Investigating the Effect of a Topical Carnosine Gel on 1,500 m Rowing Performance in Experienced Club-Level Rowers

A thesis

submitted in fulfilment

of the requirements for the degree

of

Master of Health, Sport and Human Performance

at

The University of Waikato

by

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THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2025

Abstract

It is well established that beta-alanine supplementation increases muscle carnosine levels and, in many cases, improves high-intensity exercise performance. Recent research has shown that applying a topical carnosine gel to the skin may be an effective alternative method to increase muscle carnosine and improve high-intensity exercise performance. However, there is currently no research on the effect of topical carnosine on rowing performance.

This thesis is separated into three chapters. Chapter One is a review of the literature regarding the physiology of muscular fatigue in high-intensity exercise, the relationship between muscle typology and buffering capacity, supplement-based interventions to elevate buffering capacity, the ergogenic properties of carnosine (with particular relevance to rowing performance), and the novel use of topical carnosine to improve high-intensity exercise performance. The literature shows that an accumulation of hydrogen ions, a by-product of anaerobic glycolysis metabolism, negatively impacts muscle function, causes pain in the muscle, and decreases muscle pH, ultimately reducing exercise performance. Multiple studies found that raising the amount of intramuscular carnosine improved performance because carnosine is a pH buffer and may increase the calcium ion sensitivity within the muscle. Due to the limitations of direct carnosine ingestion, an increase in muscle carnosine is traditionally achieved via beta-alanine supplementation, which can cause small performance improvements in high-intensity exercise, such as rowing. Topical carnosine is a novel, more efficient alternative to elevate muscle carnosine; however, there is limited, conflicting evidence of its effect on high-intensity exercise performance, thus, more research is needed.

Chapter Two investigated the effect of a topical carnosine gel on 1,500 m rowing ergometer time trial (TT) performance. Thirteen club-level rowers (11 male, 2 female) were randomly allocated into Group A or B, from which a crossover design was used to measure the effect of the intervention. In a double-blind fashion, participants applied 15 mL of a topical carnosine gel (CAR) or an ultrasound placebo gel (PLA) to their back and limb muscles 45 minutes before each TT, which were separated by one week. The paired dependent t-test and Cohen's effect sizes found that topical carnosine had a *trivial,* non-significant effect on TT performance (CAR 300.5 ± 23.1 s; PLA 300.4 ± 24.2 s, $p = 0.945$, $d = 0.004$), 500 m split times 500 m ($p = 0.808$, $d = 0.009$; 1000 m $p = 0.830$, $d = 0.011$; 1500 m $p = 0.849$, $d = 0.020$), and rated perceived exertion ($p = 1.000$, $d = 0.000$). These results suggest that topical carnosine does not affect 1,500 m rowing performance. After the intervention was deemed ineffective, the reliability of the 1,500 m TT was calculated using Trial 1 vs Trial 2 (Trial 1: 301 ± 23.8 s, Trial 2: 300.0 ± 24.0 s; $p = 0.114$; $d = 0.074$; *trivial* effect size; TE = 2.8 s; ICC = 0.985). The reliability statistics suggest that the 1,500 m TT performed on a Concept 2 rowing ergometer is a reliable measure of rowing performance.

Chapter Three summarises the findings from Chapter Two, identifies the strengths and weaknesses of our research, and provides recommendations for future research. Overall, this thesis identified gaps in the literature regarding carnosine and high-intensity exercise, and found links between papers on muscle typology, buffer capacity, beta-alanine, and topical carnosine that can be used to develop rationales for future research. It was clear that, under the conditions tested in this experimental protocol, topical carnosine does not affect performance in the 1,500 m rowing ergometer TT in experienced club-level rowers.

Acknowledgements

I want to thank several people for helping me throughout the process of my thesis.

Thank you, Dr Martyn Beaven, for being my primary supervisor and keeping me on track throughout this thesis. Your expertise, knowledge, and approachability made me confident to analyse and conduct sports science research critically.

Thank you, Steven Finlayson, for sharing your networks within the New Zealand rowing community. Without your help in participant recruitment, this research would not have been possible.

Thank you to the rowers who participated in this research. The 1,500 m test is mentally and physically demanding, so I am grateful for your effort and commitment.

Thank you to my family and friends for supporting me throughout this thesis process.

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List of Abbreviations

1RM – One repetition maximum

ADP – Adenosine diphosphate

ATP – Adenosine triphosphate

BA – Beta-alanine

CAR – Carnosine

Ca^{2+} – Calcium ion

CI – Confidence interval

CV – Coefficient of variation

d – Cohen's *d*

GXT – Graduated exercise test

H^{+} - Hydrogen ion

ICC – intraclass correlation coefficient

IOC – International Olympic Committee

K^{+} - Potassium ion

L/min – Litres per minute

mmol – Millimole

n/s – Non-significant

PCr – Phosphocreatine

P_i – Inorganic phosphate

PLA – Placebo

RPE – Rated perceived exertion

ROS – Reactive oxygen species

SD – Standard deviation

SEM – Standard error of measurement

TE – Typical error

TT – Time trial

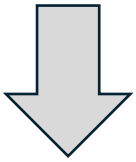
VO_{2max} – Maximal oxygen consumption

wk – Week

Thesis Outline

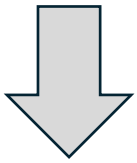
Chapter One – Literature Review

Introduction to the mechanisms of muscular fatigue during high-intensity exercise. Review the current literature on carnosine, its properties, and its ability to be increased in the muscle to benefit exercise performance. Review the physiological demands of rowing.



Chapter Two – Experimental Study

Investigating the effect of topical carnosine on 1,500 m rowing ergometer TT performance in experienced club-level rowers.



Chapter Three –Summary and Future Directions

Thesis summary, strengths, limitations, and future directions.

Background

Many competitive sports contain short-duration, high-intensity efforts where athletes experience muscular fatigue caused by the by-products of anaerobic metabolism. Sports that require considerable anaerobic energy contribution can be particularly susceptible to the by-products of anaerobic metabolism, such as 400 m track running (Duffield et al., 2005), 2,000 m rowing (Volianitis et al., 2020), and the multiple repeated sprints involved in rugby (Duthie et al., 2003). Historically, lactate ions were thought to be the main anaerobic by-product that causes fatigue, however, research from Brooks (2001) has largely refuted this. Rather, it is now understood that muscular fatigue during high-intensity exercise is caused by a range of factors, including, but not limited to, an accumulation of hydrogen ions (a different anaerobic by-product) in muscle and blood, which can decrease blood pH to ~ 6.5 (Westerblad et al., 2010).

Research has shown that increasing pH buffering capacity improves high-intensity exercise performance (de Salles Painelli et al., 2018; Grgic et al., 2020; Saunders et al., 2017; Suzuki et al., 2004; Weston et al., 1996). One method to raise buffering capacity is to elevate the carnosine content within skeletal muscle (Sale et al., 2010). Carnosine is a cytoplasmic dipeptide with a pKa of 6.8, making it an ideal intracellular buffer (Sale et al., 2010). Additionally, research in vitro shows that carnosine can improve the calcium sensitivity within the muscle, which may positively influence muscle function (Dutka & Lamb, 2004; Dutka et al., 2011; Lamont & Miller, 1992). Direct ingestion of carnosine is inefficient due to its rapid hydrolysis by carnosinase in the blood, making beta-alanine supplementation preferable as it serves as the rate-limiting precursor in carnosine synthesis (Sale et al., 2010). A systematic review and meta-analysis showed that beta-alanine supplementation can improve exercise performance and capacity in various high-intensity tests (Saunders et al., 2017). Despite the

effectiveness of beta-alanine, it can be considered inconvenient as it requires large dosages per day and can cause paresthesia (Rezende et al., 2020).

Topical carnosine gel is a relatively new, little-researched method to increase muscle carnosine. Nevertheless, topical carnosine is commercially available with a product named LactiGo™ , which claims to improve performance by up to 15% (LactiGo, n.d.-b). In an equine model, topical carnosine raised muscle carnosine by 46±17% % in 60 minutes. However, research investigating the ergogenic effects of topical carnosine is limited and conflicting. Sharpe and Macias (2016) found topical carnosine to improve performance in two high-intensity intermittent tests by 0.94% and 4%, respectively. More recently, evidence from Harnish and Miller (2023) showed that topical carnosine did not affect the performance of cyclists in a repeated 30-s Wingate test. Currently, no research has investigated the effect of topical carnosine on a single bout of high-intensity exercise.

A rowing ergometer time trial has seen athletes reach post-event lactate values up to 32 mmol/L and blood pH as low as 6.74 (Nielson, 1999), making it a suitable single-bout exercise test to measure the effects of topical carnosine. Additionally, four papers have investigated the effect of elevated muscle carnosine on rowing performance, often resulting in a small, non-significant improvement in 2,000 m rowing ergometer performance (Baguet et al., 2010; Ducker et al., 2013; Hobson et al., 2013; Jaques et al., 2019). However, at the 2028 Olympic Games, athletes will compete on a shortened course of 1,500 m. This shortened distance may place more stress on the body's buffering systems because the proportion of energy production from anaerobic glycolysis will likely be higher. Therefore, this thesis aimed to investigate the effect of topical carnosine on 1,500 m rowing performance.

Chapter One – Literature Review

Review Aim

This literature review will first provide a brief overview of the physiological mechanisms underlying muscular fatigue during high-intensity exercise, establishing the foundational context for the main discussion on interventions to improve performance by delaying the onset of muscular fatigue. Next, an analysis of current research on nutritional supplementation aimed at increasing intramuscular carnosine concentrations will be presented, with a particular focus on ergogenic benefits on exercise performance. More specifically, this section will compare the highly studied beta-alanine supplementation method with the less-researched topical carnosine. Lastly, this review will examine the potential efficacy of topical carnosine gel as a novel intervention to enhance short-distance rowing performance.

