDF). This suggests that there may be no difference in central motor commands measured peripherally through EMG and techniques that are more detailed are required to evaluate these results. Overall, these discoveries may play an important role in sports performance, as small performance effects can have significant outcomes in real-world competitions.

3 Effect of carbohydrate mouth rinse on performance after prolonged sub-maximal cycling [2]

3.1 Abstract

Previous studies have shown improved shorter-duration (~1 h) performance with carbohydrate (CHO) mouth-rinsing (WASH), especially in overnight fasted / non-fuelled subjects. **Purpose:** to determine the effect of WASH on cycling time-trial (TT) performance and muscle activity (EMG) after 2 h of sub-maximal cycling while receiving CHO. **Methods**: In a double-blind, placebo-controlled crossover design, 10 well-trained males cyclists ($\dot{V}O_{2max}$: 65 $mL \cdot kg^{-1} \cdot min^{-1}$) completed two experimental trials. Each trial consisted of a standardized pre-trial snack (2-h prior) followed by 120 min of steady-state (SS) cycling (~60% $\dot{V}O_{2max}$) followed by a ~30 min TT, randomized as follows: 1) WASH: 30g $CHO \cdot h^{-1}$ during SS + CHO-rinse during TT (every 20% of TT); 2) PLA: 30g $CHO \cdot h^{-1}$ during SS + placebo-rinse during TT. **Results**: While WASH was not significantly different than PLA ($p = 0.51$), there was a 1.7% (+6.4 to -3.2% 90% CI; ES = 0.21) decrease in TT time (35s) for WASH compared to PLA, with qualitative probabilities of a 60% positive and 23% trivial outcome. For EMG, Soleus showed a significant increase while medial gastrocnemius showed significant decrease in muscle recruitment from the beginning 20% TT segment to the last 20% only in the PLA condition, which coincided with a slower ($p = 0.01$) last 20% of the TT in PLA vs. WASH. **Conclusions:** Contrary to previous studies, this investigation utilized conditions of high ecological validity including a pre-trial snack and CHO during SS. Significant changes in muscle recruitment and time over the last 20% of the TT, along with an average 1.7% improvement in TT time, suggest CHO mouth rinse helps maintain power output late in TT's compared to placebo. Although marginal gains were achieved with a CHO mouth rinse (35 s), small performance effects can have significant outcomes in real-world competitions.

[2]Jensen, M. P., Klimstra, M. D., Sporer, B., & Stellingwerff, T. (2018). Effect of carbohydrate mouth rinse on performance after prolonged submaximal cycling. Medicine & Science in Sports & Exercise, 50(5), 1031-38.

3.2 Introduction

Carbohydrate (CHO) is the primary substrate for high-intensity exercise (Hawley & Leckey, 2015), and CHO intake has a positive effect on endurance performance (Stellingwerff & Cox, 2014). The impact of CHO supplementation on metabolism and subsequent performance is multi-factorial and depends on; length and intensity of exercise, CHO intake rate, subject training status and even the type of exercise (Jeukendrup, 2004, 2010). Glycogen stores can become limiting during high-intensity/prolonged duration exercise (≥2 h), and the current consensus statement recommendations are for the consumption of ~30-60 g CHO·h^{-1} (of ~4-8% CHO solution) during an endurance event (T. Thomas et al., 2016). Importantly, it has been consistently shown that 30-50% of endurance athletes experience some level of gastrointestinal (GI) issues during endurance exercise (de Oliveira et al., 2014). Additionally, GI problems have been shown to be increased and more likely to occur in athletes with a history of GI problems (Pfeiffer et al., 2012), with CHO intake itself (de Oliveira & Burini, 2014) and as exercise time increases (Peters et al., 1993). Therefore, prolonged and intense endurance exercise with CHO intake provides the ideal context to induce an ergogenic effect, but also the potential conditions for GI upset.

It is proposed that CHO supplementation during exercise contributes to performance in several ways: 1) direct contribution of CHO energy via exogenous CHO oxidation and/or 2) mental/cognitive stimulation of the central nervous system (CNS) probably due to a stimulation of the pleasure and reward centers of the brain by CHO exposure to the oral cavity (for review see: (Stellingwerff & Cox, 2014)). Carter et al. (2004) were the first to demonstrate that 1 h TT performance could be improved by 2.9% by simply rinsing the oral cavity with a CHO solution and no ingestion. This study was mechanistically supported several years later by functional magnetic resonance imaging (fMRI) data showing that CHO mouth rinse stimulates the pleasure and reward centres of the brain (Chambers et al., 2009). Since this work, there has been a multitude of CHO mouth rinse studies (>15), with most studies (>10) demonstrating that CHO mouth rinse can improve performance (~1 h), especially when subjects are in the fasted state (for review see: (Jeukendrup, 2013)). During prolonged exercise (>2 h), with or without CHO supplementation, CHO depletion will occur, with more muscle glycogen depletion occurring in favour of preserving hepatic glucose levels (McConell, Fabris, Proietto, & Hargreaves, 1994b). However, the amount of muscle versus liver glycogen depletion is complex and depends on the exercise intensity and exogenous CHO intake rates. Accordingly, Ataide-Silva et al. (2016) demonstrated the largest performance benefits of CHO mouth rinse when subjects were both muscle and liver glycogen depleted, compared to fed or just liver glycogen depleted (via an overnight fast). During a performance with depleted glycogen, there is evidence that muscle activation and power changes reflect a fatigued state more so than during non-depleted conditions (Ataide-Silva et al., 2016; Lane et al., 2012). Taken together, this suggests that CHO mouth rinse may improve performance by modifying the perception and/or motor response to fatigue, which may be augmented with low endogenous CHO availability (liver and muscle glycogen), and thus allow athletes to work at higher power outputs compared to placebo conditions. However, CHO mouth rinsing to help late endurance performance (>2 h) is in its infancy, and to our knowledge, only one study has examined the effect CHO mouth rinse on late endurance performance with combined measures of muscle activity and performance (Luden et al., 2016).

In order to properly evaluate the effectiveness of CHO mouth rinse on performance, it is necessary to replicate the nutritional conditions before and during actual endurance race scenarios. To our knowledge, no study to date has looked at the effect of a CHO mouth rinse during late endurance exercise in conditions of high ecological validity. These conditions would include a typical CHO-rich standardized pre-competition snack and CHO intake during prolonged endurance exercise leading into an intense time-trial (similar to the last 30 min of a marathon or cycling race when potential GI upset will be the greatest). Therefore, this study aims to determine if CHO mouth rinse during a ~30 min cycling TT, after prolonged exercise (2 h), will enhance performance as compared to placebo (artificial sweetener). We hypothesized that CHO mouth rinse would improve power output and TT performance compared to placebo.

3.3 Methods

3.3.1 Participants

Ten well-trained male cyclists (age: 29 ± 9 years, body mass: 79.1 ± 9.9 kg, stature: 179.9 ± 8.5 cm, Maximal Oxygen Uptake ($\dot{V}O_{2max}$): 64.5 ± 6.5 $mL \cdot kg^{-1} \cdot min^{-1}$, Maximal Power Output ($W_{max}$): 405 ± 38 W) were recruited and gave verbal and written consent under Human Ethics at the University of Victoria. All subjects were regional to national level category 1 or 2 with Cycling British Columbia.

3.3.2 General experimental design

This study featured a double-blind, randomized, placebo-controlled crossover design, in which subjects made 4 visits to the laboratory (21.9 ± 0.5°C, 34.1 ± 10.4% relative humidity) with all cycling testing carried out using the same electronically braked ergometer (Velotron Pro, RacerMate Inc., USA). During visit 1, the subject performed a graded test to exhaustion to determine their W_{max} and $\dot{V}O_{2max}$. During visit 2 the subject performed an augmented practice session to familiarize to the experimental protocol and the TT and to confirm the ~60% $\dot{V}O_{2max}$ power output (208 ± 20 W) achieved during the 1 h steady-state (SS) session. Visits 3, and 4 were experimental trials, featuring 2 h at ~60% $\dot{V}O_{2max}$ followed by a TT in which a set amount of work

(Total work = 0.75 * W_{max} * 1800 s = 552 ± 48 kJ; (1996)) had to be performed as quick as possible over ~30 min (Figure 3-1).

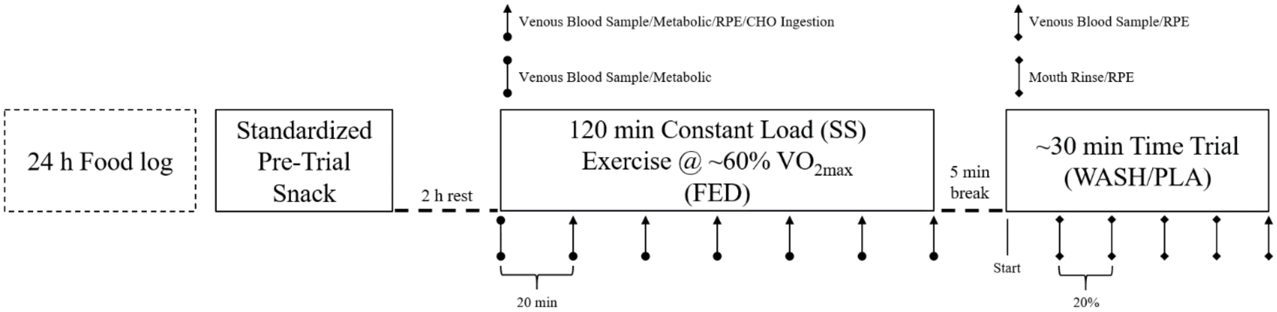

Figure 3-1: Experimental schema for visits 3-4. FED, 30 g·h^{-1} CHO; WASH, carbohydrate mouth rinse (4 x 25 mL 8% maltodextrin solution); PLA, placebo mouth rinse (taste and colour matched).

Body mass was measured using a standard scale (Avery Berkel HL120, Avery Weigh-Tronix Inc, Fairmont, MN, USA) prior to start of SS and upon completion of TT, which percent body mass loss was calculated as the difference between starting mass and mass upon completion of TT. Subjects received 1500 mL of liquid during each session and were not allowed water ad libitum. All urine samples were measured for Urine Specific Gravity (USG) using an Atago PAL-10S Refractometer (Atago, Bellevue, WA, USA).

The two randomized experimental interventions, all following a standardized CHO pre-trial snack 2 h prior (details below), were as follows:

1) WASH: 30 g CHO·h^{-1} during SS + CHO-mouth rinse during TT;

2) PLA: 30 g CHO·h^{-1} during SS + Placebo-mouth rinse (PLA) during TT;

During the SS and TT, heart rate (HR) (Polar T31, Polar Electro) and ratings of perceived exertion (RPE; Borgscale (6-20)) were taken according to the schedule outlined in Figure 3-1. During the SS ventilation, oxygen uptake ($\dot{V}O_2$) and carbon dioxide production were recorded (TrueOne 2400 metabolic cart, Parvo Medics, USA) according to the schedule outlined in Figure

3-1. Using indirect calorimetry calculations, energy expenditure, fat, and CHO oxidation rates during the SS were estimated. Blood glucose (Contour® Next EZ, USA) and lactate (Lactate Pro, Akray, Japan) were taken during the SS and immediately following the TT according to the schedule outlined in Figure 3-1. Upon laboratory arrival, each participant was prepared for EMG electrode placement by shaving hair and cleaning the skin with 70% alcohol. EMG electrodes (Delsys Trigno, USA) were then placed on anatomical landmarked sites over the *soleus* (SOL), *medial gastrocnemius* (MG), *tibialis anterior* (TA), *vastus lateralis* (VL), *rectus femoris* (RF), *vastus medialis* (VM), *biceps femoris* (BF) and *gluteus maximus* (GLUT) (Barbero et al., 2012). All measurements for electrode placement were recorded so that on subsequent trials all electrodes could be positioned in the same place. EMG was recorded at 2000 Hz through a 16-bit analog/digital converter (NI 6034E; National Instruments, Austin, TX). EMG signals resolved into EMG intensities using 10 wavelets (Von Tscharner, 2000), which act as a band-pass filter (11-432 Hz). The median frequency (MDF) and total intensity of the EMG signal was compared between conditions (20 pedal revolutions) and normalized to the first hour of the SS for each individual muscle. Total EMG intensity is a positive envelope quantifying the EMG and is equivalent to twice the square of the root-mean-square (RMS) of the EMG (Von Tscharner, 2000). Urine samples were collected prior to the start of SS. The experimental trials were separated by at least 7 days and each subject conducted their sessions at the same time (±1 h) to minimize the effect of diurnal variation.

3.3.3 Maximal workload capacity

After a 5-min warm-up at 150 W, the test started at a workload of 180 W and was then increased by 30 W every 3 min until the subject could no longer maintain the required power output. Ventilation, oxygen uptake ($\dot{V}O_2$) and carbon dioxide production were recorded continuously (TrueOne 2400 metabolic cart, Parvo Medics, USA). $\dot{V}O_{2max}$ was determined by the largest 60 s mean $\dot{V}O_2$ value. W_{max} was determined by: $W_{max} = W_{out} +((t/180)*30)$, W_{out} is the workload of the last completed stage and t is the time in seconds spent in the final stage (Jeukendrup et al., 1996). Heart rate, RPE and lactate were collected/recorded at the completion of each workload.

3.3.4 Pre-experimental testing and preparation

Participants were instructed to maintain regular training for the duration of the study and to abstain from any intense exercise, caffeine and alcohol in the previous 24-h before each session. Participants recorded their food and liquid consumption for 24-h prior to each experimental trial and repeated these diets for each subsequent trial. All-subjects received a standardized pre-trial snack that was to be consumed 2 h prior to the start of the experimental sessions (158 g CHO, 18 g Protein), to be inline with current position stand recommendations on pre-event fueling (2.1 ± 0.3 $g \cdot kg^{-1}$BM CHO, 0.24 ± 0.03 $g \cdot kg^{-1}$BM protein) (T. Thomas et al., 2016). Subjects were instructed to collect first urine void in the morning.

3.3.5 Time-Trial

There was no verbal encouragement and subjects were void of any distractions by being situated behind a barrier. The only visual feedback the subjects received was the percent work complete. At every 20% of work completed the subjects reported RPE and then received either CHO or PLA mouth rinse for 5 to 10 s, before expelling the entire amount (Figure 3-1), while HR was recorded continuously. Lactate and glucose were measured immediately following the TT. Following each trial, subjects were asked to rate how they felt during the SS and TT portions on a scale of 1 to 5 (1=Horrible to 5=Amazing) and were also probed as to whether they could identify which trial/solution they received to ensure blinding. Following the last experimental session, subjects were asked two additional questions: 1) Which session do you think you had the best performance? and; 2) In the future would you self-select a CHO mouth rinse during a TT or during the last 30 minutes of a road race? A urine sample was collected after completion of the TT and before any additional liquids were consumed.

3.3.6 Mouth rinse protocol / steady state solution

Each sample consisted of 25 mL of either 8% maltodextrin (GLOBE® 10 DE Maltodextrin, Ingredion, Canada) solution or taste matched placebo (PLA) which was weighed before and after mouth rinsing (Mettler PC 400, Switzerland) to account for any solution that may have been ingested. Both the WASH and PLA solutions (distilled water) were supplemented with 0.2% artificial sweetener (Crystal Light, Kraft Food, USA) to make the solutions indistinguishable. The mouth rinse solutions were coded by a non-affiliated researcher to ensure double-blinding. During SS, subjects were given 30 g CHO·h^{-1} in a 4% custom CHO solution (0.32 g·100 mL^{-1} Sucrose, 1.09 g·100 mL^{-1} maltodextrin, 0.2 g·100 mL^{-1} artificial sweetener, distilled water). This was equivalent to 1500 mL fluid during the 2 h SS, and 250 mL was consumed every 20 min (following removal of the metabolic mouthpiece) (Figure 3-1).

3.3.7 Statistical analysis

For measures of time to completion and power, a two-way ANOVA with repeated measures was used to examine main effects of 1) trial (WASH, PLA), 2) percentage of time-trial completed (time), and 3) trial x time interactions. Additionally, *a priori* planned orthogonal contrasts were made between the second (20-40%) and fourth (60-80%) intervals and between the third (40-60%) and fifth (80-100%) intervals to signify changes in performance between mid and late components of the TT respectively. For median EMG frequency and total EMG intensity, paired t-tests were used to compare the difference between the beginning (first 20%) and end (last

20%) of the time trial for each condition. Additionally, magnitude-based inference analysis was used to examine the influence of the CHO mouth rinse on time to completion, within conditions and paired t-tests (Sidak correction $p = 0.0169$) were used to compare between conditions (Hopkins et al., 2009). Log-transformed data were used to estimate the effect of CHO mouth rinse as the difference in mean percentage (with 90% confidence intervals (CI)) between PLA and WASH, trials. A value of 1% was used as the smallest worthwhile change in time to completion of a cycling TT (Malcata & Hopkins, 2014), with further magnitude-based inferences (Batterham & Hopkins, 2015) used to provide clinical insights into performance outcome effects representing a true change (benefit, harm or trivial). Chances of benefit or harm were determined as follows: 1-5%, very unlikely; 5-25% unlikely; 25-75%, possible; 75- 95%, likely; 95-99%, very likely; >99%, almost certain (Hopkins et al., 2009). All data is presented as mean (± standard deviations (SD), and significance is set at $p \leq 0.05$ unless otherwise stated. All statistics performed via Statistica (Version 13).