1. Muscular Fatigue During High-Intensity Exercise

Anaerobic metabolism rapidly generates adenosine triphosphate (ATP) during high-intensity exercise. The amount of ATP a person can generate from anaerobic metabolism is referred to as their anaerobic capacity, which is determined by the levels of phosphocreatine (PCr) in the muscle, the muscle's buffer capacity, and the volume of muscle mass (Sahlin, 2014). Aerobic metabolism also contributes to ATP production during high-intensity exercise; however, the ratio of ATP generated from each energy system depends on the duration and intensity of exercise (Baker et al., 2010).

1.2 Limitations of Anaerobic Metabolism

Muscle fatigue that occurs 5 to 10 seconds into maximal intensity exercise is thought to be caused by a substantial decrease in readily available muscle PCr, which decreases the rate of ATP production (Sahlin et al., 1998). Following this initial onset of muscle fatigue, anaerobic metabolism continues to support high-intensity exercise via anaerobic glycolysis. Anaerobic glycolysis metabolises carbohydrates without oxygen, enabling a high rate of ATP production to sustain high relative power output (Baker et al., 2010). The predominant use of anaerobic glycolytic energy production is unsustainable for long durations.

Historically, it was thought that the accumulation of lactate ions, a by-product of anaerobic glycolysis, was a leading cause of muscular fatigue, but research from Brooks (2001) has largely refuted this and suggests that lactate ions are beneficial during high-intensity exercise by protecting against the accumulation of extracellular potassium ions (K^+). Furthermore, lactate ions can be transported via the bloodstream to other muscles and organs to be oxidised as a fast and efficient fuel source, or undergo gluconeogenesis in the liver and kidneys (Hall et al., 2016).

Limitations of anaerobic glycolysis arise from limited muscle glycogen stores and the accumulation of hydrogen ions (H^+). These hydrogen ions are a by-product of anaerobic glycolysis that is naturally accounted for by the body's pH buffering systems; however, when buffering capacity is exceeded, approximately 0.001% of the H^+ ions produced are released into the cytosol (Sahlin, 2014). This release of H^+ can cause muscle pH to decrease by as much as 0.5, from ~ 7.0 to ~ 6.5 (Westerblad et al., 2010). An influx of H^+ into the muscle cell is thought to cause pain (Mense, 2008), impair the activity of key enzymes involved in the glycolytic process (glycogen phosphorylase and phosphofructokinase) (Walsh et al., 2002),

affect excitation-contraction coupling in the muscle by decreasing myofibrillar calcium ion (Ca^{2+}) sensitivity, and reduce the ability of the sarcoplasmic reticulum to release Ca^{2+} (Calderón et al., 2014).

1.3 Buffer Capacity and Muscle Typology

The human body has buffering systems that resist changes in pH. Buffer capacity has the most influence on continuous maximal exercise lasting 1 to 10 minutes or exercise containing multiple repeated sprints, as this is where the largest decline in muscle pH is seen (Cairns, 2006). Buffer capacity appears to be influenced by muscle fiber typology. Research by Nakagawa and Hattori (2002) found that buffer capacity was higher in fast twitch (type IIa) dominant participants, compared to slow twitch (type I) dominant participants during 60 seconds of exercise in ischemic conditions. This outcome makes logical sense when considering the metabolic properties of muscle fibers. Slow-twitch fibers are primarily aerobic, whereas fast-twitch fibers differ: Type IIb fibers are primarily anaerobic, while Type IIa fibers have a mix of aerobic and anaerobic properties (Bourdeau Julien et al., 2018).

Buffer capacity can improve with training. Parkhouse et al. (1985) found that trained rowers had a significantly higher buffer capacity than untrained participants, despite having a similar percentage of fast-twitch muscle fibres in the vastus lateralis muscle. This difference was not concluded as a training adaptation because the study did not involve a training intervention. To clarify the effect of training on buffer capacity, Weston et al. (1996) studied six well-trained cyclists who completed six high-intensity interval sessions over 28 days. This intervention significantly increased buffer capacity, which has been supported by further research, where buffer capacity increased after 12 weeks of high-intensity interval training in vegetarian males

(de Salles Painelli et al., 2018), and after an 8-week sprint training program in untrained males (Suzuki et al., 2004).

1.4 Alternative Determinants of Muscle Fatigue Beyond pH Shifts

A decline in pH is not the only cause of muscle fatigue. Increased levels of inorganic phosphate (P_i), adenosine diphosphate (ADP), and reactive oxygen species (ROS) play a role in muscle fatigue, but the mechanisms need clarification (Allen et al., 2008; Cady et al., 1989; Keyser, 2010). Westerblad et al. (2002) suggest that a build-up of P_i released from the hydrolysis of PCr may be the leading cause of fatigue during high-intensity exercise, not muscle acidosis. This argument is supported by the findings of Sahlin and Ren (1989) who showed that from a state of muscular fatigue, force production can recover faster than pH; thus, pH must not be the sole cause of reduced force production. Westerblad et al. (2002) suggested that a build-up of P_i in the muscle can affect cross-bridge force and is the leading cause of reduced myofibrillar Ca^{2+} sensitivity. This claim stems from research using muscle fibers from mice that were genetically modified to prevent the accumulation of P_i during repeated muscle contractions (Dahlstedt et al., 2001; Dahlstedt et al., 2000). The genetic modification caused P_i to be higher at rest, resulting in decreased force production (Ca^{2+} activated) in a non-fatigued state; however, after 100 fatiguing tetanic contractions, there was no significant decline in force production in the genetically modified muscle fibers, compared to the natural muscle fibers where force production declined to < 30% of starting force.

Westerblad et al. (2002) argue that pH has a very small or irrelevant effect on muscular fatigue when skeletal muscle operates at physiological temperatures, suggesting that a decrease in muscle pH may be a case of correlation, not causation. Instead, Westerblad et al. (2002)

hypothesised that pH's main effect on muscle fatigue is indirect and acts via the nervous system's activation of a pain sensation local to the muscle. This hypothesis somewhat aligns with the central governor model (Noakes, 2011), which proposes that the central nervous system monitors metabolic by-products and oxygen saturation during exercise, regulating muscle activation to prevent long-term harm, ultimately reducing exercise motivation and performance. However, evidence to support this theory is conflicting (Inzlicht & Marcora, 2016).

In favor of pH influencing muscle fatigue, Cairns (2006) combined peak tetanic force results from two previous studies (Cady et al., 1989; Spriet et al., 1987) and showed that as pH decreases, the rate of muscular fatigue increases; that is, muscle fatigue was exponentially affected as the pH decreases. This finding must be interpreted cautiously because the studies used electrical stimulation and blood flow restriction to elicit anaerobic metabolism and reduce aerobic contribution. These protocols may limit the natural process of energy production and buffering under regular conditions, considering that the accumulation of H^+ in muscle does not appear to affect aerobic metabolism (Walsh et al., 2002). Despite the limitations of these protocols, Cairns' (2006) findings suggest that decreases in pH should be delayed or avoided if muscular performance is to be maintained. These findings are supported by evidence where high-intensity exercise performance was improved after raising buffering capacity (Grgic et al., 2020; Saunders et al., 2017), suggesting that acidosis does play a role in muscle fatigue.

1.5 Mitigating Anaerobic Fatigue: Supplement-Based Interventions

Although the exact mechanisms need clarification, the commonly accepted limitations of anaerobic metabolism mean that muscular fatigue is inevitable when power output is constantly higher than what can be supported by aerobic metabolism, or when H^+ production exceeds pH

buffering capacity. In the context of competitive sports, it is evident that anaerobic glycolysis is an important energy system for multiple disciplines: continuous single-bout intense sports such as 400 m track running (Duffield et al., 2005) and 2,000 m rowing (Volianitis et al., 2020) as well as in sports with multiple repeated sprints, for instance, rugby (Duthie et al., 2003). These sporting contexts provide a rationale for coaches and athletes to use training, nutrition, supplementation, and recovery methods to reduce the impact of the by-products of anaerobic glycolysis on muscular performance and delay muscular fatigue. Concerning supplementation, two oral supplements commonly researched for high-intensity exercise performance are sodium bicarbonate and beta-alanine because they improve the buffering mechanisms in the blood and in the muscle, respectively (Maughan et al., 2018).

A recently published systematic review and meta-analysis concluded that sodium bicarbonate supplementation improves muscular endurance ($p = 0.001$), but not muscular strength ($p = 0.725$) (Grgic et al., 2020). This improvement in muscular endurance is attributed to an increased bicarbonate concentration in the blood, which enhances extracellular pH buffering (Sahlin, 2014). The International Society of Sports Nutrition has deemed sodium bicarbonate an effective ergogenic aid for single-bout and intermittent high-intensity sports lasting for 0.5 to 12 minutes, for example, judo, boxing, high-intensity cycling, and rowing (Grgic et al., 2021).

Beta-alanine supplementation elevates muscle carnosine and improves high-intensity exercise performance in a variety of sports, for example, 2,000 m rowing (Hobson et al., 2013), and repeated upper- and lower-body 30-s Wingate tests (de Salles Painelli et al., 2014; Tobias et al., 2013). An increase in intramuscular carnosine appears to be most effective at improving exercise performance of 0.5 to 10 minutes in duration (Saunders et al., 2017), which aligns

with the exercise time frame where buffer capacity has the greatest impact (Cairns, 2006). However, carnosine may have additional ergogenic effects aside from intramuscular buffering, with evidence showing that carnosine can influence Ca^{2+} handling in human and animal skeletal muscle fibres in vitro (Dutka & Lamb, 2004; Dutka et al., 2011; Lamont & Miller, 1992), suggesting that a rise in muscle carnosine levels may improve muscle function. The remainder of this review will discuss the ergogenic properties of carnosine, evaluate the methods currently available to increase muscle carnosine, and identify areas where future research is needed.