3.4 Results

3.4.1 Steady-State

All SS data is presented in Table 3-1. All exercise-induced metabolic, respiratory responses throughout the 2 h of SS exercise were as expected, and there were no significant differences between or within conditions for any variable (Table 3-1).

Table 3-1 :Metabolic, respiratory and perceptual responses during steady-state cycling with ingestion of CHO (30 g·h^{-1}; PLA & WASH). Mean ± SD, N = 10.

	Steady-State Cycling at ~60% $\dot{V}O_{2max}$						
	18-20 min	**38-40 min**	**58-60 min**	**78-80 min**	**98-100 min**	**118-120 min**	**Mean**
Lactate (mmol·L^{-1})							
PLA	1.1±0.2	1.3±0.5	1.1±0.2	1.2±0.4	1.2±0.3	1.3±0.5	1.2±0.2
WASH	1.5±0.6	1.5±0.6	1.2±0.4	1.3±0.4	1.4±0.7	1.6±0.7	1.4±0.3
Glucose (mmol·L^{-1})	2,3	1,4,5,6	1,4	2,3	2	2	
PLA	4.8±0.6	5.5±0.8	5.2±0.6	5.1±0.6	5.1±0.7	4.9±0.6	5.2±0.5
WASH	4.6±0.4	5.6±0.5	5.5±0.5	4.6±1.7	4.9±0.3	5.0±0.5	5.2±0.3
RPE	4,5,6	4,5,6	5,6	1,2	1,2,3	1,2,3	
PLA	11±2	11±2	11±2	11±2	12±2	12±2	11±2
WASH	11±2	11±2	11±2	12±2	12±2	12±2	11±2
Fat Ox (g·min^{-1})	2,4,5,6	1,5,6	5,6	1,5,6	1,2,3,4,6	1,2,3,4,5	
PLA	0.48±0.16	0.53±0.18	0.52±0.17	0.56±0.20	0.62±0.23	0.71±0.27	0.57±0.49
WASH	0.45±0.11	0.51±0.10	0.49±0.16	0.51±0.13	0.59±0.15	0.70±0.17	0.54±0.12
CHO Ox (g·min^{-1})	4,5,6	5,6	5,6	1,5,6	1,2,3,4,6	1,2,3,4,5	
PLA	2.53±0.44	2.45±0.48	2.45±0.42	2.39±0.54	2.23±0.57	1.99±0.63	2.34±0.49
WASH	2.60±0.39	2.49±0.40	2.51±0.52	2.43±0.34	2.24±0.36	1.99±0.28	2.38±0.34

$^{1\text{-}6}$ Collapsed trial significant time difference between SS intervals, ($p < 0.05$), i.e.: 1 represents 18-20 min interval, 2 represents 38-40 min

RPE (Rating of Perceived Exertion), Fat Ox (Fat Oxidation), CHO Ox (Carbohydrate Oxidation)

3.4.2 Time-Trial performance

There was a significant improvement in the time to complete the final 20% (interval 80-100%) vs interval 40-60% (Figure 3-2) during the WASH trial ($p = 0.01$), whereas PLA showed no significant changes in interval time ($p > 0.05$) (Figure 3-2). For time to complete the TT, there was no significant difference between WASH and PLA ($p = 0.51$) (Figure 3-3). Although not significant, subjects completed the WASH TT 1.7% (+6.4 to -3.2% 90% CI: Cohen ES=0.21) faster compared to PLA treatment (35 s), with qualitative probabilities demonstrating a 60% and 23% chance of a likely positive or trivial outcome, respectfully (Figure 3-3). The individual differences and group mean in time to complete TT across both trials are shown in Figure 3-3.

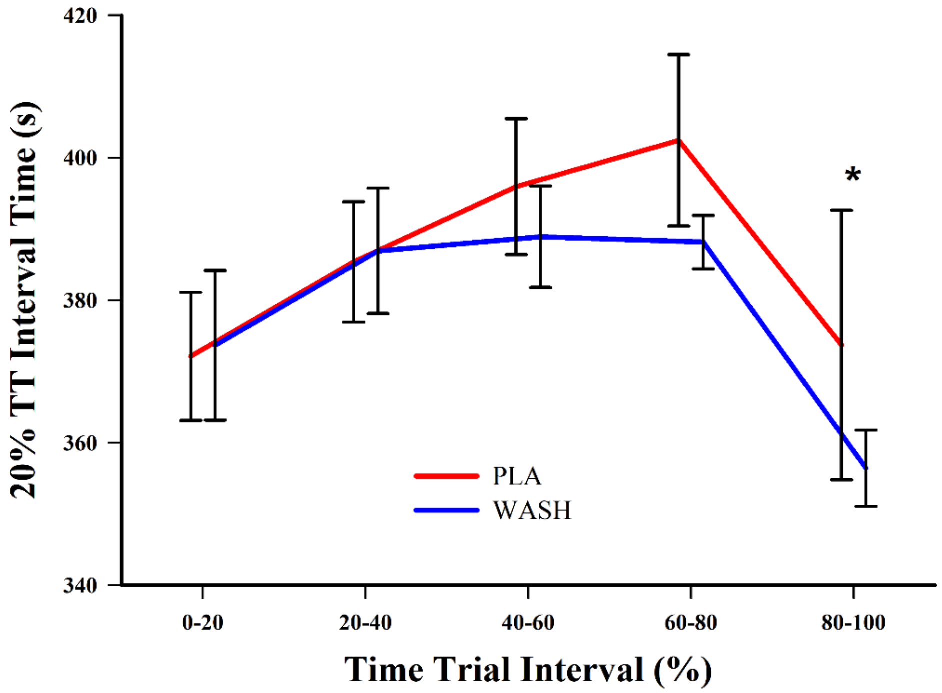

Figure 3-2: Mean performance interval time for every 20% of the required workload for WASH, and PLA trials (Mean ± SE). * Indicates a significant difference between interval times 40-60% and 80-100% for WASH (*p* = 0.01).

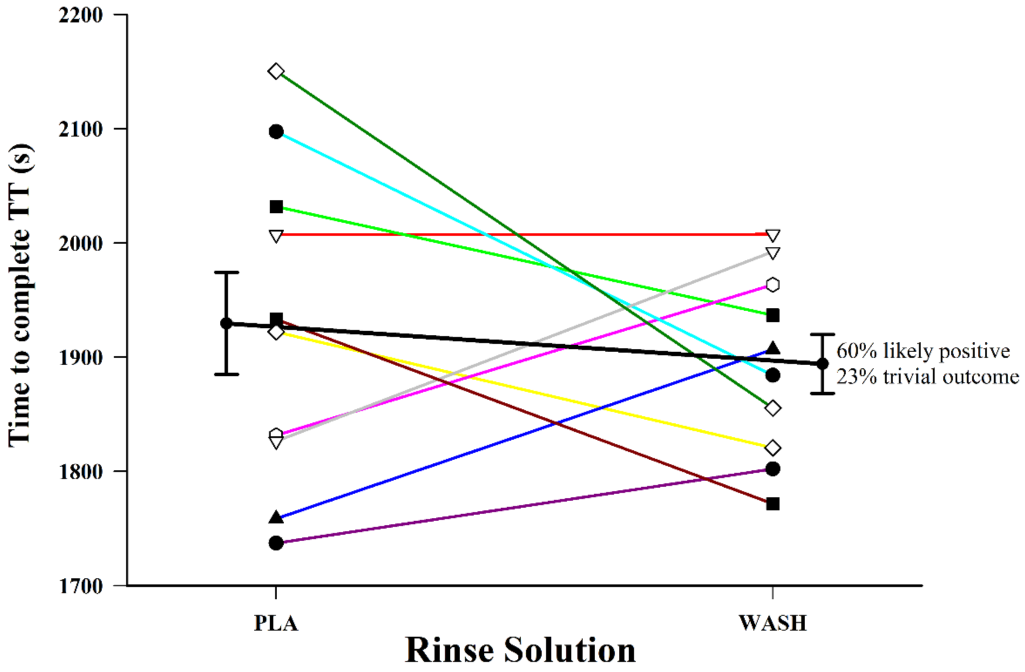

Figure 3-3: Individual subject (coloured lines) and mean (black line) time to complete time trial (TT) in the WASH and PLA trials (Mean ± SE). Qualitative probabilities of CHO mouth rinse effect are shown.

For EMG frequency analysis between the beginning (first 20%) and the end (final 20%) of the TT, there was a significant increase in MDF for SOL only during WASH ($p = 0.006$; Figure 3-4A). Conversely, there was a significant decrease in MDF of MG only during PLA ($p < 0.001$; Figure 3-4B). The remaining lower extremity muscles measured did not show significant changes for MDF or total EMG intensity, therefore, only the MDF results from the SOL and MG muscles are presented. There were no significant between trials differences for TT cadence, HR and RPE but typical temporal changes for each parameter throughout the TT (Table 3-2). There were no trial differences for mouth rinse time (7.2 ± 1.0 s), rinse volume (24.56 ± 0.4 mL) and average spit volume (24.14 ± 0.6 mL). Post TT lactate ($mmol \cdot L^{-1}$) was 7.5 ± 3.7 and 6.8 ± 2.3; glucose ($mmol \cdot L^{-1}$) was 5.4 ± 1.2 and 5.6 ± 0.9 for PLA, and WASH respectively.

Table 3-2: Metabolic measures, cycling power and perceptual measures during cycling time-trial. Mean ± SD, N = 10.

	Time Trial Interval (%)					
	20	**40**	**60**	**80**	**100**	**Mean**
Heart Rate (bpm)	2,3,4,5	1,5	1,5	1,5	1,2,3,4	
PLA	153±10	162±10	162±11	163±11	168±13 [b]	161±10
WASH	152±9	160±8	162±8	165±10 [a]	170±10 [b]	162±9
Cadence (rpm)	3,4,5	4,5	1	1,2	1,2	
PLA	100±6	99±8	97±9	96±9 [a]	96±9	98±8
WASH	99±7	97±7	96±7	96±7	95±8	97±7
RPE (Borg)	2,3,4,5	1,4,5	1,5	1,2,5	1,2,3,4	
PLA	16±2	16±1	17±1	17±1 [a]	19±1 [b]	17±1
WASH	16±1	16±1	17±1	17±1 [a]	19±1 [b]	17±1
Power (W)	3,4	5	1,5	1,5	2,3,4	
PLA	297±25	287±29	280±29	275±35	303±47 [b]	287±29
WASH	297±33	286±27	284±25	284±20	310±26 [b]	291±23

[a] Significant effect of time for 40% vs 80% within a given trial ($p < 0.05$).
[b] Significant effect of time for 60% vs 100% within a given trial ($p < 0.05$).
[1-5] Collapsed trial significant time difference between TT intervals, ($p < 0.05$), i.e.: 1 represents 20% interval, 2 represents 40% interval.
RPE (Rating of Perceived Exertion)

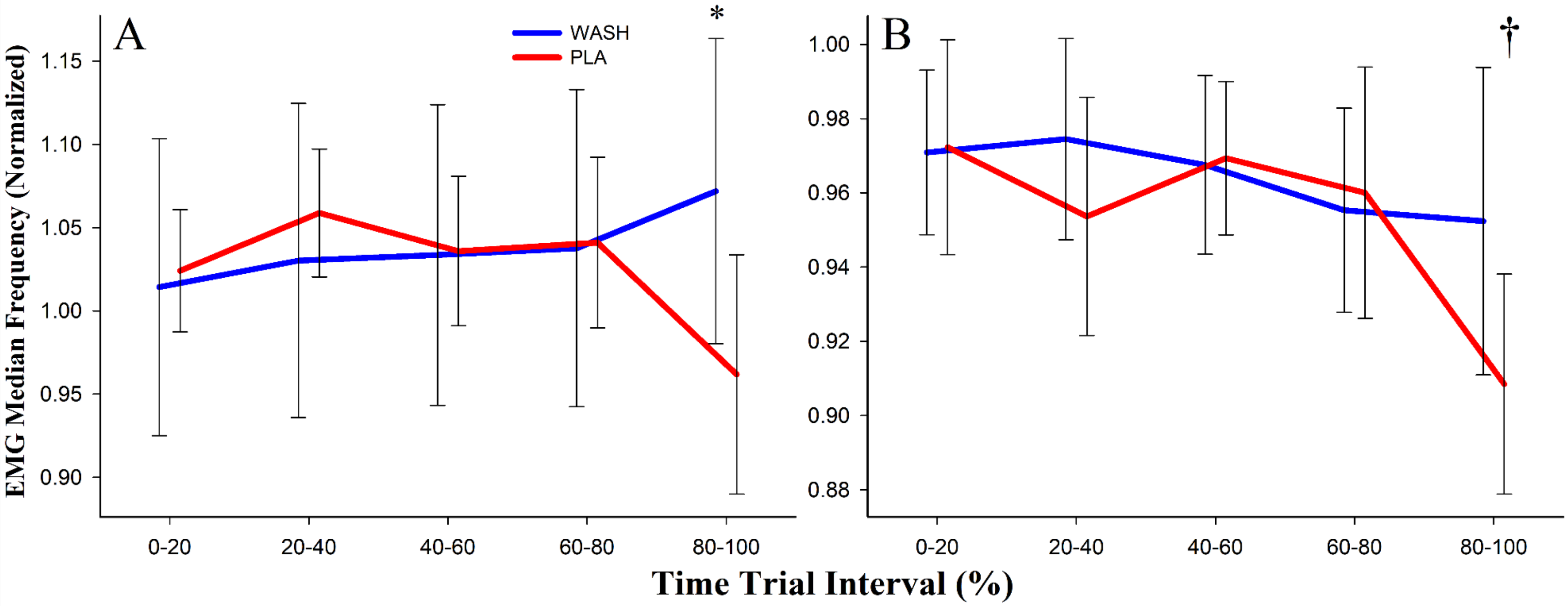

Figure 3-4: Median frequency (MDF) for A) Soleus and B) Medial Gastrocnemius, normalized to mean of the first hour of steady state. (Mean ± SE). Significant difference between 0-20% and 80-100% interval of time trial indicated by * for Soleus WASH and † for Medial Gastrocnemius PLA ($p < 0.05$).

3.4.3 Pre/Post trial hydration status and fluid intakes

The total SS fluid consumed (mL) was 1500 ± 1.4 and 1500 ± 1.8 for PLA, and WASH, respectively. There was a significant decrease in body mass (kg) for PLA ($p < 0.001$) and WASH ($p < 0.001$), 3.16 ± 0.56 and 2.94 ± 0.49, respectively. There were no differences in hydration status between trials assessed via USG or % loss in body mass (USG: average morning void 1.018 ± 0.007 and pre-trial 1.009 ± 0.004).

3.4.4 Perception and blinding questionnaires

From the 20 trials, subjects indicated they could distinguish a taste difference 10 times. However, subjects identified the correct solution only 15% of the time indicating blinding was effective. There was no significant difference on how the subjects felt on the 5-point feeling scale during SS (3.6 ± 0.5 and 3.5 ± 0.9 for PLA and WASH, respectively). There was a very strong trend on how subjects felt during the TT between the WASH and PLA trials ($p = 0.051$) (Feeling scale were 2.6 ± 1.2 and 3.2 ± 1.1 for PLA and WASH, respectively).

3.5 Discussion

Overall, the results of this study support a small positive performance benefit of CHO mouth rinse during the late stages of high-intensity cycling TT, after prolonged sub-maximal cycling in a nutritionally ecologically valid condition. Specifically, we observed that when a CHO mouth rinse was used, both time to completion and power (during different 20% portions) did not change throughout as opposed to a noticeable decrement in performance when a CHO mouth rinse was not used. On average, this resulted in a 1.7% (35 s) faster TT for WASH compared to PLA treatment, with qualitative probabilities demonstrating a 60% and 23% chance of a likely positive or trivial outcome, respectfully. It is important to note that the performance improvement was not observed in all participants suggesting that the response may be dependent on the individual. This is the first study to observe differences in measures of muscle activity associated with CHO mouth rinse from multiple leg muscles. These results mechanistically support modification in muscle activation in lower leg muscles elicited by CHO mouth rinse. The positive performance effect coupled with the change in muscle activity support a potential reduction of neuromuscular fatigue with CHO mouth rinse during a TT after a prolonged endurance task.