2. Carnosine

Carnosine is a cytoplasmic dipeptide formed by carnosine synthase bonding beta-alanine and histidine (Sale et al., 2010). It is stored throughout the body but is most abundant in skeletal muscle and brain tissue (Sale et al., 2010). Carnosine has many different functions. Firstly, the cytosol has a pH of ~ 7 (Street et al., 2001), making carnosine an effective intracellular buffer with a pKa of 6.8 (Sahlin, 2014). Sahlin (2014) suggested that 5-10% of muscle buffering capacity can be attributed to carnosine. Secondly, findings in both animal and human studies demonstrate that an increase in muscle carnosine can improve the Ca^{2+} sensitivity of the contractile apparatus of skeletal muscle, possibly improving muscle function (Dutka & Lamb, 2004; Dutka et al., 2011; Lamont & Miller, 1992). Thirdly, carnosine is an ingredient in many anti-ageing topical gels and creams for its antioxidant effects. Carnosine reduces reactive oxygen species (Jukić et al., 2021), acts as a divalent metal ion chelator (Matthews et al., 2019), and protects proteins from glycation (Hipkiss et al., 1995).

2.1 Beta-Alanine to Increase Muscle Carnosine

Oral carnosine supplementation is ineffective at raising muscle carnosine levels because carnosinase, an enzyme found in the blood, liver, and kidneys, hydrolyses carnosine before it reaches skeletal muscle (Sale et al., 2010). For an effective supplementation strategy, beta-alanine can be consumed orally. Beta-alanine naturally exists in skeletal muscle and is the rate-limiting precursor for carnosine synthesis (Sale et al., 2010). Greater beta-alanine availability allows more carnosine to be synthesised in skeletal muscle, with the saturation point yet to be identified (Rezende et al., 2020). Beta-alanine is included on the International Olympic Committee's (IOC) list of supplements containing good to strong evidence of improving performance (Maughan et al., 2018).

The typical dosage of beta-alanine is 3.2 to 6.4 g per day, divided into multiple smaller doses over at least 28 days (Rezende et al., 2020). Supplementing with 6.4 g of beta-alanine per day for four weeks has been reported to increase muscle carnosine by 40-60% in type II fibers and 60-70% in type I fibers (Kendrick et al., 2009); however, as discussed above, type I muscle fibres have lower initial carnosine values than type II muscle fibres. Paresthesia, a common side effect of beta-alanine ingestion, is thought to be caused by the ability of beta-alanine to bind to Max-related G-protein coupled receptor member D (MRGPRD), a peripheral neuronal receptor (Rezende et al., 2020). More recently, slow-release formulas have been produced to prevent the sensation of paresthesia.

2.2 The Role of Carnosine as an Intracellular Buffer

As mentioned previously, carnosine's intracellular buffering properties mean it plays a key role in buffer capacity. A 2017 systematic review (Saunders et al.) consolidated 40 individual

articles investigating the effect of beta-alanine supplementation on high-intensity exercise performance. The authors concluded that beta-alanine supplementation has a small ergogenic effect on exercise lasting 0.5 to 10 minutes, which aligns with the time frame where pH decline is the largest (Cairns, 2006).

Elevated muscle carnosine improved performance in tests such as 100 and 200 m swimming (de Salles Painelli et al., 2013), repeated 30 s upper body Wingate's (Tobias et al., 2013), Yo-Yo Intermittent Recovery Test Level 2 (Saunders et al., 2012), 2,000 m rowing (Hobson et al., 2013), a Judo-specific fitness test (de Andrade Kratz et al., 2017), and repeated 30 s lower body Wingate's (de Salles Painelli et al., 2014). Interestingly, beta-alanine can also improve 10 km running time trial performance (Santana et al., 2018), where the duration is much greater than the time frame of 0.5 to 10 minutes, where beta-alanine is considered most effective. Elevated muscle carnosine levels have also been reported to improve exercise capacity in protocols such as cycling capacity at high relative power output (Hill et al., 2007) and cycling time to exhaustion at 120% VO_{2max} (Glenn et al., 2015).

Increased muscle carnosine tends to have a greater effect on exercise capacity than performance (0.4998, medium effect (95% CI 0.246 to 0.753) vs 0.1078, small effect (95% CI −0.201 to 0.416)) (Saunders et al., 2017). This observation makes logical sense when considering that performance tests require pacing, so they may be less sensitive to H^+ than capacity tests that go to failure. Performance tests may also have a smaller window for improvement because of time and distance parameters, whereas in capacity tests, the participants' goal is to go as long as possible. It is important to consider that although the changes in performance tests are smaller, they may be more relevant in an applied context.

Another consideration, is that elevated muscle carnosine may have a training workload effect. After five weeks of strength training using a protocol that required anaerobic glycolytic energy production, beta-alanine supplementation showed a greater increase in power output than placebo (Maté-Muñoz et al., 2018). This outcome may have been achieved from a higher workload during each training session, enabled by an increased buffer capacity. This, in turn, could lead to greater stimulus and subsequently enhance adaptations. Further research is needed to confirm this training workload effect.

Despite the research showing the positive effects of beta-alanine on high-intensity exercise performance, there is also research where beta-alanine had no effect, including protocols such as time to exhaustion in supramaximal sprints (Jagim et al., 2013), high-velocity intermittent running (Smith-Ryan et al., 2012), and competitive swimming ranging from 50 to 400 m (Chung et al., 2012). Of note, in the research by Jagim et al. (2013) and Smith-Ryan et al. (2012), the training schedules were not controlled over the 5-week and 28-day supplementation periods, respectively. Considering that each participant experienced the initial testing session before supplementation, and therefore had knowledge of the post-testing procedure, it is possible that the volume, intensity, and specificity of the participants' training between tests influenced the post-test result to a greater extent than the effect of beta-alanine supplementation.

2.3 Carnosine and Ca^{2+} Sensitivity

Many of the studies discussed above focused on how elevated muscle carnosine influences buffering capacity and the ability to delay the decline in muscle pH during high-intensity

exercise. However, the mechanisms of carnosine as an ergogenic aid are not limited to pH buffering capacity. Calcium ions play an important role in muscle contraction, particularly in conditions of muscular fatigue, where the release and reuptake of Ca^{2+} from the sarcoplasmic reticulum is reduced, and myofilament Ca^{2+} sensitivity is lower (Allen et al., 2008). These processes associated with Ca^{2+} handling can be positively influenced by carnosine. Muscle carnosine can act as a cytoplasmic regulator because it binds to H^+ and Ca^{2+} (Jones et al., 2017). When H^+ accumulates in the muscle during exercise, carnosine has been demonstrated to reduce the competition of H^+ and Ca^{2+} at the troponin-c binding site by taking H^+ and unloading Ca^{2+}, allowing muscle contraction to continue effectively (Matthews et al., 2019). Further, findings in both animal and human studies demonstrate that increasing muscle carnosine can increase the calcium sensitivity of the contractile apparatus of skeletal muscle, possibly improving muscle function (Dutka & Lamb, 2004; Dutka et al., 2011; Lamont & Miller, 1992).

Elevated muscle carnosine does not appear to affect maximum voluntary force output but can positively influence muscle relaxation speed (Hannah et al., 2015; Matthews et al., 2019). Faster muscle relaxation time can improve performance by improving muscular efficiency (Jones et al., 2017). The effect carnosine has on muscle relaxation time can most likely be attributed to the Ca^{2+} binding ability of carnosine, providing a greater opportunity for Ca^{2+} to dissociate from troponin during the relaxation period (Westerblad et al., 1997). The extent of increasing relaxation speed and its contribution to performance from elevated muscle carnosine content is currently unclear. This lack of clarity is likely attributed to the multifaceted nature of exercise performance and difficulty in isolating a performance improvement to one mechanism of carnosine when multiple mechanisms may be operating simultaneously.

3. Topical Carnosine

Beta-alanine increases muscle carnosine, enhances buffering capacity, and improves performance, but it still has limitations. As mentioned above, beta-alanine supplementation requires high doses of 3.2 to 6.4 g daily over a 28-day period, which can be inconvenient. Although beta-alanine has proven successful in increasing muscle carnosine, it is inefficient. Stegen et al. (2013) found that only 2.8% of beta-alanine consumed in a five-week supplementation protocol was converted to muscle carnosine. The recent formulation of topical carnosine, also called transdermal carnosine, may be a more efficient method to increase muscle carnosine. At the time of writing, there is little research on the effects of topical carnosine on muscle carnosine content and high-intensity exercise performance.

3.2 Topical Carnosine to Increase Skeletal Muscle Carnosine

In a study by Dieter et al. (2021), a topical carnosine gel was applied to the middle gluteal muscle in ten thoroughbred racehorses. The researchers chose to investigate carnosine uptake in horses because horses naturally have up to eight times more muscle carnosine than humans, so, logically, if muscle carnosine increased in horses, it would also work in humans. After the gel was applied, muscle carnosine increased by 35±11% (mean ± standard error) in 30 minutes ($p = 0.001$), 46±17% in 60 minutes ($p = 0.044$), and 76±45% in 120 minutes ($p = 0.20$). Within these results, there was large individual variation amongst horses, with high responders having muscle carnosine increases of 200 to 300% by 120 minutes, whereas low responders only had 0 to 15% changes.

In another study, horses fed beta-alanine for 30 days showed large individual variation, with muscle carnosine increasing by 5.7-46.9% in type IIa and IIb fibers (Dunnett & Harris, 1999).