3.5.1 Time-trial performance effects of CHO mouth rinse after prolonged sub-maximal exercise

The maintenance of a consistent time to completion and power with the use of CHO mouth rinse during different portions of a time trial observed in the present study is similar to other studies examining shorter durations of exercise (<1 h) (Chambers et al., 2009; Lane et al., 2012). For example, Chambers et al. (2009) showed that power was better maintained during the CHO mouth rinse compared to placebo, particularly in the late stages of the exercise and there was no difference in their subjects RPE. Our findings suggest that while the CHO mouth rinse in WASH did not result in a statistical ($p > 0.05$) increase in power and decrease in time to completion as compared to PLA, it does not result in the same decrement in performance during the last half of the TT observed when CHO mouth rinse is not used as in the placebo condition. The current results would suggest that during prolonged activity (>1 h) CHO mouth rinse may partially mitigate the fatiguing effect of an endurance bout for some athletes (6 out of 10 athletes of the current study). As an example of the impact that an intervention might have on the minute differences in elite sport, a 35 s improvement in performance is the difference between gold and 7th place in the ~45 min women's 2016 Olympic cycling TT (IOC, 2016a) and the difference between gold and 15th place in the ~27 min men's 2016 Olympic 10,000m event (IOC, 2016b). These results are consistent to previous studies examining CHO mouth rinse on performance and are especially relevant to endurance sport as this study utilized a protocol that incorporates the longest, most ecologically valid exercise paradigm to date to investigate the effects of CHO mouth rinse on performance.

3.5.2 Ecological validity considerations of CHO mouth rinse studies- in field application

When interpreting the utility of a CHO mouth rinse for endurance sport, it is important to consider the experimental protocols used to examine the effects of CHO mouth rinse and the degree to which these study protocols replicate the real world of elite performance endurance sport. Investigations of the performance effects of CHO mouth rinse have mostly been focused on shorter duration activities (<1 h) where endogenous CHO is not a limiting factor (Carter, Jeukendrup, & Jones, 2004; Gam et al., 2013). Prolonged exercise requires the nearly continuous ingestion of CHO to allow for sustained and high CHO oxidation rates to enhance performance (Stellingwerff & Cox, 2014). However, CHO intake has been known to cause GI problems in some athletes during later stages of a race (Pfeiffer et al., 2012). As glycogen starts to deplete during prolonged exercise, the positive effect of CHO intake significantly increases performance (r = 0.356; p = 0.004; (Stellingwerff & Cox, 2014)) and the potential benefit of a CHO mouth rinse may have added benefit (Stellingwerff & Cox, 2014).

Athletes who experience GI upset related to CHO ingestion may benefit from the use of CHO mouth rinse to limit the amount of CHO that is required to be ingested during the later stages of an event and also during very high-intensity exercise (e.g. interval workouts) when blood shunting away from the GI tract can cause GI issues (Neufer, Young, & Sawka, 1989). Beyond Luden et al. (2016), this is only the second study to add to the current knowledge of the effect of CHO mouth rinse on high-intensity performance towards the end of prolonged exercise (≥2 h). This is particularly important to some athletes participating in endurance sport as for example, commonly the last 30 min of a marathon or cycling race is when potential GI upset related to ingestion of CHOs will be the greatest and ultimately the most limiting to performance (Peters et al., 1993).

Previously, Luden et al. (2016) reported that CHO mouth rinse used during a ~3 min TT after 3 h of exercise with no CHO ingestion resulted in a 3.8% improvement in performance. Although it is important to establish a potential improvement during late endurance exercise in a state where nutritional depletion and neuromuscular fatigue is prevalent, the level of nutritional depletion utilized by Luden et al. (2016) is not representative of real-world conditions, as some ingestion of CHO is required during prolonged exercise situations (T. Thomas et al., 2016). In the current study, we tested endurance-trained athletes following typical race conditions or prolonged training sessions. During these sessions athletes would commonly consume a meal ~2 h prior to competition, followed by consumption of CHO during early to mid stages of exercise and potential use of the CHO mouth rinse during the later stage of exercise to mimic the last sustained effort (push) to the finish line. To our knowledge, no other study to date has looked at the effect of a CHO mouth rinse during the late stages of endurance activity in conditions of high ecological validity that include a typical CHO-rich standardized pre-trial snack and CHO intake during prolonged steady-state exercise (2 h), followed by a ~30 min TT. Therefore, these results are applicable to athletes in sports with demands of prolonged racing (>1 h) which culminates in a final maximal exertion such as a sustained period (~30 min) of high-intensity exercise followed by a sprint to the finish line.

It has been shown that CHO stimulates receptors in the mouth that activate areas of the brain associated with motor control and reward (Chambers et al., 2009). It has been suggested that CHO mouth rinse may be more effective when performed in a post-absorptive overnight fasted state (when the oral receptors have a greater sensitivity to CHO) (Ataide-Silva et al., 2016; Lane et al., 2012; Trommelen et al., 2015). Fasting is thought to sensitize CHO receptors and therefore result in greater central drive to improve performance in response to CHO (Lane et al., 2012). Additionally, the effect of a CHO mouth rinse on performance has been reported to be greater where muscle glycogen in diminished (Ataide-Silva et al., 2016). Therefore, the majority of previous studies have incorporated a fasting protocol and were designed to fatigue/deplete athletes in an attempt to maximize the potential observed benefit of a CHO mouth rinse (Ataide-Silva et al., 2016; Chambers et al., 2009; Lane et al., 2012; Trommelen et al., 2015). The present results may also support the beneficial effect of CHO mouth rinse during a late endurance bout when muscle glycogen stores are diminished as the effect is only noticed during the later portions of the protocol as the TT component may start with reduced muscle glycogen.

3.5.3 Muscle fatigue response

Examination of the MDF content of the EMG signals suggests that fibre type/motor unit recruitment may differ during a fatiguing bout of exercise performed with or without CHO mouth rinse. The decreasing MDF of the MG EMG signal during PLA may demonstrate fatiguing muscle activity with decreased recruitment of type II fibres and increased representation of type I fibres active within the muscle (Ataide-Silva et al., 2016). Although not significant, a similar decrease in MDF is visually evident in SOL EMG signal during the PLA condition when looking at the last 20% of the TT (Figure 3-4A). These findings of muscle activation may suggest the potential of modification of central neural drive related to fatigue in the triceps surae muscle group during PLA. This potential evidence of muscle fatigue and a concomitant decrease in power output is thought to be an internal neural protective strategy that results from reduced CHO availability (Ataide-Silva et al., 2016). The increase in MDF of the SOL EMG signal and the maintenance of MDF of the MG EMG signal during the WASH condition suggests that both an increase and maintenance of recruitment of type II fibres may be present to reduce negative effects of neuromuscular fatigue to maintain constant cycling power output (Duc & Grappe, 2005). In summary, these findings show that fatigue of the plantar flexor muscles may only be present during PLA, while during WASH this fatigue is not evident, possibly supporting the role of CHO mouth rinse in delaying the onset of type II motor unit de-recruitment to maintain performance (Jeffers et al., 2015).

However, this beneficial performance effect observed during WASH may only be short-lived as greater recruitment of type II fibres would result in a greater ability to maintain power output, but also greater chance of failure due to fatigue as a result of glycogen depletion and the greater reliance of type II fibres on anaerobic metabolism (Beaven et al., 2013; Chong et al., 2011). Evidence of this short-term centrally mediated increase in performance with CHO mouth rinse, with a later erosion in power outputs / performance, has been observed during cycling sprint performance (Beaven et al., 2013). Additionally, this short-term benefit is similar to that observed during an acute performance bout where CHO mouth rinse reduced the attenuation in maximal leg extension force in a fatigued state, but later bouts were not benefited by CHO mouth rinse (Jensen et al., 2015). Thus, the effect of CHO mouth rinse may be to prolong the onset of fatigue to some muscles while allowing other muscles to recruit more motor units, to enable an acute enhancement of power output and performance. These results are also very intriguing considering the different physiological fibre compositions (Johnson, Polgar, Weightman, & Appleton, 1973) and biomechanical roles of the triceps surae muscles observed in our study. That is, SOL has a greater percentage of Type I fibres compared to MG (Johnson et al., 1973) and is a single joint muscle preferentially recruited for high resistances, whereas MG is a multi-joint muscle preferentially recruited for actions that require high shortening velocities (Wakeling & Horn, 2009). Taken together the differing results in MG and SOL during the study conditions could support complex muscle recruitment strategies dependent on CHO availability.

3.5.4 Conclusion

Under conditions of high ecological validity, the current study suggests CHO mouth rinse appears to have a marginal impact on TT performance after a prolonged endurance task in a fed state for some participants. This performance effect occurs concurrently with evidence of changes in muscle recruitment potentially associated with fatigue. In real-world competitions, this may have a meaningful impact on performance outcomes. These findings are encouraging for endurance athletes and may be particularly meaningful to those who demonstrate GI intolerance of CHO ingestion at later stages of the race.

4 The effect of carbohydrate mouth rinse on cycling neuromechanics in late endurance performance

4.1 Abstract

Studies have shown improved performance during high-intensity short duration exercise (~1 h) with the use of CHO mouth rinse (WASH); however, the impact of CHO mouth rinse on performance during longer endurance event (>90 min) is limited. **Purpose:** To identify the impact on muscle coordination and cycling biomechanical parameters when using WASH during a cycling time-trial (TT) completed after 2 h of sub-maximal cycling using advance neural and biomechanical analysis. **Methods**: Each trial consisted of 120 min of steady-state (SS) cycling (~60% $\dot{V}O_{2max}$) followed by a ~30 min TT, randomized as follows: 1) WASH: 30g $CHO{\cdot}h^{-1}$ during SS + CHO-rinse during TT (every 20% of TT); 2) PLA: 30g $CHO{\cdot}h^{-1}$ during SS + placebo-rinse during TT. Principal component analysis was used to establish muscle coordination, and power output profile between conditions. **Results**: Only the PLA condition showed an increase in muscle excitation in TA, RF and GLUT between the start and end of the TT ($p < 0.01$). This increase in activation was suggestive of increased muscle fatigue during the PLA condition but not in the WASH condition. Additionally, the phase shift in power output profile was more pronounced in PLA, resulting in the later application of power, while WASH had an earlier onset of power in the pedal cycle. **Conclusions:** These findings suggest that CHO mouth rinse played a significant role in mitigating the effects of fatigue that are often associated with a decrease in cycling efficiency. This shows for the first time that CHO mouth rinse may have a substantial effect on the maintenance of power while mitigating the impact of neuromuscular fatigue, in late endurance performance.

4.2 Introduction

Recent studies have shown that simply rinsing the mouth with a CHO solution (CHO mouth rinse), without consumption, can improve performance in high-intensity endurance exercise (~1 h) (see review: Peart, 2017). Additionally, early findings in late endurance performance (>2 h) suggest that, without CHO supplementation, CHO mouth rinse may be beneficial when glycogen stores start to diminish (Luden et al., 2016). During a prolonged performance with associated glycogen reductions (fasted), evidence from muscle activation and cycling power output reflect a fatigued state more so than during fed states (non-glycogen limiting) (Ataide-Silva et al., 2016; Lane et al., 2012). Reductions in muscle activation and power production during fatigue may be a neural protective strategy to limit force production to maintain finite fuel resources and limit tissue damage (St Clair Gibson et al., 2001). This is consistent with investigations in rodent models which suggest that reduction in muscle glycogen results in impairment in exercise performance due to central fatigue as opposed to peripheral mechanisms (Williams, Batts, & Lees, 2013). Interestingly, during exercise in energy-depleted conditions, a CHO mouth rinse has shown the effect of recovering activation and cycling power output to non-depleted levels compared to a placebo condition (Ataide-Silva et al., 2016). A possible mechanism for this potentially short lasting improvement is through the enhanced neural drive to motor units from a supraspinal source (Chambers et al., 2009), also referred to as central motor drive (CMD) (Gandevia, 2001). While not entirely understood the mechanism of performance improvement attributed to CHO mouth rinse is thought to be at least partly due to mental/cognitive stimulation of the central nervous system (CNS) probably due to a stimulation of the pleasure and reward centers of the brain by CHO exposure to the oral cavity (Chambers et al., 2009). Overall, while there is evidence of a beneficial effect of CHO mouth rinse on performance, the impact and mechanism are unclear, and greater understanding of the effect, especially during endurance performance is required.

The pattern of pedal force application is remarkably consistent among cyclist (Hug, Drouet, Champoux, Couturier, & Dorel, 2008). The use of higher resolution data collection throughout the pedal cycle has allowed the creation of advanced biomechanical parameters that could distinguish slight differences in the pattern of pedal force application, such as differences between elite and recreational riders (Bertucci et al., 2008). This more detailed analysis of the pattern of pedal force application, together with advances in the neuromechanical analysis of cycling (Blake et al., 2012; Wakeling & Horn, 2009) has improved the potential to investigate kinetic and kinematic changes between interventions such as the use of ergogenic aids during cycling. Novel approaches to EMG decomposition have been developed to allow the amplitude, timing and frequency content of an EMG signal to be simultaneously resolved using non-linear scaled wavelets of specified resolution (Von Tscharner, 2000). Changes in the EMG signal, in both frequency and time, provide evidence of modifications of muscle fibre recruitment strategies (Von Tscharner, 2002; Wakeling, Kaya, Temple, Johnston, & Herzog, 2002; Wakeling, Pascual, et al., 2002) and therefore may be used to distinguish muscle recruitment differences in states of CHO depletion (e.g. glycogen utilization) or due to the effect of a CHO mouth rinse. Further, principal component analysis (PCA) has been used to both extract wavelets specifically tuned to isolate the predominant high- and low-frequency bands of the EMG signals (Lee, de Boef, Arnold, Biewener, & Wakeling, 2011), as well as determine the muscle recruitment patterns associated with higher efficiencies (Blake & Wakeling, 2015). Additionally, PCA of cycling dynamics can be useful to isolate the fundamental mechanical components and has been used to provide evidence that muscle coordination, power output and overall mechanical efficiency are dependent on the distribution of power (Blake & Wakeling, 2012; Wakeling et al., 2010). Only a few studies have looked at EMG in multiple muscles during CHO mouth rinse in conditions where fatigue and energy depletion may impact performance, and no studies have attempted to use advanced neuromechanical analysis to assess the neurophysiological and biomechanical effect of CHO on performance. While some studies have shown neural evidence to support important changes because of CHO mouth rinse on performance (Ataide-Silva et al., 2016; Jeffers et al., 2015; Jensen et al., 2015), the use of advanced EMG analysis across multiple muscles in endurance cycling may enable the ability to determine modifications in muscle recruitment and fatigue related to CHO mouth rinse. This may provide further insight into the potential mechanisms of performance improvements as well as help with guidelines around its proper utilization in athletes.

Therefore, the purpose of this study was to apply advanced neuromechanical analysis to late endurance cycling with and without CHO mouth rinse to determine the changes in cycling dynamics and lower limb muscle activation associated with this intervention. The role of CHO mouth rinse is to act as an ergogenic aid that will aid in isolating mechanisms associated with fatigue during prolonged cycling. This study aims to understand the changes in activation of lower limb muscles that may occur during the late stages of an endurance cycling protocol related to the use of CHO mouth rinse.

4.3 Methods

4.3.1 Participants

Ten well-trained male cyclists (age: 29 ± 9 years, body mass: 79.1 ± 9.9 kg, stature: 179.9 ± 8.5 cm, Maximal Oxygen Uptake ($\dot{V}O_{2max}$): 64.5 ± 6.5 $mL\cdot kg^{-1}\cdot min^{-1}$, Maximal Power Output at $\dot{V}O_{2max}$ (W_{max}): 405 ± 38 W) were recruited and gave verbal and written consent under Human

Ethics at the University of Victoria. All subjects were regional to national level category 1 or 2 with Cycling British Columbia.

4.3.2 General experimental design

Each cyclist attending four separate sessions. The first two sessions included a graded test to exhaustion to determine W_{max} and $\dot{V}O_{2max}$ and an augmented practice session to familiarize subjects to the experimental protocol. Following this, each subject completed two experimental trials (each on separate days) in a double-blind, randomized, placebo-controlled crossover design. Each experimental trial featured 2 h cycling at 60% $\dot{V}O_{2max}$ followed by a time trial (TT) in which a set amount of work (Total work = 0.75 * W_{max} * 1800 s = 552 ± 48 kJ; (Jeukendrup et al., 1996)) had to be performed as quick as possible over ~30 min (Figure 3-1). All data during the TT was split into 5 equal intervals (TT1-5) based on 20% of the total work done. All cycling testing carried out using the same electronically braked ergometer (Velotron Pro, RacerMate Inc, USA) and ergometer geometry was matched to each subject's own bicycle as close as possible.

The two randomized experimental interventions, both following a standardized CHO pre-trial snack 2 h prior (details below), were as follows:

1) WASH: 30 g $CHO \cdot h^{-1}$ during SS + CHO-rinse every 20% during TT;

2) PLA: 30 g $CHO \cdot h^{-1}$ during SS + Placebo-rinse every 20% during TT;

Upon laboratory arrival, each participant was prepared for EMG electrode placement by shaving hair and cleaning the skin with 70% alcohol. EMG electrodes (Delsys Trigno, USA) were then placed on the right leg over anatomical landmarked sites of the *soleus* (SOL), *medial gastrocnemius* (MG), *tibialis anterior* (TA), *vastus lateralis* (VL), *rectus femoris* (RF), *vastus medialis* (VM), *biceps femoris* (BF) and *gluteus maximus* (GLUT) (Barbero et al., 2012). The entire right leg was then covered with a tubular net bandage to reduce motion artifacts. All anatomical landmarks and associated measurements for electrode placement were recorded so that on subsequent trials all electrodes could be positioned in the same place. EMG was recorded at 2000 Hz through a 16-bit analog/digital converter (NI 6034E; National Instruments, Austin, TX).

A custom device was designed to collect the torque signal from a cycling power meter (Science PowerMeter, Schoberer Rad Meβtechnik (SRM), Julich, Germany) at high temporal resolution, along with measurement of angular velocity every 10 degrees (°) of crank rotation (Figure 1-3). The custom device also sent out a pulse signal at top dead centre (TDC) (0°) and bottom dead centre (BDC) (180°) which was used to time align the EMG signal with cycling power output. Instantaneous crank torque and angular velocity from each 10° segment were used to calculate power output for each 10° segment.

The dataset used for Chapter 3 and Chapter 4 was the same, for complete experiment design, please refer to methods 3.3 (p. 64).

4.3.3 EMG analysis

The EMG signals from the TT were resolved into intensities in both time and frequency space with a set of EMG-specific wavelets (Von Tscharner, 2000). Intensity (I_{EMG}) of the EMG signal at a given time for each individual muscle was given by the sum of intensities across all 10 wavelets domains (k=1-10), which is a measure of power within the signal. The selected wavelets give a frequency bandwidth of ~11 – 432Hz and act as a band-pass filter. The EMG intensities were interpolated to 360 points per muscle per pedal cycle starting at TDC (12 o’clock; see Figure 4-7) of the right crank arm. The intensities were normalized to the mean for each muscle for each subject from the average EMG intensity during the first hour of their steady-state cycling bout. The total EMG intensity (I_{tot}) was calculated for each individual muscle and represents the sum of the interpolated intensities over 20 complete pedal cycles. From each time trial interval, 20 pedals from the end of the interval were used in the analysis. Cycles, where the first wavelet (wavelet 0; centre frequency ~6.9 Hz) had the highest intensity of any wavelet for a particular muscle, were discarded due to potential contamination of movement artifact. In addition, some muscles were identified as having a large amount of signal (more than ten times the intensity of any other cycles) at the first two wavelets (wavelets 0 and 1; centre frequencies ~6.9 and 19.3 Hz) with the intensity in the second wavelet larger than the first wavelet. Therefore, we additionally discarded any cycles where the intensity of the first two wavelets for a specific muscle were both larger than any other wavelet (except for GLUT).

4.3.4 Cycling biomechanical parameters

To explore the effect of condition (PLA/WASH) on cycling power output during the TT, the high-resolution data output was interpolated to 10 evenly spaced data points for every 10° segment. These data points were joined to create 360 data points per revolution. This resulted in one data point per degree and accurate to each 10° segment. Biomechanical parameters used for this analysis are presented in Table 4-1, and each metric was calculated separately for the left and right leg based on top and bottom dead centre.

Table 4-1: Cycling Biomechanical Parameters.

Metric	Description
AVG-P	The average power in watts from TDC to BDC for each leg
MAX-P	The maximum power that occurred during the downstroke of each leg
EPR	Effective Power Range = Range of pedal stroke for each leg where the power was greater than the average power calculated for the whole pedal stroke

4.3.5 Principal Component analysis

Principal component analysis (PCA) was used to transform the EMG and power data sets to a reduced number of dimensions that describe the major features of the data (Wakeling & Horn, 2009). The reduced set of principal components (PC) used in the analysis make up the most common features of both the EMG intensity signal and power output profile, and therefore explain most of the dataset variance. EMG intensities from all 8 muscles and 360 interpolated cycling power output data were used to form separate data matrices. For cycling power output, a 360 (interpolated power samples) X 1800 (total number of pedal cycles [9 subjects’ × 20 pedals × 5 TT intervals × 2 Experimental Conditions]) data matrix was constructed. For EMG intensities, a 2800 (8 muscles × 360 interpolated data points per pedal cycle) × 1200 (total number of pedal cycles [6 subjects’ × 20 pedals × 5 TT intervals × 2 Experimental Conditions]) data matrix was constructed. The principal components were calculated from the EMG intensity and power data without prior subtraction of the mean, to ensure the whole signal was described and not just its variance (Wakeling & Rozitis, 2004). A Covariance matrix was calculated from each data matrix, and the power output (P_w) and EMG intensity (I_w) PC weightings were determined from the eigenvectors of the covariance matrix. The relative proportion (%) explained by each PC was given by eigenvector x covariance matrix. The product of the transpose of the EMG and power eigenvector matrices and the original EMG and power matrices produced the loading scores of each pedal cycle on each PC ($I_{PC,LS}$ = EMG Intensity loading score; $P_{PC,LS}$ = Cycling Power Profile loading score).

4.3.6 EMG optimized wavelets

The EMG intensities at high- and low-frequencies were calculated using optimized wavelets for each muscle (Hodson-Tole & Wakeling, 2007). The sum of EMG intensities for each wavelet creates an intensity spectrum (one intensity per wavelet) for each cycle that is similar to the power spectrum. PCA was calculated on these intensity spectra, and two wavelets were constructed that were optimized for the high- and low-frequency bands of each muscle. Linear combinations of the first two PCs that yielded the highest and lowest mean frequencies, while still generating positive intensities at all frequencies, were calculated and used to construct the two wavelets using least-squares minimization of a wavelet function (Hodson-Tole & Wakeling, 2007). The calculated parameters of the optimized wavelets are presented in Table 4-2.

EMG intensities for the high- and low-frequency bands were calculated from the optimized wavelets using the same method as described above (see EMG analysis 4.3.3), for the entire wavelet bank, and interpolated to 360 intensities per wavelet per cycle. PCA was calculated on these EMG intensities using 720 intensities per cycle (360 EMG intensities for each of the high and low-frequency bands). For each muscle, the high- and low-frequency components of the EMG signal were reconstructed using the linear combination of the first 10 PCs and the loading scores for each respective condition-trial combination (Figure 4-4). In addition to the reconstructed EMG intensities for the high-frequency bands and low-frequency bands, the difference between the last time trial interval (TT5) and the first time trial interval (TT1) for each frequency band was calculated to identify 1) intensity amplitude changes, and 2) phase shift in intensity during the pedal cycle (Figure 4-4).The total of each high EMG intensity band ($I\mathrm{H}_{\mathrm{tot}}$) and low EMG intensity band ($I\mathrm{L}_{\mathrm{tot}}$) were calculated for each muscle and pedal cycle as the sum of the interpolated intensities.

Table 4-2: Parameters of optimized wavelets.

	Centre frequency (Hz)	Scale	r with reconstructed spectra
SOL			
Low	81.00	0.103	0.925
High	160.97	0.048	0.922
MG			
Low	91.38	0.068	0.977
High	196.61	0.071	0.966
TA			
Low	79.33	0.084	0.980
High	174.96	0.071	0.954
VL			
Low	69.01	0.116	0.990
High	124.14	0.097	0.972
RF			
Low	69.50	0.117	0.988
High	145.34	0.048	0.921
VM			
Low	72.80	0.097	0.990
High	143.51	0.068	0.975
BF			
Low	63.78	0.063	0.984
High	185.34	0.051	0.898
GLUT			
Low	37.29	0.143	0.997
High	92.60	0.066	0.962

4.3.7 Statistical analysis

For measures of I_{tot}, a three-way ANOVA with repeated measures was used to examine main effects of 1) condition (WASH, PLA) 2) time-trial intervals (time; 20% segment) 3) muscle, and 4) trial x time x muscle interactions. For $I_{PC1-5,LS}$, a three-way ANOVA with repeated measures was used to examine main effects of 1) condition (WASH, PLA; COND) 2) time-trial interval (TT1-TT5;TT) 3) loading score (LS), and 4) COND × TT × LS interactions. When a significant main effect or interaction was identified from the ANOVAs, post hoc Tukey tests were used to distinguish specific significant differences.

To examine the individual frequency bands of $I\mathrm{H}_{\mathrm{tot}}$ and $I\mathrm{L}_{\mathrm{tot}}$, for each condition, a three-way ANOVA with repeated measures was used to examine main effects of 1) frequency band (HIGH, LOW; HZBAND), 2) time-trial interval (TT1-TT5; TT), 3) muscle and 4) HZBAND × TT × muscle interactions. For measures of median frequency (MDF), a three-way ANOVA with repeated measures was used to examine main effects of 1) condition (WASH, PLA; COND) 2) time-trial interval (TT1-TT5; TT) 3) muscle, and 4) COND × TT × muscle interactions.

For average power output, a two-way ANOVA with repeated measures was used to examine main effects of 1) trial (WASH, PLA), 2) percentage of time-trial completed (time), and 3) trial × time interactions. Additionally, *a priori* planned orthogonal contrasts were made between TT2 (20-40% work done) and TT4 (60-80% work done) intervals and between TT3 (40-60% work done) and TT5 (80-100% work done) intervals to signify changes in performance between mid and late components of the TT respectively.

The power data were reconstructed for WASH and PLA separately, based on the relationship between specific biomechanics parameters and the PC loading scores. The power output profile was reconstructed for each condition for TT1 and TT5, using the first 5 PCs that describe the major features of power output (99.6%). The biomechanical parameters were tested individually as the dependent variable with subject as a random factor, time trial interval and solution as fixed factors and the first 5 cycling power output loading scores as covariates using ANCOVAs. Cadence was included as a covariate in each statistical analysis, except when it was the dependent variable. For each biomechanical parameter, if the $\mathrm{P}_{\mathrm{PC,LS}}$ from the ANCOVA had a significant effect on the power output, then the product of the PC weightings and $\mathrm{P}_{\mathrm{PC,LS}}$ were used to reconstruct the power output, with rank order of the top 100 (best) and bottom 100 (worst) pedal cycles based on each biomechanical parameter. If the $\mathrm{P}_{\mathrm{PC,LS}}$ had no significant effect on the factor then the mean $P_{PC,LS}$ for each PC weighting was used to reconstruct the power output profile (Blake, 2012). Thus, the main power output profile that occurred with the best and worst values for given biomechanical parameter was used to reconstruct the power output profile. Pairwise Pearson correlations coefficients were also determined for all biomechanical parameters and first 5 $P_{PC1\text{-}5,LS}$. For $P_{PC1\text{-}5,LS}$, a three-way ANOVA with repeated measures was used to examine main effects of 1) condition (WASH, PLA; COND) 2) time-trial interval (TT1-TT5; TT) 3) loading score (LS), and 4) COND × TT × LS interactions. All biomechanical parameters between WASH and PLA were assessed using paired t-tests.

Additionally, magnitude-based inference analysis was used to examine the influence of the CHO mouth rinse on time to completion, within conditions and paired t-tests (Sidak correction $p = 0.017$) were used to compare between conditions (Hopkins et al., 2009). Log-transformed data were used to estimate the effect of CHO mouth rinse as the difference in mean percentage (with 90% confidence intervals (CI)) between PLA and WASH, trials. A value of 1% was used as the smallest worthwhile change in time to completion of a cycling TT (Malcata & Hopkins, 2014), with further magnitude-based inferences (Batterham & Hopkins, 2015) used to provide clinical insights into performance outcome effects representing a true change (benefit, harm or trivial). Chances of benefit or harm were determined as follows: 1-5%, very unlikely; 5-25% unlikely; 25-75%, possible; 75- 95%, likely; 95-99%, very likely; >99%, almost certain (Hopkins et al., 2009).

All data are presented as mean (± standard deviations (SD)), and significance is set at $p \leq 0.05$ unless otherwise stated. All statistics performed via Statistica (Version 13) and SPSS (Version 24).

4.4 Results

4.4.1 EMG

Visualizations of mean total EMG intensities of WASH indicates a larger intensity for MG, VL and BF compared to PLA during TT1, while SOL and VM had a larger intensity compared to WASH during TT5 (Figure 4-1). For I_{tot} there was a significant effect of COND × TT × Muscle (F(28,140)=1.69; $p = 0.03$), which yielded significant differences between conditions (PLA/WASH) for the GLUT muscle at each of the time point of TT1 ($p < 0.01$), TT2($p < 0.01$), TT3($p = 0.02$), TT5($p < 0.01$), and for the TA muscle at TT4 ($p = 0.02$). There were significant differences between time trial intervals TT1 and TT5, within the PLA condition for I_{tot} for both the TA and GLUT muscles' ($p < 0.01$), and no significant differences in the I_{tot} of any muscle between time points in the WASH condition (Figure 4-1). The largest total EMG intensity for TA, RF and GLUT muscles was during TT5 in the PLA condition, with TA and GLUT being significantly higher than during TT1.

The first 20 principal components (PCs) explained 95.5% of the EMG intensity signal, with the first 5 accounting for 79.2% ($I_{\mathrm{PC1\text{-}5}}$). $I_{\mathrm{PC1,W}}$ is the main PC, representing the mean of the EMG intensity. Notably, of the remaining four PCS, $I_{\mathrm{PC2,W}}$ explains 21.0% of the variance of the EMG intensity. $I_{\mathrm{PC2,W}}$ shows an increase in activation of GLUT immediately after TDC and a decrease in activation of TA leading into BDC and approaching TDC (Figure 4-2). The first 5 PCs can be visualized through the summation of the $I_{\mathrm{PC1\text{-}5,W}}$ and the $I_{\mathrm{PC1\text{-}5,LS}}$ specific to each condition (PLA and WASH)(Figure 4-2). The mean loading scores of $I_{\mathrm{PC2,LS}}$ were significantly different between the WASH and PLA conditions for all TT intervals (1-5) ($p < 0.01$, Figure 4-2) with $I_{\mathrm{PC2,LS}}$ during WASH being negative for all TT intervals and positive during TT intervals of the PLA condition. For $I_{\mathrm{PC,LS}}$ there was a significant effect of COND × TT × LS (F(16,1904) = 9.86; $p < 0.01$). $I_{\mathrm{PC1,LS}}$

was significantly lower for TT1 ($p < 0.01$), TT2 ($p < 0.01$) and TT5 ($p = 0.01$) during the WASH condition compared to PLA. For $I_{PC4,LS}$ and $I_{PC5,LS}$ there was significant differences between conditions during TT5 only. During the WASH condition, $I_{PC4,LS}$ was significantly lower ($p < 0.01$) and $I_{PC5,LS}$ was significantly higher compared to during the PLA condition ($p < 0.01$) (Figure 4-2).

The MDF of the EMG showed a significant effect of COND × TT × Muscle (F(28,112)=1.71; $p = 0.03$). The MDF of the EMG of the SOL muscle showed a significant decrease from TT1 (first 20%) to TT5 (final 20%) of the time trial during the PLA condition ($p < 0.01$.

The EMG intensity bands, IH_{tot} and IL_{tot}, showed a significant main effect of HZBAND × TT × MUSCLE (F(28,149)=2.26; $p < 0.01$). During the PLA condition only, total high-frequency bands and low-frequency bands showed a significant increase in intensity from TT1 to TT5 for the TA ($p < 0.01$), RF ($p < 0.01$), and GLUT ($p < 0.01$) muscles (Figure 4-3). Examination of the optimized wavelets for the high-intensity bands and the low-intensity bands showed that the first 10 PCs explained over 95% of the EMG intensity profile for all muscles. Visualization of the reconstructed EMG intensities for the optimized wavelets is shown in figure (Figure 4-4).