These results suggest that topical carnosine can be equally, if not more effective than beta-alanine for raising muscle carnosine, at least in an equine model. As such, topical carnosine may be more cost-effective and time-convenient than beta-alanine supplementation because it increases muscle carnosine within two hours of application and does not require a loading phase.

3.3 Topical Carnosine and High-Intensity Exercise Performance

Currently, only two studies have investigated the effect of topical carnosine on high-intensity exercise performance in humans. Both papers used a commercially available topical carnosine gel named LactiGo™. LactiGo™ claims to increase muscle carnosine levels within one hour of application and improve high-intensity exercise performance by up to 15% (LactiGo, n.d.-b). LactiGo™ states that menthol 1.25% is an active ingredient, but it also contains ethoxdiglycol, glycerine, L-carnosine, magnesium sulphate, phenoxyethanol, water, and xanthan gum. Of note, Dieter, an author in the previously discussed paper investigating the effect of topical carnosine on muscle carnosine in thoroughbred horses, was involved in the formulation of LactiGo™ (Pardi, 2020). Based on this connection, it is reasonable to assume LactiGo™ has a similar formula and effectiveness as the topical carnosine used in the thoroughbred study.

Sharpe and Macias (2016) investigated the effect of topical carnosine on performance in 11 elite male football players who completed two high-intensity intermittent tests. It is worth noting that one of the researchers, Macias, is the director of research development for LactiGo™ (Macias, 2016). This study used a non-blinded sequential design. Forty-five minutes before testing began, each participant applied warm-up cream, or warm-up cream + topical

carnosine, to their arms, legs, and torso. There was a three-day washout period between each trial. On average, topical carnosine increased the distance covered during a Yo-Yo intermittent recovery test by 84 m (0.94%) and improved the total time to complete a test consisting of 3 x [1000 m run, 90 s rest] by 9 s (4%). It cannot be concluded with certainty that these performance gains were from elevated muscle carnosine because it was not measured. That said, the 4% improvement in the 3 × 1000 m run is comparable to the 2.85% median performance increase reported with beta-alanine supplementation, a value calculated by Hobson et al. (2012) based on 15 studies that used a range of exercise tests. Other factors that may have influenced performance are learning effects between trials and the fact that LactiGo™ also contains menthol, which elicits cooling and analgesic effects (Pergolizzi Jr et al., 2018).

In the second study, Harnish and Miller (2023) investigated the effect of topical carnosine on the performance of 15 trained male cyclists in a repeated Wingate test. This study used a more robust design than Sharpe and Macias (2016), with a randomised control double blind placebo crossover design. The participants applied topical carnosine or placebo gel to their legs at least 60 minutes before each testing session started. The repeated Wingate testing consisted of 5 x 30 s sprints with 4 to 5 minutes of recovery between efforts. Topical carnosine did not affect peak 1 s power or mean 30 s power during repeated Wingate sprints, compared to the placebo. Again, muscle carnosine values were not measured.

To date, the available research investigating the effect of topical carnosine on high-intensity exercise performance is conflicting. Thus, more research on this topic is needed. The studies discussed above both involved intermittent exercise tests in trained participants. Future research using a single bout performance test lasting 0.5 to 10 minutes in trained participants

would be novel and contribute to our understanding of the effect topical carnosine has on high-intensity exercise performance.

4. Topical Carnosine: A Potential Ergogenic Aid for Short-Distance Rowing

Rowing requires a large aerobic and anaerobic capacity. Elite rowers have recorded VO_{2max} up to ~ 6.9 L/min (Volianitis & Secher, 2009), post-event lactate values as high as 32 mmol/L, and post-event pH as low as 6.74 (Nielson, 1999). The high metabolic demand of rowing means that energy production from anaerobic glycolysis is inevitable at race intensities. Previous studies have recognised these demands and found that beta-alanine supplementation has a small, positive effect on 2,000 m rowing performance (Baguet et al., 2010; Ducker et al., 2013; Hobson et al., 2013; Jaques et al., 2019). Table 1 summarises the current research on interventions used in rowing populations to elevate muscle carnosine and affect exercise performance.

Table 1. Interventions Designed to Elevate Muscle Carnosine and Improve Exercise Performance in Rowing Populations.

Authors	Participants	Duration	Supplementation Protocol	Experimental Design	Testing Protocol	Results
Baguet et al. (2010)	18 elite rowers. 1 female & 17 males.	7 wk	5 g/day BA or PLA.	Randomised, double blind control trial.	2,000 m TT pre-& post 7-wk BA.	• BA ↑ 2.7 ± 4.8 s • PLA ↓ 1.8 ± 6.8 s. n/s vs BA (p = 0.07). • Significant, correlation between change in muscle carnosine and 2,000 m performance improvement (p = 0.042, r = 0.498).
Hobson et al. (2013)	20 trained, club-level rowers. Sex not stated.	4 wk	6.4 g/day BA or PLA.	Randomised, double blind control trial.	2,000 m TT pre-& post 4-wk BA.	• BA ↑ 2,000 m TT performance 6.4 ± 8.1 s more than PLA. p value not stated. BA rated *very likely* of a positive effect.
Ducker et al. (2013)	16 competitive male rowers.	4 wk	80 mg/kg BA or 10 g glucose per day.	Randomised control trial.	2,000 m TT pre-& post 4-wk BA.	• BA ↑ 2.9 ± 4.1 s • PLA ↓ 1.2 ± 2.9 s • Small effect size (d = 0.20, p = 0.55). • Post supplementation, BA ↑ 750 m & 1,000 m splits compared to PLA (d = 0.57, p = 0.01).
(Beasley et al., 2018)	27 healthy male rowers.	4 wk	3 groups: BA1 had 2.4 g BA/day, BA2 had 4.8 g BA every second day, and PLA had 2.4 g cornflour/day.	Randomised, double-blind control trial.	30-min TT & 3 x 30 s sprint with 60 s recovery. 3 trials, day 0, 14, & 28.	• ↔ distance covered in the 30 min TT or 30 s average power.
Jaques et al. (2019)	25 division 3 collegiate rowers. 14 females and 11 males.	4 wk	3.2 g/day BA or PLA.	Randomised, double-blind control trial.	2,000 m TT. 3 trials, wk 1, 3, & post BA.	• BA ↑ 4 s vs PLA ↑ 2 s (n/s) • Small effect size (d = 0.29).
Suszter et al. (2020)	23 high performance male rowers.	5 wk	50 mg/kg/day BA or PLA.	Randomised, double-blind control trial.	Time to exhaustion in a treadmill GXT pre-& post 5 wk BA.	• Greater ↑ time to exhaustion for BA (n/s).

Abbreviations: Beta-alanine (BA), Placebo (PLA), Rowing ergometer time trial (TT), Week (wk), Improved (↑), Decreased (↓), No difference (↔), Graduated exercise test set at a constant speed of 11 km/h with the gradient ↑ by 3% every 2 min (GXT), non-significant (n/s)

The trend from Table 1 shows that elevated muscle carnosine levels lead to a small, non-significant improvement in rowing performance. Among the studies using a 2,000 m time trial to assess performance, the most noticeable improvement occurred in Hobson et al. (2013), which used the highest fixed daily dose of beta-alanine (not adjusted for body weight). It is possible that this larger performance improvement was due to the dose-response relationship that occurs between beta-alanine and muscle carnosine (Rezende et al., 2020). Speculatively, the higher daily dose of beta-alanine may have resulted in more muscle carnosine accumulation, which potentially led to greater buffering capacity compared to studies using lower daily dosages. Table 1 also shows that more research is needed to investigate how female rowers respond to increased muscle carnosine, as it appears that possibly only 14 of the 131, or 10.6% of the participants across all studies in Table 1, were female. The lack of female-specific research is problematic, considering female participation in rowing has been steadily increasing globally (Keenan et al., 2018; World Rowing, 2018), and female athletes make up ~ 50% of the total rowing competitors at the Olympic Games (Oronova-Hristova, 2024).

The rowing distance in the 2028 Los Angeles Olympic Games will be shortened to a 1,500 m course rather than the traditional 2,000 m due to venue constraints. Based on previous Olympic and World Championship rowing data, the 25% reduction in course length will shorten race times to approximately 3.75 to 5.25 minutes, depending on the event. A shortened race duration will likely increase the relative energy production from anaerobic glycolysis during the race, requiring a higher buffering capacity. This metabolic demand is seen in other events of similar durations, such as the 1,500 m run and 400 m swim with relative anaerobic energy system contributions of 15-25% and 10-55%, and post-event lactate values of ~ 18 and 12 mmol/L, respectively (Campos et al., 2017; Gupta et al., 2021; Haugen et al., 2021; Rodríguez & Mader, 2011). Evaluating the effect of increasing muscle carnosine for the 1,500 m distance is

warranted. Of note, rowers with high muscle carnosine had statistically significantly faster 500 m split times at 1,000 and 1,500 m within a 2,000 m rowing ergometer time trial compared to participants with low muscle carnosine (Baguet et al., 2010).

Based on the metabolic demands of rowing and previous beta-alanine and topical carnosine research, it is feasible to suggest that applying topical carnosine to rowers' legs, arms, and trunk muscles may positively influence short-distance rowing performance. There is currently no research investigating the effect of topical carnosine on rowing or any other single-bout performance test.