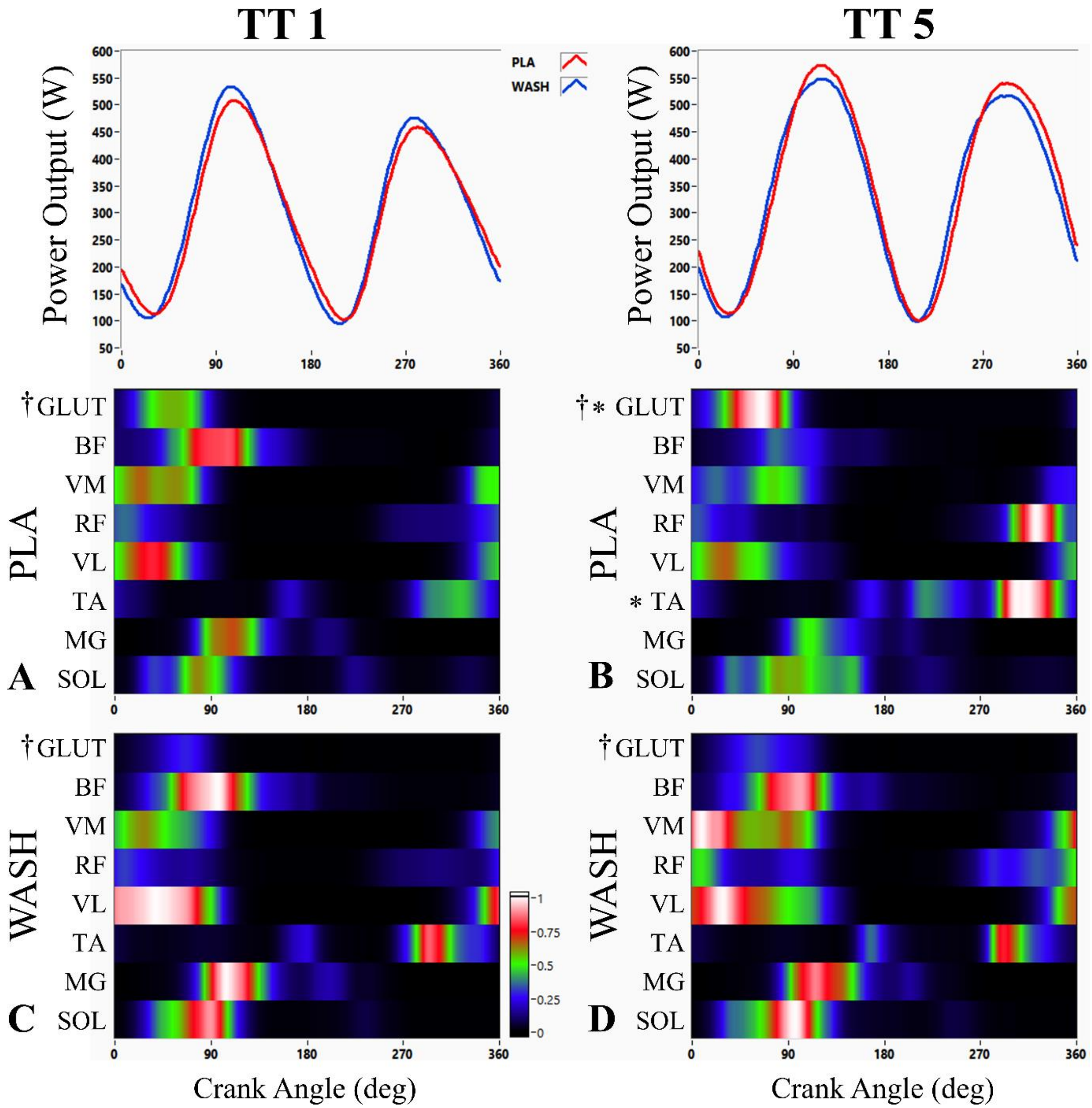

Figure 4-1: Average power output for PLA (red) and WASH (blue) and muscle coordination patterns for A) PLA TT1, B) PLA TT5, C) WASH TT1, and D) WASH TT5; mean EMG intensity pattern for each condition is normalized to the maximum of each muscle across all conditions. * denotes a significant difference in muscle total EMG intensity between TT1 and TT5 for PLA condition. † denotes significance in muscle total EMG intensity between PLA and WASH, $p < 0.05$. SOL, soleus; MG, medial gastrocnemius; TA, tibialis anterior; VL, vastus lateralis; RF, rectus femoris; VM, vastus medialis; BF, biceps femoris; GLUT, gluteus maximus.

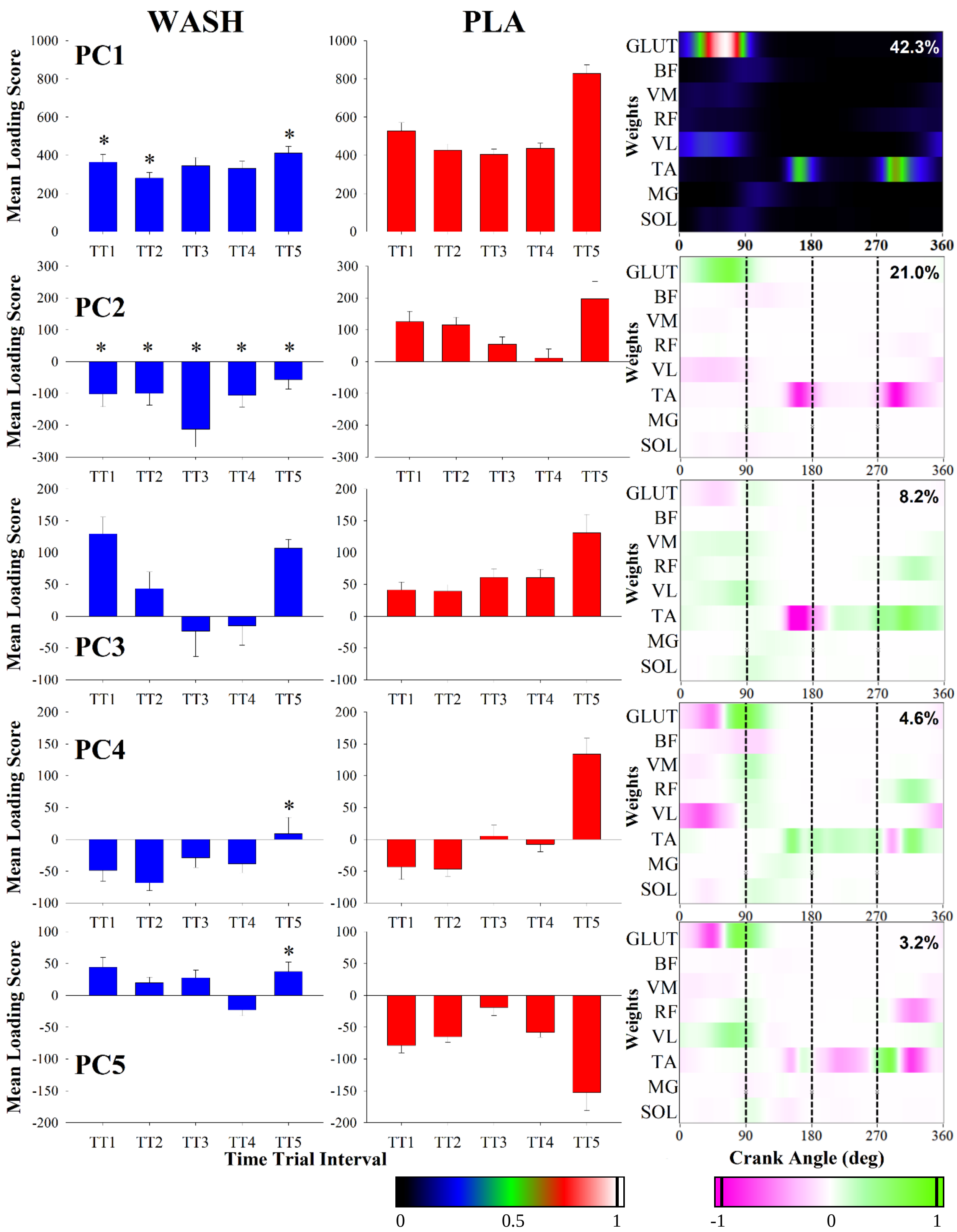

Figure 4-2: Mean loading scores for EMG intensity (*I*) principal components ($I_{PC1\text{-}5,LS}$) and weightings ($I_{PC1\text{-}5,W}$) (eigenvector) including relative proportion of the total signal they describe. Bars show mean ± SE. * denotes significance between PLA and WASH, $p<0.05$.

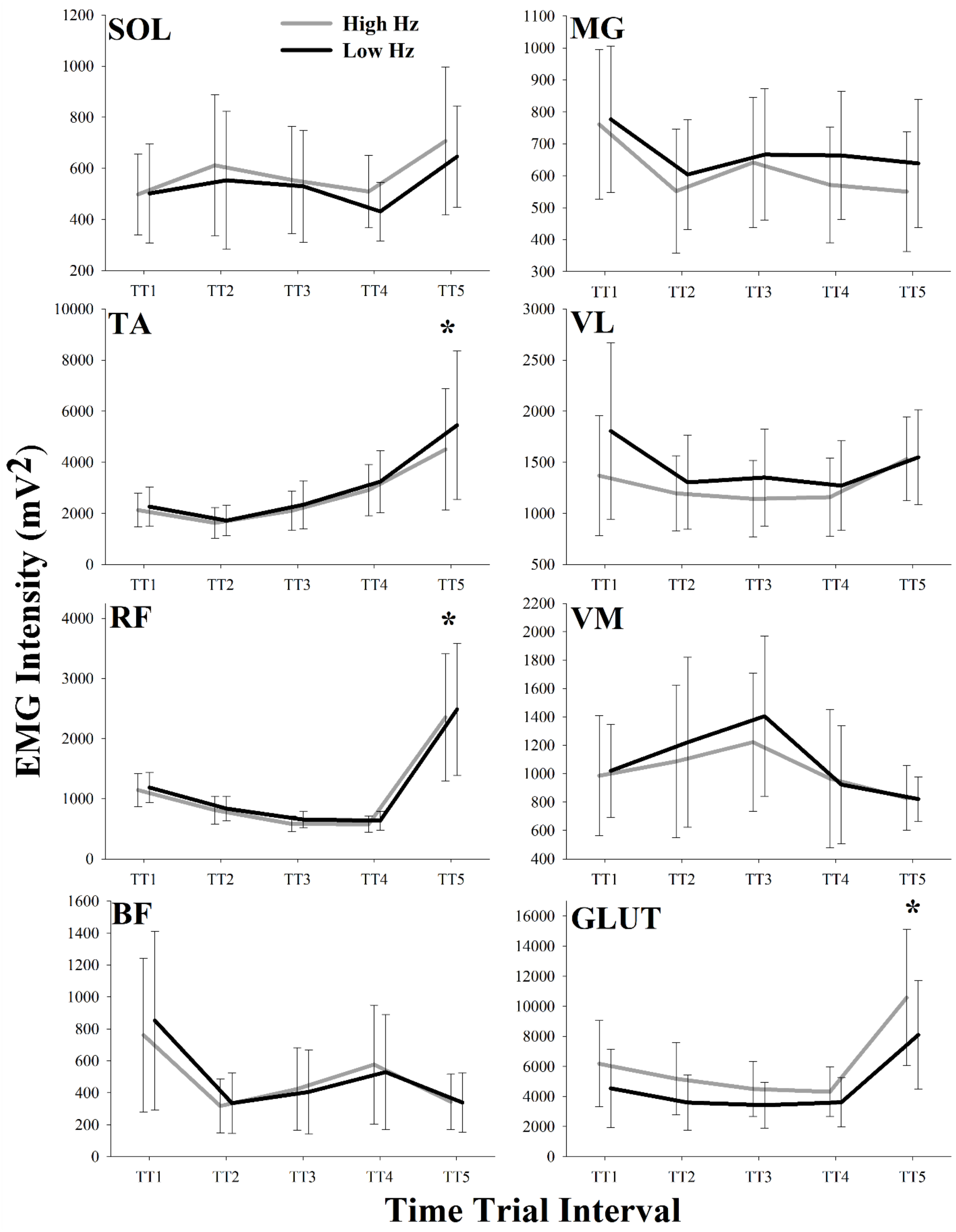

Figure 4-3: High (IH_{tot}) and Low(IL_{tot}) optimized wavelet EMG intensity for each time trial interval for PLA condition (Mean ± SE). * denotes significant difference between TT1 and TT5, $p<0.05$.

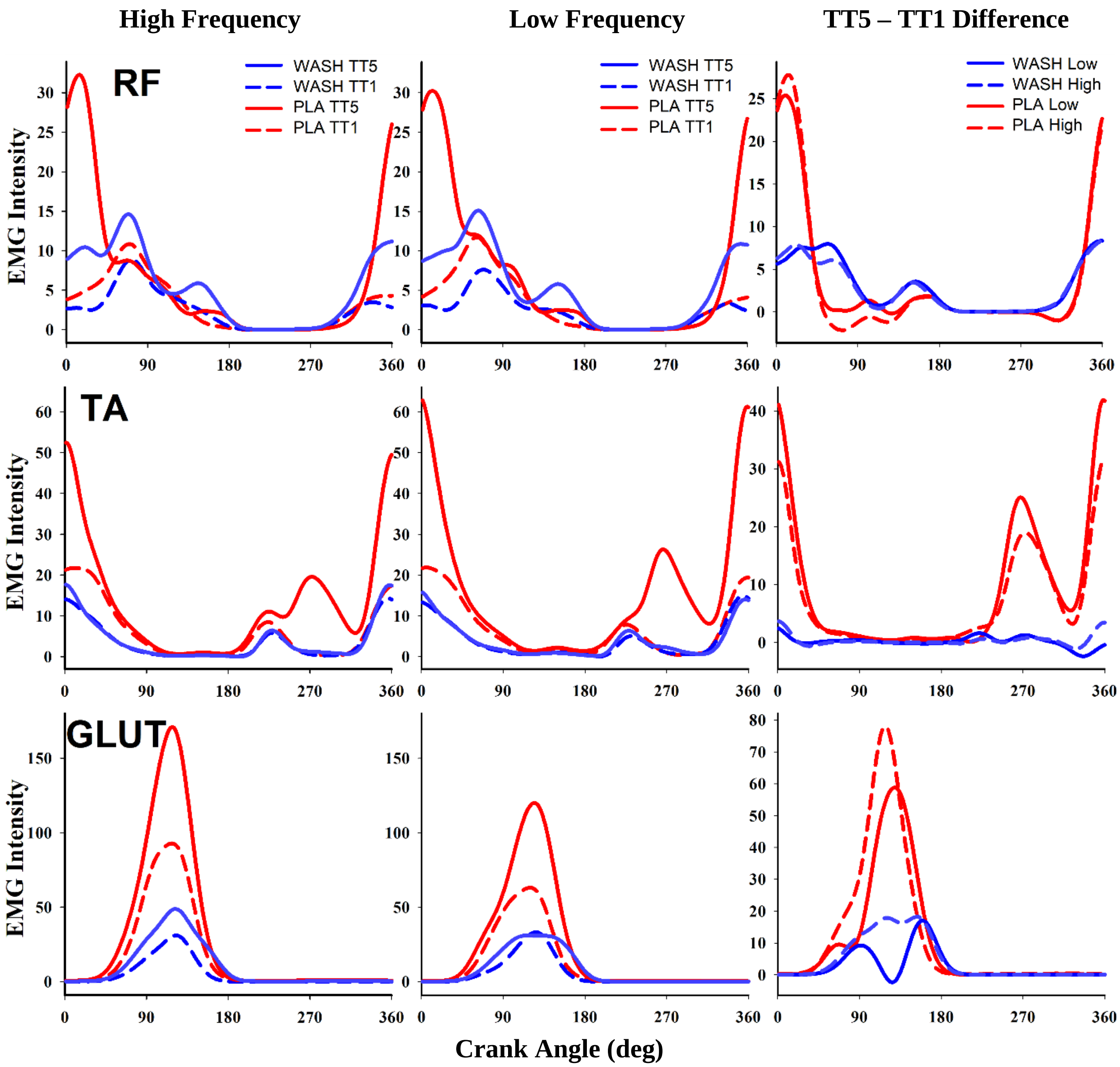

Figure 4-4: Reconstructed mean EMG intensities for High- and Low-frequency band using optimized wavelets for RF, TA and GLUT for TT1 and TT5. Difference between TT5 and TT1 for each frequency band is represented in the last column for each muscle.

4.4.2 Time-Trial performance

There was a significant improvement in the time to complete the final 20% (TT5) vs TT3 during the WASH trial (*p* = 0.01), whereas PLA showed no significant changes in interval time (*p* > 0.05) (Figure 3-2). For time to complete the TT, there was no significant difference between WASH and PLA (*p* = 0.51) (Figure 3-3). Although not significant, subjects completed the WASH

TT 1.7% (+6.4 to -3.2% 90% CI: Cohen ES=0.21) faster compared to PLA treatment (35 s), with qualitative probabilities demonstrating a 60% and 23% chance of a likely positive or trivial outcome, respectfully (Figure 3-3).

The mean power output for the time trial was 286.8 ± 52.7 W and 291.5 ± 47.1 W for PLA and WASH respectively. However, for mean power output during the TT, there was no significant difference between WASH and PLA ($p = 0.96$). There was a significant improvement in the average power output during the final 20% of the time trial, TT5 vs TT3 during the WASH trial ($p < 0.01$) and PLA trial ($p = 0.01$) (Figure 4-5). For the PCA reconstructed cycling power output profile, the first 5 PCs explained 99.6% of power profile, with the first PC explaining 95.4%, an approximate equivalent to the mean of the signal $P_{PC1,W}$ (Figure 4-7). The changes in power output profile can be visualized by their PC loading scores and eigenvectors (weightings) (Figure 4-6). $P_{PC2,W}$ highlights a shift in power production, with a shift to earlier in the pedal cycle, while $P_{PC2,LS}$ for PLA is negative for all TT intervals, while for WASH only TT3 and TT5 have a negative mean loading score. $P_{PC3,W}$ indicates a pedal stroke asymmetry between left and right leg, with greater reliance on the right leg that is associated with the positive $P_{PC3,LS}$ that is only observed during TT5 for both WASH and PLA. $P_{PC4,W}$ demonstrates a pedal asymmetry related to the condition, with a decrease in left leg power production associated with a negative mean loading score. There was no significant difference between WASH and PLA for $P_{PC4,LS}$. However, PLA had a greater decrease in mean LS throughout the TT, indicating a greater decrease in left leg power production the last half of the TT. $P_{PC5,W}$ only explains 0.18% of the power profile signal; however, the oscillation in the signal may indicate a decrease in power profile smoothness during the progression of the TT as $P_{PC5,LS}$ decreases for both WASH and PLA (Figure 4-6).

For $P_{PC,LS}$ there was a significant effect of COND × TT × LS (F(16,2866) = 2.72; $p < 0.01$), which yielded significant differences between conditions (PLA/WASH) for $P_{PC1,LS}$ TT3($p < 0.01$), TT4($p < 0.01$), and $P_{PC2,LS}$ TT4 ($p < 0.01$). There was a significant correlation between average power output and $P_{PC1,LS}$ ($r = 0.98$). The correlations between the loading score that are significantly associated with the biomechanical parameters are shown in Table 4-4. To visualize the impact each biomechanical parameter had on the overall power output profile, the top (best) and bottom (worst) from each parameter were used to reconstruct the power output profile (Figure 4-8).

Table 4-3: Correlation coefficients between PC loading scores and biomechanical parameters.

Loading Score	L Max-P	L EPR	L AVG-P	R Max-P	R EPR	R AVG-P	Average
$P_{PC1,LS}$	0.838*	0.196*	0.926*	0.844*	-0.026	0.938*	0.980*
$P_{PC2,LS}$	0.059*	-0.060*	0.172*	-0.072*	-0.190*	0.150*	0.170*
$P_{PC3,LS}$	0.383*	0.317*	0.432*	0.054*	-0.347*	0.153*	0.313*
$P_{PC4,LS}$	-0.410*	-0.434*	-0.301*	0.103*	0.418*	0.200*	-0.063*
$P_{PC5,LS}$	-0.052*	0.051	0.018	-0.257*	-0.150*	-0.133*	-0.057*

* Correlation is significant at the $p < 0.05$ level

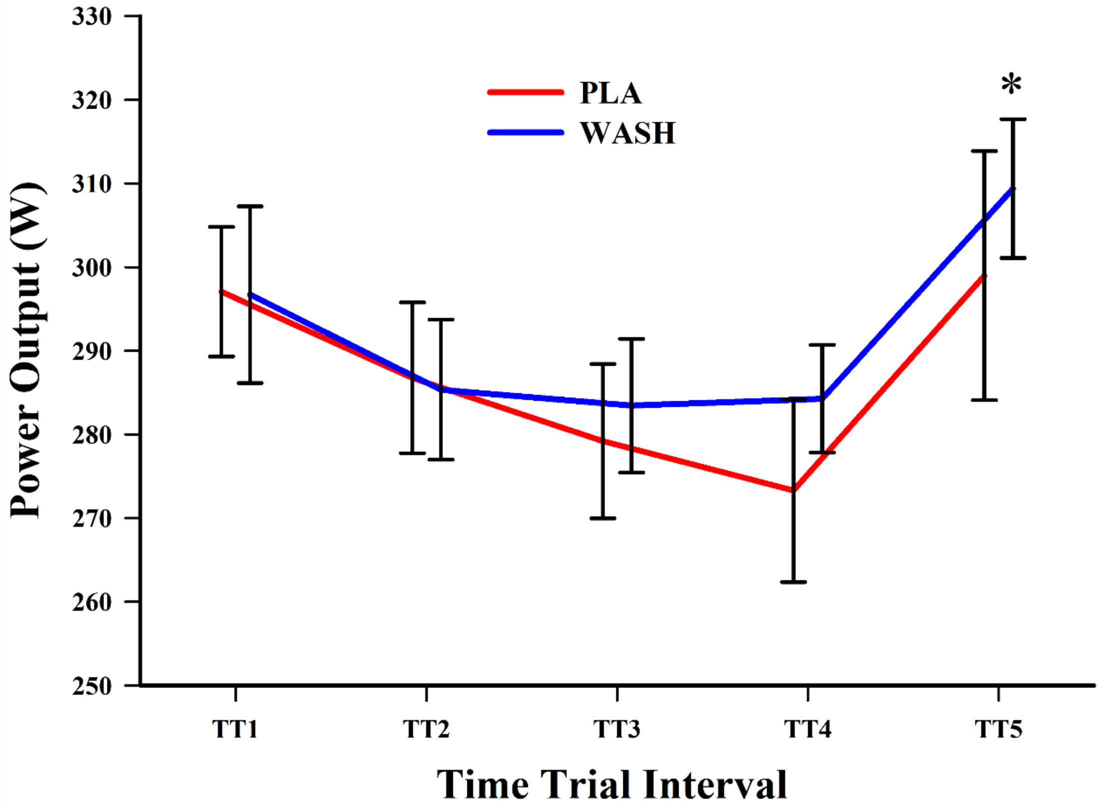

Figure 4-5: Mean performance power output during each 20% of the required workload for WASH, and PLA trials, (n = 9; Mean ± SE). * Indicates a significant difference between TT3 and TT5 for WASH (*p*= 0.01).

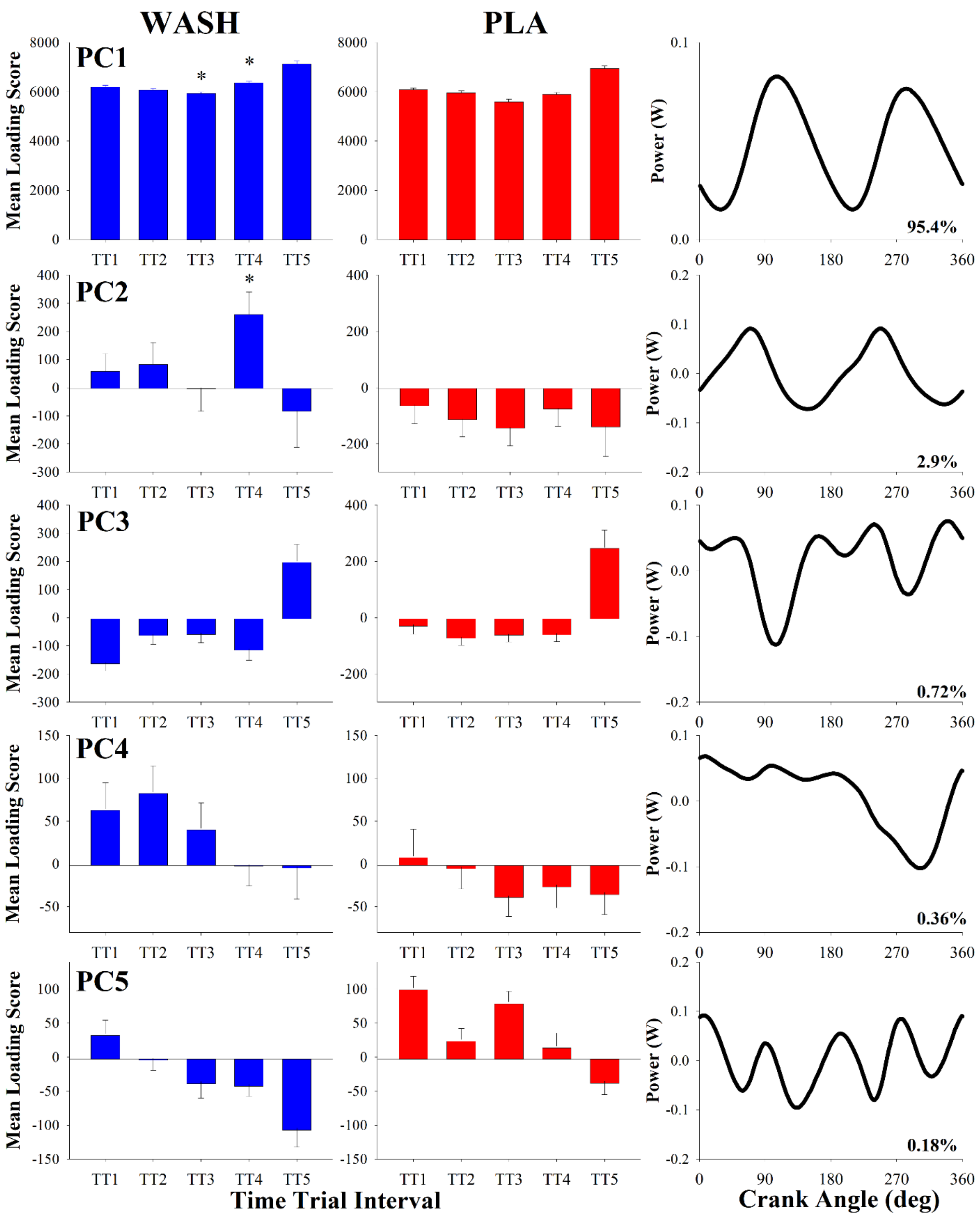

Figure 4-6: Mean loading scores for the first five power profile principal components (P_{PC}) and weightings (eigenvector) for the power output profile (including relative proportion of the total profile they describe). Bars show mean ± SE. * denotes significant difference between PLA and WASH, $p<0.05$.

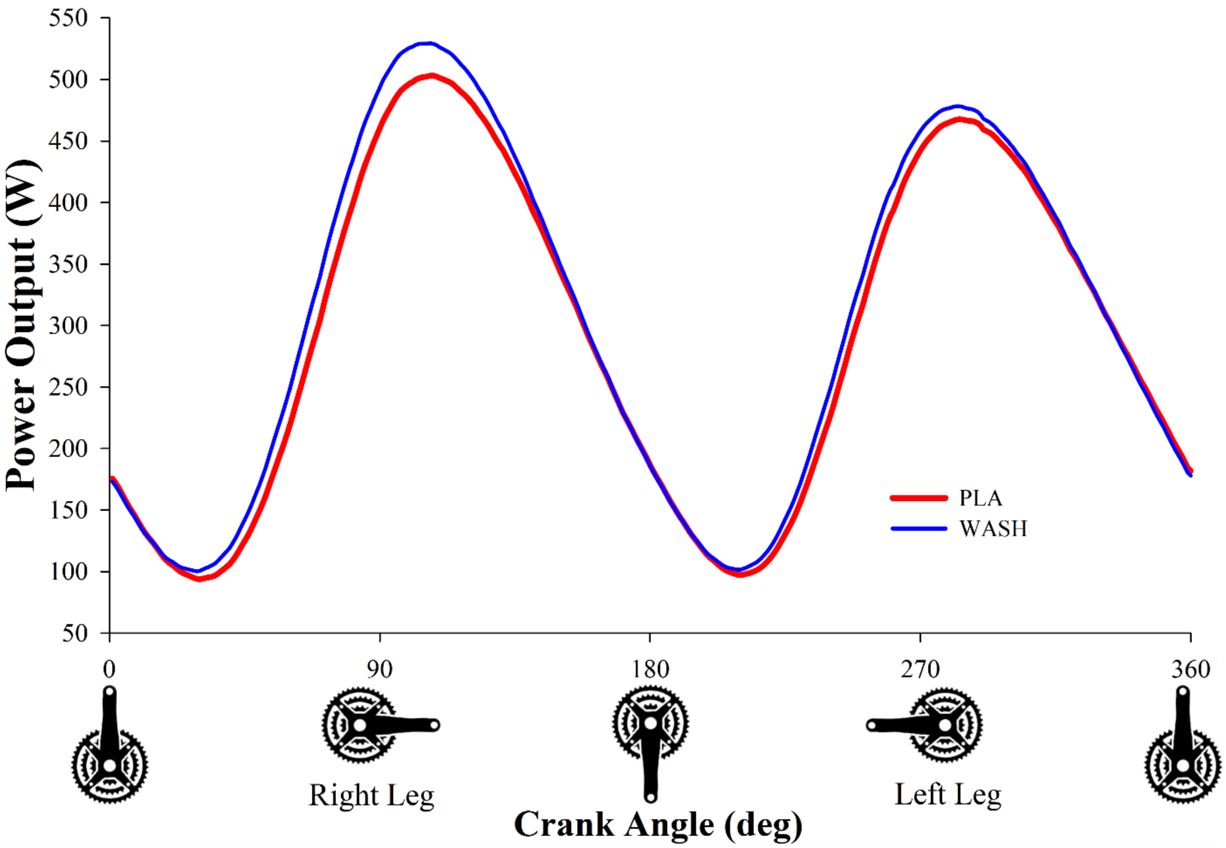

Figure 4-7: Power profile reconstruction from PC analysis using mean loading score for the first 5 Pcs for WASH and PLA separately.

4.4.3 Cycling biomechanical parameters

The PC's contributing significantly ($p < 0.05$) to biomechanical parameters based on PC loading scores from an ANCOVA were $P_{PC1-5,LS}$ for L-Max-P, R-Max-P, L-Avg and R-Avg, $P_{PC2-5,LS}$ for R-EPR, and $P_{PC1-4,LS}$ for L-EPR. The significant PC's for each biomechanical parameters were used to reconstruct the cycling power profile, and show the top and bottom 100 pedal cycles that relate to each parameter (Figure 4-8). For all biomechanical parameters, except right EPR, there was a significant difference between WASH and PLA ($p < 0.05$). Summary of biomechanical parameters for WASH and PLA are displayed in Table 4-4.

Table 4-4: Cycling Biomechanical parameter results, n = 9. * denotes significant difference between PLA, *p* < 0.05 (Mean ± SD).