Conclusion

Anaerobic glycolysis is required to support the energy demands of high-intensity exercise, but it is limited by the release of H^+ into the cytosol, causing pain (Mense, 2008), impairing the activity of key enzymes involved in glycolysis (Walsh et al., 2002), and affecting Ca^{2+} handling in the muscle (Calderón et al., 2014). These effects lead to muscular fatigue. Improving the body's buffering capacity by increasing the amount of bicarbonate in the blood or carnosine in the muscle can delay the onset of muscular fatigue and improve exercise performance (Maughan et al., 2018). Beta-alanine supplementation is the most common method to raise intramuscular carnosine (Saunders et al., 2017); however, beta-alanine requires a high daily dosage (3.2 to 6.4 g) for 28 days or more, which requires continuous daily application and commitment and can cause side effects of paresthesia (Rezende et al., 2020).

Recently, research has investigated the effect of topical carnosine on high-intensity exercise performance. Topical carnosine improved performance in two high-intensity intermittent exercise tests (Sharpe & Macias, 2016), but not in a series of five repeated 30-s Wingate's (Harnish & Miller, 2023). Thus, more research is needed to evaluate the effect of topical carnosine on high-intensity exercise performance. Previous research shows that beta-alanine supplementation has a small, positive effect on 2,000 m rowing performance (Baguet et al., 2010; Ducker et al., 2013; Hobson et al., 2013; Jaques et al., 2019). Based on the existing literature, it appears reasonable and practical to investigate topical carnosine's effect on a single bout of rowing, such as the 2,000 m test. However, a shortened rowing course distance for the 2028 Los Angeles Olympic Games of 1,500 m will likely increase the relative energy production from anaerobic glycolysis during the race, requiring a higher buffering capacity. This provides a twofold rationale to investigate the effect of topical carnosine on the shortened race distance: firstly, to improve the understanding of how topical carnosine affects continuous high-intensity exercise performance, and secondly, to provide research for coaches and athletes in the current Olympic cycle to evaluate and make informed decisions regarding supplementation. Therefore, Chapter Two was designed to investigate the effect of topical carnosine on 1,500 m rowing ergometer time trial performance in experienced male and female club-level rowers.

Chapter Two – Experimental Study

Investigating the Effect of Topical Carnosine on 1,500 m Rowing Performance in Experienced Club-Level Rowers

Abstract

Background: The shortened rowing distance (1,500 m) in the 2028 Olympic Games will increase the proportion of anaerobic glycolytic energy production during the race, placing more stress on the body's buffering systems. Previous research has shown that elevating intramuscular carnosine concentrations may improve buffering capacity and high-intensity exercise performance, but there is little research investigating these effects via topical carnosine application. The purpose of this study was to investigate the effects of topical carnosine on 1,500 m rowing performance. ***Methods:*** In a double blind, placebo-controlled crossover study, 13 experienced rowers (> 3 years' experience, 11 male, 2 female) completed two 1,500 m time trials (TT) on a Concept 2 rowing ergometer separated by one week. Forty-five minutes before each TT started, 15 mL of topical carnosine (CAR) or ultrasound placebo (PLA) gel was applied to the participants' back and limb muscles. Time (s) to complete TT, 500 m split times, and rated perceived exertion (RPE) were measured. Data was analysed using a dependent paired t-test and Cohen's effect sizes. ***Results:*** There were trivial, non-significant differences in performance between conditions for 1,500 m TT performance (CAR 300.5 ± 23.1 s; PLA 300.4 ± 24.2 s; $p = 0.945$; $d = 0.004$), 500 m split times (500 m $p = 0.808$, $d = 0.009$; 1000 m $p = 0.830$, $d = 0.011$; 1500 m $p = 0.849$, $d = 0.020$), and RPE ($p = 1.000$, $d = 0.000$). It was evident that the intervention did not affect TT performance, so the reliability of the 1,500 m TT was calculated using Trial 1 vs Trial 2 (Trial 1 301 ± 23.8 s, Trial 2 300.0 ± 24.0 s; $p = 0.114$; $d = 0.074$; *trivial* effect size; TE = 2.8 s; ICC = 0.985). ***Conclusion:*** Topical carnosine does not affect 1,500 m rowing ergometer TT performance in experienced club-level rowers.

Key words: Beta-alanine, carnosine, rowing, high-intensity exercise

Introduction

Rowing requires a large aerobic capacity, demonstrated by elite rowers reaching VO_{2max} values of up to 6.9 L/min (Volianitis & Secher, 2009). However, the anaerobic contribution to rowing performance should not be underestimated. Nielson (1999) found that post-event blood lactate values can reach up to 32 mmol/L, and blood pH can decrease as low as 6.74. This decline in pH is caused by an accumulation of hydrogen ions (H^+) in the muscle or blood, which are released as a by-product of anaerobic metabolism (Westerblad et al., 2010). A decline in pH is detrimental to performance because it can disrupt key enzymes required in the glycolytic process (Walsh et al., 2002), cause pain in the muscle (Mense, 2008), and decrease force production (Cairns, 2006; Metzger & Moss, 1987). High-intensity training can improve the body's buffering systems that combat against H^+ accumulation (de Salles Painelli et al., 2018; Suzuki et al., 2004; Weston et al., 1996), along with sodium bicarbonate supplementation, which raises bicarbonate levels in the blood (Price & Singh, 2008), or beta-alanine supplementation, which raises carnosine levels in the muscle (Rezende et al., 2020).

Carnosine is a cytoplasmic dipeptide with a pKa of 6.8, making it an effective intracellular buffer (Sahlin, 2014). Carnosine improves the Ca^{2+} sensitivity of the muscle, potentially improving muscle function (Dutka & Lamb, 2004; Dutka et al., 2011; Lamont & Miller, 1992). Direct oral supplementation of carnosine is impractical because carnosinase hydrolyses carnosine in the bloodstream (Sale et al., 2010). Instead, beta-alanine supplementation is used so that carnosine synthase can bond beta-alanine with histidine to form carnosine in the muscle (Sale et al., 2010). Supplementing with 3.2 to 6.4 g/day of beta-alanine for 4 to 7 weeks has a small, but often non-significant improvement in 2,000 m rowing ergometer performance (Baguet et al., 2010; Ducker et al., 2013; Hobson et al., 2013; Jaques et al., 2019). However,

looking deeper into these studies, Baguet et al. (2010) neared statistical significance ($p = 0.07$), Hobson et al. (2013) concluded that beta-alanine was *very likely* to improve performance, and Ducker et al. (2013) found that the 750 and 1,000 m split times were significantly faster with beta-alanine.

Recently, topical carnosine, a novel method to increase muscle carnosine, has become commercially available. In an equine study, topical carnosine increased muscle carnosine by 46±17% in 60 minutes (Dieter et al., 2021). There is limited and conflicting evidence regarding the effects of topical carnosine on high-intensity exercise performance. Topical carnosine improved performance in two high-intensity intermittent tests by 0.94% and 4%, respectively (Sharpe & Macias, 2016) but did not affect performance in a repeated 30-s Wingate cycling test (Harnish & Miller, 2023). Further research into the efficacy of topical carnosine to improve high-intensity exercise performance is warranted because, arguably, the single application of a topical carnosine gel 45 minutes before exercise is more convenient than beta-alanine supplementation, where a standard protocol requires multiple doses per day for 28 days or more (Rezende et al., 2020). A rowing ergometer time trial (TT) would be an ideal way to test topical carnosine in a single bout of high-intensity exercise because the results can be compared against previous research in rowing that used beta-alanine to raise intramuscular carnosine levels. Rowers at the 2028 Olympic Games will race on a shortened race distance of 1,500 m, likely leading to a higher proportion of anaerobic glycolytic energy system contribution and more stress on the body's buffering systems. Therefore, this current study aims to investigate the effect of topical carnosine on 1,500 m rowing ergometer TT performance. Based on the research regarding beta-alanine supplementation and rowing performance, it was hypothesised that topical carnosine would improve 1,500 m TT performance.

Methods

Participants

Thirteen experienced (> 3 years rowing) rowers competing at club level or higher volunteered for the study (11 male, 2 female). Participant characteristics are presented in Table 2. Participants were excluded if they had used beta-alanine supplementation within 30 days of the first testing session (n = 0). Participants were recruited through expression of interest emails and text messages sent to rowing clubs in the Waikato and Auckland regions of New Zealand. Participants were asked to refrain from high-intensity exercise (heavy breathing, > 80% max heart rate) for 24 hours before each TT. The sample size calculated for a paired t-test (80% power, $\alpha = 0.05$) determined that 13 participants were required for this study. Participants were randomly allocated into group A or B (Group A: Week 1 = Carnosine, Week 2 = Placebo. Group B: Week 1 = Placebo, Week 2 = Carnosine). All participants were given information on the tasks, associated risks, and their rights before and at the first scheduled session. Informed consent was given at the first session before any experimental tasks began (including height and weight data collection). This study gained ethical approval from the University of Waikato Human Research Ethics Committee HREC(Health)2024#29 and conformed to the guidelines for professional practice and community contact in the conduct of university research or related activities.

Table 2. Participant Characteristics (Mean ± SD)

	Group A (n = 7)	Group B (n = 6)
Age (y)	21 ± 2.4	21 ± 3.2
Height (cm)	183.1 ± 8.5	184.3 ± 6.2
Weight (kg)	83.7 ± 11.7	82.1 ± 7.6
Males	5	6
Females	2	0

Research Design

This study used a double-blind, randomised crossover design. All rowers attended two testing sessions and received ~ 15 mL of topical carnosine or placebo gel. Each participant applied the gel to their arms and legs after being given ~ 5 mL of gel per leg and ~ 1.5 mL of gel per arm. The researcher applied ~ 2 mL of gel to the participant's back. This dose was selected based on previous research (Harnish & Miller, 2023; Sharpe & Macias, 2016) and the recommendations provided by the carnosine gel manufacturer adapted to full-body application (LactiGo, n.d.-a). The topical carnosine used in this study was Lactigo™ (Lactigo™, Henderson, USA), a commercially available gel containing menthol 1.25%, ethoxdiglycol, glycerine, L-carnosine, magnesium sulphate, phenoxyethanol, water, and xanthan gum. The placebo was Parker Aquasonic Ultrasound Gel with 1.25% menthol added to mimic the smell of Lactigo™ so that both gels presented as being as identical as possible. The double-blind procedure was implemented by transferring each gel into an unlabelled red or yellow bottle. Both the researcher and the study participants were unaware of which gel was in each bottle. Group A received topical carnosine on their first session (n = 7), and Group B received the placebo (n = 6). In the second session, the participants received the gel they did not receive in the first session. The two testing sessions were separated by one week to ensure washout occurred (Sharpe & Macias, 2016) and were completed at the same time of day. The 1,500 m TT is shorter than the standard 2,000 m TT the rowers are familiar with, however, a crossover design meant that any learning effect from the first to second trial was accounted for.

Experimental procedures

In the first testing session, participants did a 5-minute skin patch test for both gels on the top of the hand before applying the gel to their legs, arms, and back. There were 45 minutes between gel application and TT start. During this time, participants adjusted the foot position

on the rowing ergometer, manually set the drag factor to New Zealand testing standards (130 for males, 110 for females), and completed a warm-up. This process, including the warm-up, was replicated in the second testing session. Testing was performed on a Concept 2 rowing ergometer (Concept 2, Morrisville, USA), a reliable machine for exercise testing, SEM = 0.5% (Smith & Hopkins, 2012) and CV = 0.6% (95% CI = 0.4-1.0%) for 2,000 m TT (Schabort et al., 2010). Participants used the identical ergometer for both trials. Rowing metrics were visible during both trials, displaying stroke rate, distance, time, and 500 m split time. Verbal encouragement was given during the TT. Within 30-s post-TT, participants reported their rated perceived exertion (RPE) using the Borg 15-grade RPE scale (6-20) (Borg, 1982). The total time to complete the 1,500 m TT and data from each 500 m split were recorded as performance measures post-TT. Following the TT, participants cooled down at their own pace.

Statistical analysis

Data was analysed using a dependent paired t-test with statistical significance being accepted at $p \leq 0.05$. The standardised differences in means were calculated as Cohen's d and Hopkins' magnitude thresholds were applied (Hopkins et al., 2009), where <0.2 (trivial); 0.2 to 0.6 (small effect); >0.6 to 1.2 (moderate effect); >1.2 to 2.0 (large effect); >2.0 (very large effect). Values are presented as means ± SD.

Results

Time Trial Performance

Table 3 presents the mean TT performance data. On average, TT performance was nearly identical in both conditions (Placebo 300.4 ± 24.2 s; Carnosine 300.5 ± 23.1 s) with no statistically significant difference ($p = 0.945$). Applying topical carnosine to the skin before exercise had a *trivial* effect on 1,500 m TT performance compared to the placebo ($d = 0.004$).

Figure 1 shows the TT results from each participant, demonstrating the variation in individual responses to topical carnosine and placebo gels.

Table 3. Difference in 1,500 m Rowing Time Trial Performance: Topical Carnosine versus Placebo (Ultrasound Gel).

	Placebo	Carnosine
Mean (± SD)	300.4 ± 24.2	300.5 ± 23.1
CV	8.00%	7.70%
Difference (s)		+ 0.08
% Change		0.03%
p Value		0.945
Cohen's *d*		0.004
Effect Size		Trivial

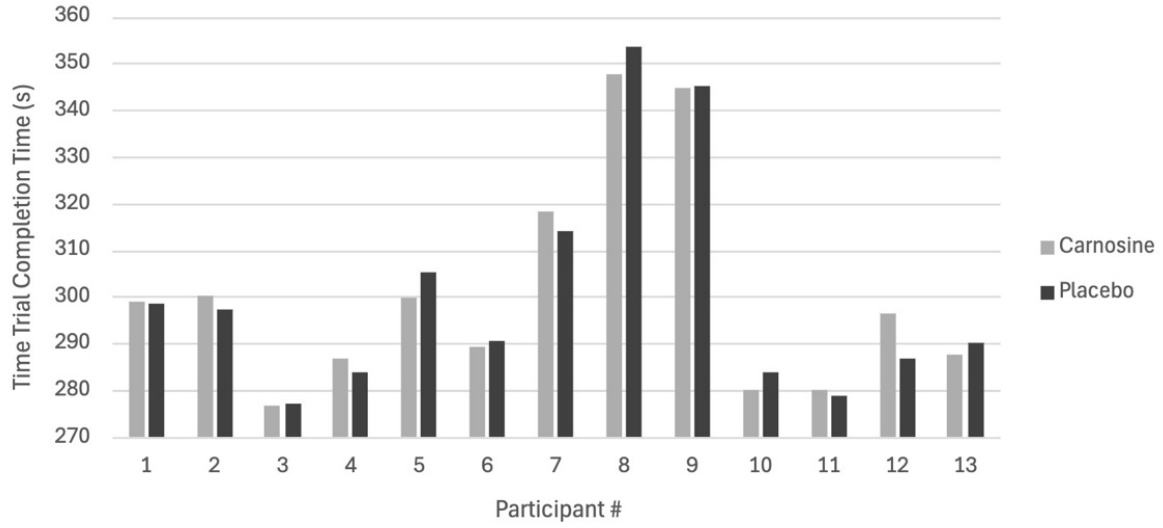


Figure 1. Individual Rowing Ergometer 1,500 m Time Trial Results: Topical Carnosine versus Placebo (Ultrasound Gel)

500 m Split Times

There were trivial differences in the 500 m split times for the two conditions, with no differences reaching statistical significance (500 m, $p = 0.808$, $d = 0.009$; 1000 m, $p = 0.830$, $d = 0.011$; 1500 m, $p = 0.849$, $d = 0.020$). Both conditions followed the same pacing trend, with the middle split (500-1000 m) being significantly slower than the first and last 500 m splits as shown in Figure 2 (500 vs 1000 m, $p = 0.002$; 1000 vs 1500 m, $p = 0.003$; 500 vs 1500 m, $p = 0.964$).

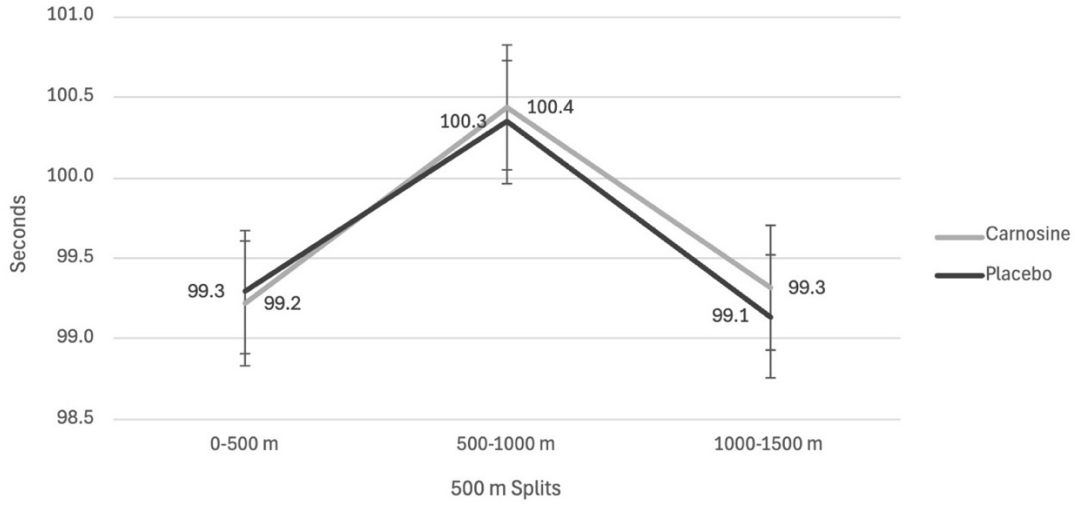


Figure 2. 500 m Split Times From 1,500 m Rowing Ergometer Time Trials Topical Carnosine vs Placebo (Ultrasound Gel).

Rated Perceived Exertion (RPE).

There was no difference in RPE between the conditions; the placebo and carnosine tests both produced a mean RPE of 18.9 (p = 1.000, d = 0.000). This result shows that the participant's perceived effort was not significantly different for each trial.

TT Learning Effect (Trial 1 vs Trial 2)

A crossover study design was used to account for learning effects between the first and second trials, but it was still of interest to determine whether learning effects occurred. Data showing the results of Trials 1 vs 2 can be seen in Table 4. On average, Trial 2 was 1.7 s faster than Trial 1, a change of -0.57% (d = 0.074, *trivial* effect size). A paired dependent t-test showed that this difference did not reach statistical significance (p = 0.114)

Inter-trial Reliability

Since there was no statistically significant effect of learning, nor a performance effect of topical carnosine, the inter-trial reliability of the 1,500 m TT was analysed. Table 4 shows that the 1,500 m rowing ergometer TT is a reliable measure of rowing performance.

Table 4. 1,500 m Rowing Performance: Trial 1 versus Trial 2

	Trial 1	Trial 2
Mean (± SD)	301.7 ± 23.8	300.0 ± 24.0
CV	7.88%	8.01%
Difference (s)		-1.7
% Change		-0.57%
p Value		0.114
Cohens *d*		0.074
Effect Size		Trivial
TE (s)		2.8
ICC		0.985
R value		0.987

Abbreviations: Standard deviation (SD), coefficient of variance (CV), typical error (TE), intraclass correlation coefficient (ICC).