		WASH	PLA
Cadence (rpm)			
		97.4 ± 7.3*	98.2 ± 8.2
AVG-P (W)			
	Right	313.9 ± 51.0*	297.8 ± 58.2
	Left	292.6 ± 55.0*	285.5 ± 63.6
MAX-P (W)			
	Right	572.7 ± 97.8*	531.9 ± 98.4
	Left	514.6 ± 103.6*	492.1 ± 110.1
EPR (°)			
	Right	87.6 ± 8.2	87.5 ± 7.1
	Left	83.3 ± 9.6*	85.6 ± 9.2

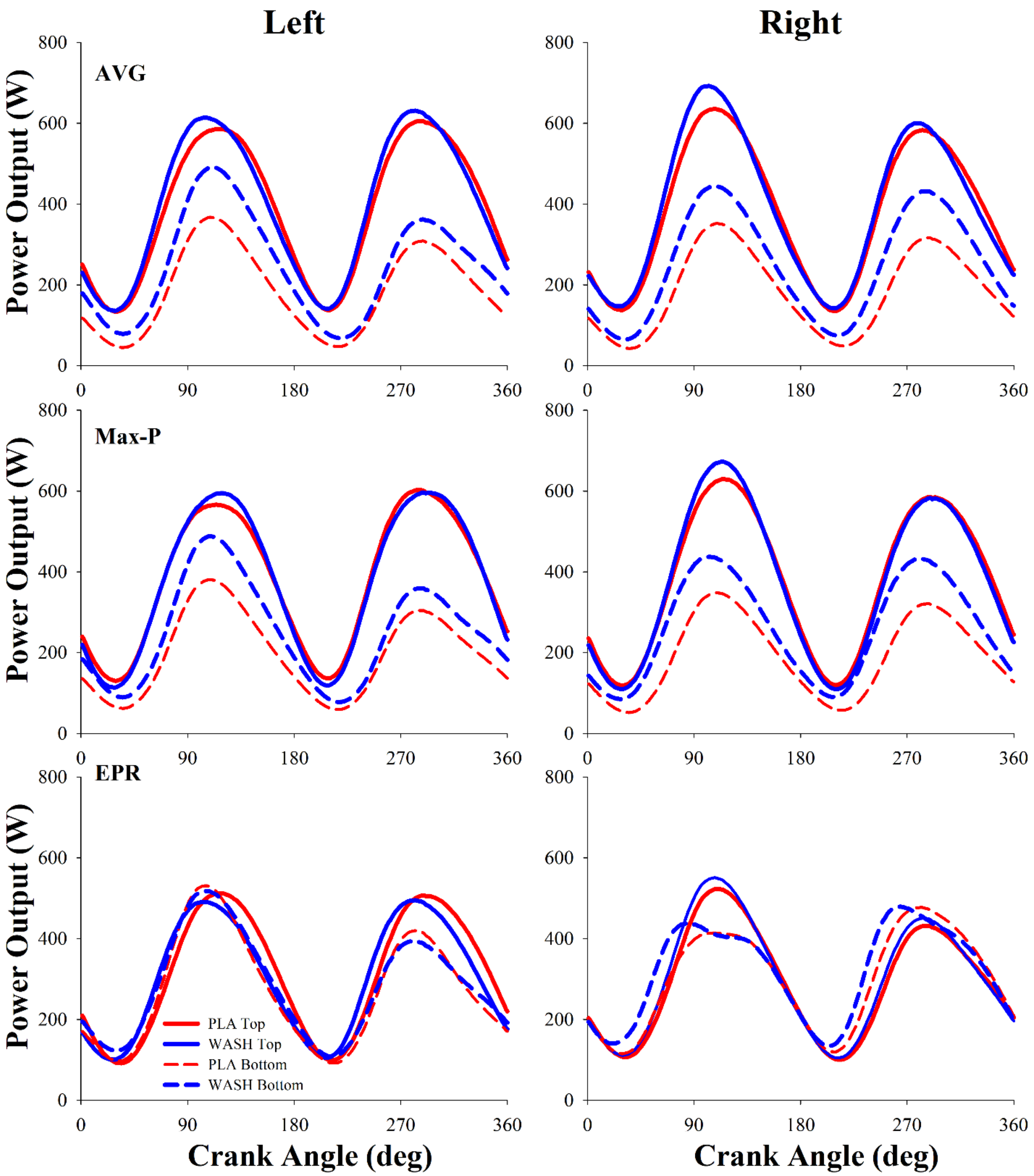

Figure 4-8: Power profile reconstructions from the principal component analysis for biomechanical parameters, average power (AVG), maximum power (Max-P), and effective power range (EPR) with respect to top 100 and bottom 100 pedal cycles for each parameter relative to the left and right side.

4.5 Discussion

This is the first study to use advanced neuromechanical analysis to examine the effect of CHO mouth rinse in a late endurance cycling trial. The results of this investigation show noticeable differences between WASH and PLA for both power output and muscle activation. The average power output profile in WASH showed an earlier onset in the pedal cycle, greater duration and higher amplitude versus PLA during the TT (Figure 4-7). Additionally, only the PLA condition showed a significant increase in muscle activation throughout the time trial, which could be evidence of fatigue. This shows for the first time that CHO mouth rinse may have a substantial effect on the maintenance of power while mitigating the impact of neuromuscular fatigue, in late endurance performance.

4.5.1 Power output profile and biomechanical parameters

In this investigation, while there was no significant improvement in average power and time to completion between WASH and PLA, there was a significant increase in cycling power output and decrease in time of completion during the last half of the TT (TT3 to TT5) only during the WASH condition (Figure 4-5). So, although there was no difference in power output throughout the entire TT, this data might suggest that in longer TT's, when fatigue is even greater, that WASH may have a significant impact on power and performance outcomes. This is similar to a comparison of TT time to completion intervals as seen in Chapter 3 (Figure 3-2). Both the PLA and WASH conditions represent a typical J-shaped pacing strategy observed during 20km cycling TT (Abbiss & Laursen, 2008), however, during WASH, there is a maintenance of power output during the middle of the TT that is not evident in PLA. This is similar to other studies that show a trend for maintenance of power based on CHO mouth rinse (Ataide-Silva et al., 2016; Jeffers et al., 2015; Lane et al., 2012).

While the reconstructed power output was similar between conditions, there were some important differences observed in PC loading scores and the reconstructed power outputs based on biomechanical parameters. The average $P_{PC1,LS}$ for each TT interval closely resembles the average power output per interval (Figure 4-5), with a significant difference in TT3 and TT4 between conditions (Figure 4-6). Additionally, while $P_{PC2,LS}$ only explained 2.9% of the variation in power output profile, the mean loading scores between conditions show a variation, with WASH having an average positive loading score and PLA having a negative loading score for all TT intervals. The greatest difference between $P_{PC2,LS}$ was between conditions for TT4. When considering the shape of the PC2 weighting (eigenvector) in relation to the power output during cycling (Figure 4-6), it seems to resemble a shift in power to earlier in the pedal cycle and greater magnitude of power during the rising push-down and pull-up phases. This difference between conditions may indicate a tendency for a shift or earlier onset of power per pedal stroke during WASH. This could require reliance upon greater relative joint powers and may result in changes in EMG activation throughout the TT (Bini, Carpes, Diefenthaeler, Mota, & Guimarães, 2008; Bini, Diefenthaeler, & Mota, 2010; Martin & Brown, 2009).

In the overall average power outputs (Figure 4-7) and five of the six power outputs reconstructed by mechanical parameters (Figure 4-8), there was a marked increase in maximum power between conditions with WASH having larger power in the right and left leg leading conditions. More apparent is that the bottom (worst) values in the reconstructed powers were substantially higher in the WASH condition and the range of values between the top and bottom values was greater for the PLA condition. This difference seen in PLA may suggest that there is greater variability in power output and a larger range in the power outputs throughout the TT. A smaller range of power outputs observed in WASH is similar to other studies who observed that during conditions of higher CHO availability (fed state) and utilizing CHO mouth rinse, there was a tendency for less variation in average power between time trial intervals (Ataide-Silva et al., 2016; Lane et al., 2012). Greater variability in the averaged power between subjects can be observed in the standard error values with PLA having a greater variability from WASH (Figure 4-5). These changes in power variability may be as a result of changes in muscle activation and coordination and may be related to internal pacing strategies associated with CHO availability and fatigue (Dorel 2009).

4.5.2 Muscle activation

The analysis of EMG in this investigation showed that the use of CHO mouth rinse impacted total intensity, median frequency and high- and low-frequency intensity bands. For total EMG intensity there was an increase in GLUT and TA muscles, and for high- and low-frequency intensity there was an increase in RF, GLUT and TA, with an increase in excitation between TT1 and TT5 only during the PLA condition. Additionally, there was a decrease in median frequency in the SOL muscle in the PLA condition only. While the increase in intensity during PLA could be linked to an increase in power output required during the later stages of the TT, this same increase in intensity is not observed with a similar increase in power during the WASH condition. Taking together, the decreased MDF in the SOL muscle during the PLA condition and the differential results in TA, RF and GLUT between conditions, could be evidence of fatigue only observed during the PLA condition (Abbiss et al., 2008; Davis & Bailey, 1997). This result is similar to other studies that suggest CHO may act to mitigate the effects of neuromuscular fatigue while maintaining performance (Ataide-Silva et al., 2016; Jensen, Klimstra, Sporer, & Stellingwerff, 2018; Jensen et al., 2015; Lane et al., 2012).

The primary muscle coordination pattern ($I_{\mathrm{PC1,W}}$) represents the mean coordination pattern for all participants for all trials, with GLUT demonstrating the greatest excitation (Figure 4-2). The mean loading scores for I_{PC1} were significantly lower for TT1, TT2 and TT5 during the WASH condition, this may suggest that there is a higher reliance on GLUT to contribute to power production during the early part of the TT and at the very end when fatigue is at its highest (Elmer et al., 2012). Blake et al. (2012) demonstrated that the early activation of GLUT would be a beneficial strategy to improve power output and efficiency as the power producing muscles (VL,VM) would be active for more of the down-stroke, thus resulting in less demand on the less efficient muscles during the up-stroke. For $I_{\mathrm{PC4,LS}}$ there is a significant increase during TT5 during the PLA condition, which along with $I_{\mathrm{PC4,W}}$ indicates a later activation of the GLUT muscle during the down-stroke, which would support the notion that during the PLA condition, there may be a decreased efficiency throughout the TT which is amplified during the final portion of the TT when subjects were making a final push to the line. The noticeable difference between the change in excitation of the GLUT and TA muscles for $I_{\mathrm{PC2,W}}$, along with the significant difference between $I_{\mathrm{PC2,LS}}$ for all TT intervals indicates a larger reliance on GLUT throughout the whole TT during the PLA condition.

4.5.3 EMG high- and low-frequency band intensity characteristics

Further details of differences between conditions for EMG can be observed when comparing the high-frequency band and low-frequency band optimized intensities and the difference between TT intervals. Using the results from total intensity (Figure 4-1) to guide the investigation, there are observable differences between TA, RF and GLUT, between conditions, showing a more substantial increase in intensity for all muscles in the PLA condition between TT1 and TT5 (Figure 4-4). The increase in EMG intensity for RF and TA during PLA occurred across the top and early part of the pedal cycle, TDC. These two muscles play a role in moving the pedal over TDC to allow for an efficient application of force to the pedal during the down-stroke (Blake et al., 2012; Blake & Wakeling, 2015; MacIntosh, Neptune, & Horton, 2000; Neptune, Kautz, & Hull, 1997) and have been identified as critical muscles for efficient cycling (Blake & Wakeling, 2015). During cycling, TA is postulated to perform essential functions to control pedal orientation throughout the pedal stroke as well as to maintain stabilization through ankle dorsiflexion across TDC (So, Ng, & Ng, 2005). RF is a bi-articular muscle performing dual functions of flexion of the hip and extension of the knee. The more considerable increase in EMG intensity for RF from TT1 to TT5 during PLA condition may be evidence that it is actively being used to control the direction of force applied to the pedal (Blake et al., 2012; Duc & Grappe, 2005). It has been hypothesized by Sarre and Lepers (2005), that an increase in RF activation is required when an increase in power is needed to facilitate the transition across TDC. This is evident only in PLA TT 5, when there is a substantial increase in intensity prior to TDC, which could indicate a diminished pedal stroke efficiency during the PLA condition, and inability for the quadriceps muscles to produce the required power during the down-stroke to assist the transition across TDC, since, during the pedal down-stroke, peak joint moments occur in sequence from knee to hip to ankle (van Ingen Schenau, Boots, de Groot, Snackers, & van Woensel, 1992). Additionally, it has been shown that the transition across TDC (up-stroke to down-stroke) is essential to producing power early in the pedal cycle, which will help maximize the power output, while maintaining minimal muscle activation and thereby maximizing efficiency during cycling (Blake & Wakeling, 2012, 2015). In the present data, we saw a slower onset of power output during PLA (Figure 4-6), while at the same time a more considerable increase in TA and RF total EMG intensity, which are both active muscles during the transition across TDC (Figure 4-1). The high-frequency band and low-frequency band reconstructions for the GLUT muscle show distinctively larger total intensity between WASH and PLA conditions. Dorel et al. (2009) had a 29% increase in EMG activity level for GLUT during exhaustive exercise, and they linked this increase in activity to 2 possible explanations: 1) progressive recruitment of additional MUs to compensate for alterations of contractile properties or 2) change of muscle coordination strategy. As this large increase in EMG intensity for the GLUT muscle was not seen in the WASH condition, a more considerable change in muscle coordination strategy might have been present with the absence of CHO mouth rinse. Therefore, during the PLA condition, there seemed to be a major reliance on GLUT to be the dominant power producer to compensate for potential fatigue and loss of force of the full knee extensors (VL, VM) during the PLA condition (Dorel et al., 2009). Taken together the results for EMG and cycling power seem to support a substantial change in cycling neuromechanics associated with CHO mouth rinse, which may be evidence of the neural mechanisms responsible for this performance change.

4.5.4 Potential neural mechanisms dependent on pacing

During cycling time trials is it well known that athletes will use pacing strategies to disperse the workload required to complete the trial (Abbiss & Laursen, 2008) and during indoor cycling time trials, subjects will commonly regulate their pacing by adjusting the resistance (Bini et al., 2008; Duc & Grappe, 2005; Lane et al., 2012). The introduction of a pacing strategy impacts both the power output and level of fatigue, which may have an effect on muscle coordination (Dorel et al., 2009). Common to all pacing strategies used during a cycling TT, there will be the sprint to the end (K. Thomas, Stone, Thompson, St Clair Gibson, & Ansley, 2012). Examination of the power output, as a descriptor of the pacing strategy, is suggestive of a steady loss of power through the middle of the TT with a sharp increase in the final 20% during PLA condition. This sharp increase in power is matched by an increase in the intensity of the RF, GLUT and TA during this final portion of the TT in the PLA condition only. In contrast, during the WASH condition, the power output depicts more of a maintenance of power through the middle of the TT and no significant increase in intensity of muscle activation during the final 20%, despite an increase in power output in keeping with the sprint to the finish goal of this point in the time trial. These between condition differences may be related to the athlete's perception of their level of fatigue during the placebo condition. In studies using fMRI, CHO mouth rinse has been shown to stimulate the pleasure and reward centres of the brain (Chambers et al., 2009). During TTs completed using CHO mouth rinse, participants had reported a similar rating of perceived exertion, even when the work rate during the CHO mouth rinse trial was greater (Lane et al., 2012). It is possible that these perceptual differences influenced by CHO mouth rinse may impact the maintenance of effort throughout a TT (Konishi et al., 2017).

Conversely, the impact of the perception of fatigue during the PLA condition resulted in a drop in power until the final motivational point of the TT, sprint to the end, at which point an altered neuromuscular patterning of muscle activation was apparent to reach these higher levels of power output. Fatigue has been considered a neuroprotective strategy to limit damage due to glycogen depletion and maintaining homeostasis to prevent ultimate failure (St Clair Gibson & Noakes, 2004). Ataide-Silva et al. (2016) found that CHO mouth rinse ameliorated the neuromuscular strategy associated with fatigue by restoring EMG amplitude and EMG frequency during exhaustive exercise. This decrease in EMG was associated with attenuation in the reduction in performance, which was not seen in the non-CHO mouth rinse trial.

4.5.5 Conclusion

This is the first study to explore the impact of CHO mouth rinse on both neural and biomechanical performance parameters in cycling. These data suggest that CHO mouth rinse may contribute to trained athletes' ability to maintain power and mitigate the impact of neuromuscular fatigue in late endurance performance. This further supports the growing body of literature demonstrating the role of CHO mouth rinse in high-performance sport.

5 Conclusion

5.1 Summary of dissertation

Since the seminal CHO mouth rinse study by Carter et al. (2004), there has been an increased interest in the ergogenic effect of this intervention, as well as using this intervention as a research tool to perturb central versus peripheral fatigue based mechanisms. Ergogencially, CHO mouth rinse is especially interesting as it has the potential positive effect of increasing performance, while also minimizing some of the detrimental performance effects that may be caused by CHO ingestion (such as GI discomfort; (Stellingwerff & Cox, 2014)). However, there is still uncertainty as to the exact performance effect during exercise at different intensities and durations, and the exact mechanism for improvement remains speculative (Jeukendrup, 2013). Equally, the majority of studies have not been performed on elite athletes or in states of ecological validity. Additionally, the level of analysis for most studies examining the effect of CHO mouth rinse on performance have employed limited neural and biomechanical analytics necessary to evaluate the central mechanistic effects of CHO mouth rinse on performance.

An important underlining issue when assessing the performance gain associated with CHO mouth rinse is the state of CHO depletion the subjects are in prior to exercise. There are various permutations of depletion, and many studies may leverage one of these permutations to increase the potential of performance gains. Listed below are a few possible examples that have been used with CHO mouth rinse studies:

1. Overnight fasted; results in low liver glycogen (about 70-80% reduced) but normal blood glucose and glycogen levels. For example our Chapter 2 experiment.
2. Overnight fasted then exercise with no exogenous glucose supplementation over shorter durations (<1 h). This results in low liver and blood glucose, while muscle glycogen is

reduced during exercise. This represents most of the ~1 h time trial cycling/running studies using CHO mouth rinse (Table 1-1).