Discussion

This study investigated the effect of topical carnosine on 1,500 m rowing ergometer TT performance. Previous research indicates that rowing performance can be improved by increasing muscle carnosine levels via beta-alanine supplementation (Baguet et al., 2010; Ducker et al., 2013; Hobson et al., 2013; Jaques et al., 2019). Therefore, we hypothesised that applying a topical carnosine gel to the participant's arms, legs, and back 45 minutes before the TT would result in a small performance improvement compared to a placebo gel. However, the results of this study show that topical carnosine did not affect 1,500 m rowing ergometer TT performance, perception of effort, nor the pacing strategy adopted by the rowers.

These results lead to the question of why no effect was seen, considering that in four previous studies, increasing intramuscular carnosine had a small effect on 2,000 m rowing performance, even if statistical significance was not reached (Baguet et al., 2010; Ducker et al., 2013; Hobson et al., 2013; Jaques et al., 2019). One key difference is the shorter TT length used in this current study, opting to use a 1,500 m TT based on the 2028 Olympic Games distance, meaning direct

comparisons to the previous research cannot be drawn. However, we believe a 1,500 m rowing TT was an adequate test for the ergogenic effects of topical carnosine, based on the metabolic demands of rowing (Nielson, 1999; Volianitis et al., 2020) and buffer capacity requirements of all-out exercise lasting 1 to 10 minutes (Cairns, 2006).

Like the two previous studies investigating topical carnosine as a potential ergogenic aid (Harnish & Miller, 2023; Sharpe & Macias, 2016), we did not measure the participants' intramuscular carnosine at any point. Therefore, all three studies have assumed that muscle carnosine increased after applying the topical carnosine gel, based on the research of Dieter et al. (2021) in thoroughbred horses that showed topical carnosine can elevate intramuscular carnosine levels by 46±17% in 60 minutes. Of these three studies, topical carnosine only had a performance effect when a sequential, non-blinded design was used, where learning effects are likely (Sharpe & Macias, 2016). This provides a strong rationale for future research to investigate the ability of topical carnosine to increase intramuscular carnosine in humans, particularly in the large muscle groups that have a significant role in exercise performance. Arising from the limitations of our research design, a speculative explanation for why topical carnosine did not improve performance is that it may have failed to elevate intramuscular carnosine altogether or failed to penetrate sufficiently into the deep musculature to have a meaningful effect on performance.

Assuming topical carnosine acutely increased intramuscular carnosine as intended, it is apparent that this increase in muscle carnosine did not influence performance in a single bout of high-intensity exercise. In theory, the performance gains seen in previous beta-alanine research may, in part, be caused by a higher training capacity in the time between pre- and

post-performance tests. This idea of a training effect is based on the fact that type IIa muscle fibres have a mix of aerobic and anaerobic properties (Bourdeau Julien et al., 2018) and have a high potential for intramuscular carnosine to be elevated (Kendrick et al., 2009). It is possible that elevating intramuscular carnosine for a continued period (such as > 28 days as seen in most beta-alanine research) improves buffer capacity and Ca^{2+} handling for this given period, causing more anaerobic training exposure for type IIa, and type IIb muscle fibres, resulting in a cumulative training effect. This theory relates to research by Maté-Muñoz et al. (2018) where, after a five-week, glycolytic dominant resistance training block, participants who consumed 6.4 g of beta-alanine per day lifted more weight than the placebo group throughout the training block without compromising lifting velocity. This higher work capacity resulted in a greater increase in back squat one repetition maximum (1RM) and greater average power at 1RM than the placebo group. There are notable differences when testing for strength and power compared to four to six-minute all-out performance, but the effects on training capacity are arguably still relevant. Investigating this training effect theory has been problematic for previous research because topical carnosine is relatively new as a potential ergogenic aid, and the time course of beta-alanine supplementation to increase muscle carnosine is slow. Thus, the effect of an acute, singular rise in muscle carnosine versus training with elevated muscle carnosine has not been evaluated. One way to address this enhanced training effect of carnosine is to test an acute, single application of topical carnosine against training with topical carnosine for many days before a performance test. However, factors such as total carnosine accumulation would need to be considered.

Another potential factor limiting the ergogenic potential of topical carnosine, or elevated muscle carnosine in general, is muscle fibre typology. Steinacker (1993) states that high-performing rowers have a higher ratio of slow-twitch (type I) muscle fibres than non-

performers. More recently, higher muscle carnosine levels (relative to the participants within the study) were found to correlate with better rowing performance in a group of elite rowers (Baguet et al., 2010). This finding is important to recognise because intramuscular carnosine levels strongly correlate with the percentage area of type II fibres in skeletal muscle (Baguet et al., 2011) and have been used to characterise muscle fibre typology as fast or slow twitch in previous research (Lievens et al., 2020). However, it is also known that intramuscular carnosine can be higher in a trained versus untrained population with the same muscle typology (Parkhouse et al., 1985), suggesting that despite the strong correlation, muscle fibre typology is not the sole determinant of muscle carnosine levels and *vice versa*. Although we did not assess muscle fibre typology and the current research is not entirely clear, it is possible that the participants in this current study were type I fibre dominant due to high aerobic demand and the training load of rowing (Volianitis et al., 2020). When compared to type II fibre dominant individuals, type I fibre dominant individuals may get less ergogenic benefit from increased muscle carnosine because the work output from type II fibres (the fibres mainly influenced by carnosine) would be proportionately lower, combined with the fact that the contractile abilities of type I fibres are less sensitive to an increase in P_i and H^+ than type II fibres (Lievens et al., 2020; Metzger & Moss, 1990). These muscle fibre typology differences may explain why there was no difference in performance in this current study and why previous research in rowing has only shown small effects, often not reaching statistical significance (Baguet et al., 2010; Ducker et al., 2013; Hobson et al., 2013; Jaques et al., 2019). Future research should aim to identify whether muscle fibre typology influences an athlete's response to beta-alanine and topical carnosine. Doing so may improve the judgment of athletes and coaches in deciding whether these ergogenic aids are likely to benefit performance.

Lastly, both conditions produced a near identical TT outcome, implying the main active ingredient, carnosine, did not influence performance, so we were able to assess the reliability of the 1,500 m rowing ergometer TT using Trial 1 vs Trial 2 *post hoc*. The coefficient of variation (CV), typical error (TE), and intraclass correlation coefficient (ICC) results suggest that a 1,500 m TT completed on a Concept 2 rowing ergometer is a reliable measure of rowing performance. However, for coaches and practitioners aiming to assess trivial changes in 1,500 m TT performance, Smith and Hopkins (2012) suggested that the TE should be less than 0.3%. Our results show the TE for the 1,500 m TT was 0.92% (2.8 s), a value that is comparable to the standard error of measurement (SEM) for the 2,000 m TT of 0.6% (Schabort et al., 2010) and 0.7% (Soper & Hume, 2004), which, as commented by Smith and Hopkins (2012) about these 2,000 m SEM values "Although this reliability is not ideal, it is unusual for tests of athletic performance to be this good" (p. 351). Thus, based on the results of this current study, it seems reasonable to suggest that performing a 1,500 m TT on a Concept 2 rowing ergometer is a reliable measure of performance and may be used as a race-distance specific benchmarking test for elite rowers training to compete in the upcoming 2028 Olympic Games. Coaches can be confident that an athlete is improving if their 1,500 m TT performance (time in seconds) improves by more than 0.92%, because the change in performance is greater than the TE expected between trials.

Conclusion

In conclusion, the application of a topical carnosine gel did not improve 1,500 m rowing ergometer TT performance in a group of experienced, club-level rowers. Despite the support from previous, similar research, an improvement in buffer capacity and calcium sensitivity in the muscle may not influence performance in the specific TT distance of 1,500 m. Topical

carnosine may have failed to elevate muscle carnosine to the levels required to see a noticeable difference in buffering capacity and calcium sensitivity. beta-alanine supplementation, which is long-term in nature, appears to be more effective at improving performance than topical carnosine. Thus, it is plausible that maintaining elevated muscle carnosine supports anaerobic training adaptations, leading to a greater improvement in performance. We note that muscle fibre typology may influence the ergogenic potential of carnosine.

More research is needed to determine how effective topical carnosine is at increasing muscle carnosine in humans, and whether different factors such as dosage, timing of dosage, and application to specific muscle groups have an influence on physiological and performance outcomes. Furthermore, future research should identify whether there is an adaptation effect from training with elevated muscle carnosine for prolonged periods, and whether muscle fibre typology influences the ergogenic potential of carnosine.

Chapter Three: Summary and Future Directions

Summary

Increasing the amount of carnosine in the muscle can be ergogenic for athletes competing in sports that highly depend on anaerobic glycolysis. Carnosine is an effective intramuscular buffer (Sahlin, 2014) and improves muscle contraction mechanics by influencing Ca^{2+} handling and sensitivity (Calderón et al., 2014; Dutka et al., 2011; Jones et al., 2017). When anaerobic glycolysis is the dominant energy system during exercise, there is a rapid onset of muscular fatigue caused by a build-up of H^+ in the cytoplasm. This H^+ accumulation results in pain (Mense, 2008) and limits further anaerobic glycolytic energy production due to the impairment of key enzymes in the glycolytic process: glycogen phosphorylase and phosphofructokinase (Walsh et al., 2002). At these high intensities, P_i accumulates in the muscle, which lowers the cross-bridge force output of the muscle (Westerblad et al., 2002). A 2017 systematic review and meta-analysis show that increasing muscle carnosine via beta-alanine supplementation improves exercise capacity and performance by minimising the negative effects associated with anaerobic glycolysis (Saunders et al., 2017).