3. Overnight fasted then exercise with no exogenous glucose supplementation over longer exercise durations (>2 h). This results in low liver and blood glucose with significantly reduced muscle glycogen.
4. Glycogen reducing exercise protocol prior to an overnight fast, resulting in lower muscle glycogen and lower liver glycogen prior to experimental exercise (Table 1-2).

These varying levels of depletion will all influence performance as muscle glycogen is reduced, however in a real-world scenario, high performance/elite endurance athletes will not typically compete in a low glycogen status.

The goal of this dissertation was to add to the literature in CHO mouth rinse investigations by addressing a number of these above-noted considerations through more detailed neuromechanical analysis of CHO mouth rinse at different exercise intensities, varying levels of fatigue, and during ecologically valid conditions.

While the exact mechanism for performance improvement is still speculative, the current consensus is that because there is no CHO absorption at the peripheral level during CHO mouth rinse, the main mechanism considered is the attenuation of centrally mediated inhibition of motor output (CNS) (Ataide-Silva et al., 2016; Chambers et al., 2009; Jeffers et al., 2015). In states of reduced CHO availability, neuromuscular fatigue is thought to be a neuroprotective strategy to maintain fuel sources (St Clair Gibson & Noakes, 2004), however, when CHO is available, it has been shown to improve performance and reduce fatigue (Jeukendrup, 2004). Equally, CHO mouth rinse restores power and muscle activity to non-depleted levels even when endogenous CHO availability is reduced (Ataide-Silva et al., 2016). In addition, Lane et al. (2012) and Jeffers et al. (2015) in a limited analysis support the premise that CHO reduces the effect of neuromuscular fatigue. Indeed in the current dissertation, in chapter 2 the use of a CHO mouth rinse mitigates the strength reduction in a fatiguing contraction and in Chapter 3 and 4 a CHO mouth rinse results in diminished effects of fatigue in both power and muscle activation during a late endurance time trial. It is also not surprising that the effect of CHO mouth rinse seems to be more readily apparent during states of fatigue and reduced glycogen (Ataide-Silva et al., 2016). Therefore, the work from this dissertation adds to the support for an overall benefit of CHO mouth rinse as well as a deeper understanding of its action during exercise.

While there is an excellent value in the work that has been previously done in CHO mouth rinse, there is often disconnect between the experimental paradigm and the cohorts that could benefit from its effect. That is, there are many studies whose protocols utilize recreationally trained athletes in a low glycogen level status during time to exhaustion trials. The effect of performance of an ergogenic aid on the level of athlete is an important consideration of its use. For example, nitrate ingestion has a substantial effect on performance for sub-elite athletes but has yet to consistently demonstrate significant effects in highly trained athletes (Jones, 2014). Therefore, it is critical to study a population-specific to that intended intervention. Additionally, while the effect of CHO on performance during fasted conditions (low liver glycogen) has been observed, it is unreasonable to expect that elite athletes would be in states of energy and neuromuscular depletion before an event.

The consensus statement for CHO consumption before an endurance event is 1-4 $g \cdot kg^{-1}$ for exercise >60 min and consumed 1-4 h prior to exercise and during an endurance event consuming ~30-60 g $CHO \cdot h^{-1}$ (T. Thomas et al., 2016). To ensure the highest degree of ecological validity, we implement various typical pre-race/race nutrition interventions for our subjects in Chapters 3 and 4, with subjects receiving 2.1 ± 0.3 $g \cdot kg^{-1}$BM CHO 2 h prior to starting, plus 30 g $CHO \cdot h^{-1}$

during the steady state portion. In chapter 2, we violated some of these ideas by utilizing a simple, single joint, isometric movement to reduce any issues that can be present during more complicated dynamics tasks (De Luca, 1997). While this does not entirely fit the standard for ecological validity as compared to elite athletic events, it did allow a simple controlled exploratory paradigm where we could investigate a simple, single joint isometric action and therefore remove some mitigating factors that could limit the ability to observe and interpret the noted performance effect. These results suggest that the use of CHO mouth rinse may produce a positive performance benefit in sports that include single explosive actions (e.g. powerlifting). In contrast, in Chapter 3 and 4 we attempted to investigate an ecologically valid cycling time trial protocol with athletes who's physiological, and cycling performance profile were fitting of an elite cohort. However, a limitation of such investigation is that the changing rate of work during different time trial intervals, as compared to steady state or time to exhaustion trials, and this inherent variability could limit the statistical approach to such an analysis and a more liberal statistical approach was required (Batterham & Hopkins, 2015; Malcata & Hopkins, 2014). As such, in addition to traditional frequentist statistical approaches, we also used magnitude-based inferences. The finding of Chapter 2 using magnitude-based inferences support a ~3% less attenuation of peak torque post-fatigue in WASH vs. PLA supported by a qualitative inference of the outcome being 55% likely to be positive. The findings of Chapter 3 using magnitude-based inferences support the 35 s faster time to completion when using WASH vs. PLA, with a qualitative probability of a 60% positive outcome, while marginal, this effect could be relevant to the elite athlete/coach. This approach, based on this paradigm enables the potential application of CHO in elite performance trials and gives a tacit probability of sharing with coaches and athletes.

A significant methodological and engineering achievement of the work in this dissertation was the development of a custom sensor that enabled a higher resolution of power measurement, angular velocity and added the ability to synchronize other analog data (i.e. EMG) at 36 points during the pedal cycle. Current systems are either limited in their measurement resolution and small, but meaningful variations in the power profile may be missed (Bertucci et al., 2008) or are very expensive. Overall this improvement in measurement alongside recent advances in cycling neuromechanical measurement (Blake & Wakeling, 2015) enabled the development of an analysis platform that was able to elucidate functional and indirect mechanistic characteristics of an ergogenic aid (CHO mouth rinse). In Chapter 4, the high-resolution power measurement and EMG combined with PCA of kinetic and muscle activation enabled a more detailed assessment of the effect of CHO mouth rinse than previous studies. For example, while Lane et al. (2012), Jeffers et al. (2015) and Ataide-Silva et al. (2016) showed similar findings, these investigations only had average measures of power and used a limited number of muscles and a very superficial analysis. The more detailed analysis in Chapter 4 was able to pinpoint modifications at the muscle level, which may be related to specific performance adjustments. This type and level of analysis is a powerful tool that can also be used to further investigate CHO mouth rinse as well as other ergogenic aids.

Overall, these results support the growing evidence that CHO mouth rinse can be used as an ergogenic aid to improve performance, and provides further evidence it can help during the later stages of an endurance event (> 2 h) and during a maximal strength effort while fatigued. The underlying mechanisms responsible for performance gains while using CHO mouth rinse are still somewhat unresolved. However, these results, together with the advanced methodology employed in these studies, help refine the toolset that can be used to measure improvement. Ultimately, these studies highlight the importance of increased resolution and ecological validity during studies of CHO mouth rinse use when looking for small, but meaningful changes that can explain performance gains.

5.2 Practical applications

5.2.1 Use of CHO mouth rinse in sport

Small performance effects can have a significant outcome in real-world sporting competitions, where even a <1% increase in performance may mean the difference between standing on the podium or not (Malcata & Hopkins, 2014). CHO mouth rinse has been shown to have a significant improvement (1.72%; Table 1-1) on performance, which makes it an ergogenic aid that should be considered by athletes during training and/or competition. More importantly, CHO mouth rinse has shown no evidence of detrimental impact on performance, so using it during competition should not cause concern to athletes. However, it is important to note that CHO mouth rinse is not a substitute for CHO ingestion during sport, however, as the current findings suggest, CHO mouth rinse can be used during specific time points of a competition when CHO ingestion may not be possible or may be detrimental for some athlete's optimal performance. The results of the studies of this dissertation add to the current knowledge of the use of CHO mouth rinse in sport.

The findings of Chapter 2 suggest that CHO mouth rinse has potential application to sports that include single explosive actions (e.g. powerlifting). Use of CHO mouth rinse in these types of activities had previously received very little attention. Our findings provide foundational knowledge to guide future lines of research (see discussion section 5.4 future directions).

The ecological validity of the study design employed for Chapters 3 and 4, speaks directly to the possible practical applications of these findings. Precisely, the data reinforce the benefits of the use of CHO mouth rinse towards the late stages of a cycling race. Athletes who experience GI upset related to CHO ingestion may benefit from the use of CHO mouth rinse to limit the amount of CHO that is required to be ingested during the later stages of an event and also during very high-intensity exercise (e.g. interval workouts) when blood shunting away from the GI tract can cause GI issues (Neufer et al., 1989). Extended periods of high-intensity exercise (>75% $\dot{V}O_{2max}$) can cause reduced blood flow to the gastric region, which makes ingestion of food/liquid more difficult. This is particularly important to some athletes participating in endurance sport, as for example, commonly the last 30 min of a marathon or cycling race when potential GI upset related to ingestion of CHOs will be the greatest and ultimately the most limiting to performance (Guillochon & Rowlands, 2017; Peters et al., 1993; Rowlands & Houltham, 2017). It may also benefit athletes who are on a caloric restricted diet where they may maintain training volume/intensity, but limit total consumed calories using CHO mouth rinse. Another possible benefit is its use during very intense interval training, when stomach upset and side ache can be increased due to the elevated intensity level (Shi et al., 2004).

5.2.2 Measurement of cycling performance with higher resolution

Most commercial power meters are limited to low-resolution readings (≤10Hz), which limit their ability to be used for the accurate representation of the cycling power profile. Another area of concern is these devices provide limited or no access to raw data, which creates a ‘black box’ situation where the accuracy of the device is somewhat speculative. Therefore it was imperative for this dissertation that a higher resolution system (>200Hz) be developed, along with the ability to sync cycling power data with muscle activity (EMG).

The measurement device created for this dissertation has continued to be developed for advancement in cycling biomechanical performance measures. The cycling performance measure now demonstrates improved resolution to 5° from the 10° utilized for this dissertation. This system of measurement in cycling drew attention from Cycling Canada and has been applied to the evaluation of athlete performance of BMX race starts for the past two competitive seasons, athlete performance monitoring in track cycling and is under development for use in on-track cycling performance measurement. The understanding of the power profile with increased resolution has been an iterative process and ever evolving. The number of new biomechanical parameters has increased to over 20, with a majority of them being used in the daily training environment for evaluation of athletes. The device created is an add-on for the SRM PowerMeter, which gives the ability to use existing hardware that is readily available. This reduces the cost associated with buying expensive research-grade equipment or the difficulty in creating your own power meter or force transducer pedals

The higher resolution of data collection during cycling has proven to be a valuable asset when examining the benefit of an ergogenic aid, and provides an accessible methodology (toolset) for future studies exploring advancement in cycling, which could include but is not limited to; 1) technique, 2) training adaptations, 3) equipment development, 4) injury status and 5) nutritional interventions.

5.3 Limitations

There are several important limitations in the interpretation of the results of these studies:

1) The participants studied in Chapter 2 included athletes with varying athletic backgrounds concerning the type of sport and level of competition. The protocol was also performed in an overnight fasted condition, which is not a typical situation for athletes. Furthermore, conditions of performance testing were constrained to a single joint movement which lacks ecological validity.

2) In Chapter 2, the study employed the use of MVCs. Mechanistically, future studies could employ the use of interpolated twitch methodology to understand the level of maximal activation achieved by the participant. This would offer further understanding of the neurophysiological impact of the use of CHO mouth rinse.
3) Subjects used for Chapter 3 and 4 had different cycling background history. However, their average $\dot{V}O_{2max}$ was 64.5 ± 6.5 $mL \cdot kg^{-1} \cdot min^{-1}$, which gives us confidence that these subject represented a semi-elite cohort. They also all had experience doing time trials, and a familiarization protocol was used to make sure their set workload was not too hard or too easy.
4) Measurement of muscle excitation in Chapters 3 and 4 included only one leg. For this reason, we cannot explore important concepts such as interlimb coordination and how the use of CHO mouth rinse affects this important motor parameter in cycling.
5) A dynamic collection of EMG during a prolonged cycling activity is challenging. It is challenging to optimize signal quality when activity is continuous and cannot be interrupted to make adjustments. For this reason, Chapter 4 did not include the data of all athletes tested.

5.4 Future directions

Understanding of the use of CHO mouth rinse remains in its infancy. The findings of this dissertation implicate important areas for future research. Chapter 2 demonstrates a positive effect of the use of CHO mouth rinse during activity requiring maximal force production. These findings call for further exploration under conditions of greater ecological validity with the inclusion of athletes competing in power sports at an elite level and development of a study protocol using a repetitive well-trained power task (e.g. lifts, sprints). Additionally, the results of this dissertation shows that force production in a fatigued state is attenuated in a single trial, however, in activity where this performance effect may be applicable the activity is commonly repeated (e.g. sprinting, weightlifting) and therefore, the repeated use of CHO mouth rinse during repeated bouts of max force production should be explored. Furthermore, the physiological rationale for the effect of CHO mouth rinse during short duration needs to be further explored.

The increased resolution of measurement of power during cycling achieved in studies of Chapter 3 and 4 facilitates more detailed investigation of performance parameters in cycling. Application of this methodology in future cycling research will allow for more detailed analysis of the entire pedal cycle. Furthermore, this device demonstrates potential to be combined with a recently developed cycling biofeedback system (Blake, 2015) to advance training tools for athletes. The potential application of this advanced training tool is currently in development, including the design of future lines of research in elite-level cycling.

The role of CHO mouth rinse and its use in the studies in this dissertation was to act as an ergogenic aid, not only to see its impact on performance, but also its effect at the neuromuscular level. In the current literature, there are many ergogenic aids and protocols that are being investigated to improve performance and reduce fatigue for athletes (i.e., beta-alanine, sodium bicarbonate) (Bellinger, Howe, Shing, & Fell, 2012). As stated previously, when looking at elite athletes and performance gains, sometimes the smallest improvement can lead to a significantly better placing during an event, even if not statistically significant (Malcata & Hopkins, 2014). However, sometimes the outcomes of these protocols and/or identified uses of ergogenic aids are not adequately substantiated or supported, which can lead to doubt in coaches, support staff and athletes. The methodology used in the current studies represents a toolset that can tease out small, but meaningful changes, which is of tremendous value not only to the elite sports world but the scientific community as a whole. One area of research that has seen mixed findings for CHO mouth rinse use is during sprinting/maximal strength exercise. Therefore the utilization of this new toolset adds an exciting opportunity to conduct future research in this area, especially during sprint cycling where the increased power collection resolution may add valuable information to performance improvements. The use of this toolset could also be used in future research that includes, but is not limited to; 1) ergogenic aids whose main mechanism of action is to reduce fatigue (antioxidants, beta-alanine, and sodium bicarbonate), 2) Warm-up strategies to increase maximal strength (plyometric exercise) (Masamoto, Larson, Gates, & Faigenbaum, 2003), and 3) Active warm-up on muscle activation (Stewart, Macaluso, & De Vito, 2003)

Mechanistically, CHO mouth rinse is suggested to affect the level of perception of fatigue centrally. The current study included a base level question of athletes with regards to their perception of fatigue during the trial (Chapters 3 and 4) and whether athletes could determine whether they were using placebo or CHO mouth rinse (Chapter 2-4). The perception of fatigue during competition and training conditions can be self-limiting to performance. Therefore, an important line of future research is to examine the impact of the use of CHO mouth rinse on the athlete’s perception of fatigue using a more detailed interview process and including questions regarding their perception of their ability to reach the performance goals of the experimental paradigm. This line of inquiry could address the important interplay of the use of ergogenic aids and the psychology of the elite athlete.

CHO mouth rinse has been suggested to be of particular benefit to athletes who experience adverse side effects related to CHO ingestion during training and competition. Therefore, it is important that future research is directed towards understanding performance benefits in these athletes specifically. Investigating how CHO ingestion versus CHO mouth rinse affects performance measures during stages of a sport specific trial when athletes are vulnerable to the adverse effects of CHO ingestion would provide necessary understanding of the potential benefit of the use of CHO mouth rinse. Potentially, the performance benefits of the use of CHO mouth rinse may be heightened when examined in this athlete group specifically.

5.5 Conclusion

This dissertation provides new insight into the use of CHO mouth rinse during short duration power activity and in the late stages of endurance activity. Importantly, these results elucidate a more comprehensive understanding of the neurophysiological and biomechanical effects of the use of CHO mouth rinse at the level of muscle during sports performance. Methodologically, this dissertation provides advancement in the field of performance measurement in cycling with the development of a system for measuring power at a higher level of resolution throughout the pedal cycle. The development of this system, together with the ecological validity employed to measure late stage cycling and the use of CHO mouth rinse, provides an important framework for future cycling research.

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