Oral carnosine supplementation is limited by carnosinase, an enzyme found in the blood, liver, and kidneys, which hydrolyses carnosine before it reaches skeletal muscle (Sale et al., 2010). Beta-alanine (the rate-limiting precursor of carnosine synthesis) supplementation is the most proven method to increase muscle carnosine and improve high-intensity exercise performance lasting 1 to 10 minutes (Saunders et al., 2017). Despite the effectiveness of beta-alanine, it requires a loading phase of 3.2 to 6.4 g per day for 28 days or more (Rezende et al., 2020), of which only 2.8% gets converted into carnosine (Stegen et al., 2013).

Recently, a topical carnosine gel applied to the skin of racehorses increased muscle carnosine by 46±17% in 60 minutes (Dieter et al., 2021), suggesting that topical carnosine may be a more convenient alternative to beta-alanine supplementation. A topical carnosine gel named LactiGo™ is commercially available and claims to improve high-intensity exercise performance by up to 15% (LactiGo, n.d.-b). However, evidence of the efficacy of topical carnosine is conflicting. Sharpe and Macias (2016) found topical carnosine to improve the distance covered in a Yo-Yo intermittent test by 84 m (0.94%) and the time to complete a repeated 1,000 m test by 9 s (4%). In contrast, Harnish and Miller (2023) provide evidence that topical carnosine does not affect performance in a repeated 30-s Wingate test.

We chose to investigate the effect of topical carnosine on 1,500 m rowing ergometer TT performance. The rationale for this was three-fold; first, previous research shows that increasing muscle carnosine causes a small improvement in 2,000 m rowing ergometer performance (Baguet et al., 2010; Ducker et al., 2013; Hobson et al., 2013; Jaques et al., 2019). Secondly, topical carnosine has yet to be investigated using a single-bout high-intensity exercise test. Lastly, all rowing events at the 2028 Olympic Games will use a shortened 1,500 m course.

The results of this current study suggest that topical carnosine does not improve performance or influence pacing in a 1,500 m rowing ergometer TT within a group of experienced club-level rowers. There are many theoretical explanations for why no difference in performance was seen, including a lack of muscle carnosine increase, a type I muscle fibre dominant group or participants, or the potential that increasing muscle carnosine for long periods (such as during beta-alanine supplementation) is more effective at improving exercise performance than

a singular acute dose. Overall, this current research adds to the body of evidence showing the effect topical carnosine has on high-intensity exercise performance. However, more research is needed to understand how topical carnosine affects muscle carnosine and exercise performance.

Limitations

The primary limitation of this research is that we did not measure the carnosine concentration in the participants' muscles at any point. As a result, we are uncertain about how the topical carnosine gel affected intramuscular carnosine, making it impossible to compare with the intramuscular carnosine changes seen in previous research using beta-alanine supplementation. Thus, all discussions around the performance results were based on assumptions of an increase in intramuscular carnosine, which is not ideal.

Time trial performance was the primary focus of this research to provide a simple, meaningful indication of the efficacy of topical carnosine. Physiological measures of work output, such as heart rate, lactate, and oxygen consumption, were not recorded. In hindsight, at least one of these measures should have been recorded to verify maximal performance from a physiological standpoint, as we relied solely on the participant's RPE combined with the researcher's presence and encouragement to facilitate a maximal effort.

Other than following the requirements of the study to avoid high-intensity exercise (>80% max heart rate) within 24 hours of each time trial, we did not control the participants' diet or training programs between trials. However, we are confident this would have had little effect because

previous research suggests that completing a strength training session 24 and 48 hours before a 2,000 m rowing TT does not affect performance (Gee et al., 2011). Limitations caused by a lack of dietary control mainly arise from caffeine and carbohydrate intake. Caffeine can improve rowing performance, particularly at a 3 to 6 mg/kg dosage (Turnes et al., 2019). Carbohydrate consumption can affect performance based on muscle glycogen availability (Vigh-Larsen et al., 2021).

In this research, we had recruitment limitations within our population that resulted in a low number of female participants. As a result it was not possible to analyse any sex-effect of topical carnosine in female populations. Lastly, topical carnosine may affect trained rowers differently than the general population.

Strengths

Our randomised, double-blind, crossover study design was strong (Spieth et al., 2016). Randomisation meant that there was no bias when allocating groups. Being double-blind minimised the placebo effect and bias from researchers or participants. A cross-over design meant that any learning effect from the first to the second trial was cancelled out through group average calculations.

Every participant used the same Concept 2 rowing ergometer for both trials. The Concept 2 rowing ergometer is a reliable machine for exercise testing, SEM = 0.5% (Smith & Hopkins, 2012) and CV = 0.6% (95% CI = 0.4-1.0 %) for 2,000 m TT (Schabort et al., 2010). Every participant was familiar with the machine.

The rationale behind the 1,500 m TT used in this research was to measure performance changes in the shortened distance for the 2028 Olympic Games. Although we did not have access to a group of elite rowers for this research, our population were still highly trained, experienced rowers who had been rowing for at least three years. Thus, the population tested in this current study may still provide applicable insights into the efficacy of topical carnosine in elite-level rowing.

Future Directions

This study aligns with Harnish and Miller (2023), where topical carnosine did not affect performance in repeated 30-s Wingate tests on a cycle ergometer. In contrast, Sharpe and Macias (2016) found topical carnosine improved performance in an intermittent Yo-Yo test and a repeated 1,000 m run. These conflicting results indicate that more research is needed to clarify the ergogenic effect of topical carnosine in intermittent and single-bout exercise tests requiring a high proportion of anaerobic glycolytic energy production.

This current study and the two studies mentioned above did not measure the participants' muscle carnosine at any point. Dieter et al. (2021) found topical carnosine to increase the amount of carnosine in the middle gluteal muscle of thoroughbred horses by 46±17% in 60 minutes, with a large degree of individual variation between horses. If the relative muscle carnosine increases seen in thoroughbred horses were to occur in human muscle, we would expect a small effect on performance (Saunders et al., 2017). However, topical carnosine did not affect performance in this current study or that of Harnish and Miller (2023), so future research needs to investigate how topical carnosine affects muscle carnosine in humans, the

rate of carnosine increase, and how much variation there is amongst individuals. Future research should assess whether single versus multiple applications of topical carnosine make a difference in performance and whether using topical carnosine regularly during a training phase affects work capacity and adaptation.

Research investigating the ergogenic effect of topical carnosine concerning physiological factors such as sex and muscle fibre typology is currently lacking. With respect to muscle typology, type I muscle fibres are less sensitive to increases in P_i and H^+ than type II muscle fibres (Lievens et al., 2020; Metzger & Moss, 1990), suggesting a person's muscle typology may influence the ergogenic potential of carnosine, seeing as the most-known benefit of increased muscle carnosine is heightened H^+ buffer capacity. Furthermore, it is unclear whether increased muscle carnosine (via beta-alanine supplementation) affects female exercise performance (Murphy et al., 2022). The effect of topical carnosine on female athletic performance goes hand-in-hand with muscle fibre typology because females tend to have a greater distribution and area percentage of Type I fibres than males (Nuzzo, 2023), which makes it plausible to suggest that females may respond differently to increased carnosine than males. Thus, future research should investigate the effect of increased muscle carnosine on exercise performance in type I vs type II muscle fibre dominant participants, with an additional focus on female populations. This research may explain the variability in the ergogenic effects between individuals when muscle carnosine increases. Additionally, this research would provide valuable insights to assist with athletes' and coaches' supplementation choices.

References

Allen, D. G., Lamb, G. D., & Westerblad, H. (2008). Skeletal muscle fatigue: cellular mechanisms. *Physiological Reviews*, *88*(1), 287-332. https://doi.org/10.1152/physrev.00015.2007

Baguet, A., Bourgois, J., Vanhee, L., Achten, E., & Derave, W. (2010). Important role of muscle carnosine in rowing performance. *Journal of Applied Physiology*, *109*(4), 1096-1101. https://doi.org/10.1152/japplphysiol.00141.2010

Baguet, A., Everaert, I., Hespel, P., Petrovic, M., Achten, E., & Derave, W. (2011). A new method for non-invasive estimation of human muscle fiber type composition. *PLoS One*, *6*(7), e21956. https://doi.org/10.1371/journal.pone.0021956

Baker, J. S., McCormick, M. C., & Robergs, R. A. (2010). Interaction among skeletal muscle metabolic energy systems during intense exercise. *Journal of Nutrition and Metabolism*, *2010*, 905612. https://doi.org/10.1155/2010/905612

Beasley, L., Smith, L., Antonio, J., Gordon, D., Johnstone, J., & Roberts, J. (2018). The effect of two β-alanine dosing strategies on 30-minute rowing performance: a randomized, controlled trial. *Journal of the International Society of Sports Nutrition*, *15*(1), 59. https://doi.org/10.1186/s12970-018-0266-3

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine & Science in Sports & Exercise*, *14*(5), 377-381.

Bourdeau Julien, I., Sephton, C. F., & Dutchak, P. A. (2018). Metabolic networks influencing skeletal muscle fiber composition. *Frontiers in Cell and Developmental Biology*, *6*, 125. https://doi.org/10.3389/fcell.2018.00125

Brooks, G. A. (2001). Lactate doesn't necessarily cause fatigue: why are we surprised? *The Journal of Physiology*, *536*(1), 1-1. https://doi.org/https://doi.org/10.1111/j.1469-7793.2001.t01-1-00001.x

Cady, E. B., Jones, D. A., Lynn, J., & Newham, D. J. (1989). Changes in force and intracellular metabolites during fatigue of human skeletal muscle. *The Journal of Physiology*, *418*(1), 311-325. https://doi.org/https://doi.org/10.1113/jphysiol.1989.sp017842

Cairns, S. P. (2006). Lactic acid and exercise performance. *Sports Medicine*, *36*(4), 279-291. https://doi.org/10.2165/00007256-200636040-00001

Calderón, J. C., Bolaños, P., & Caputo, C. (2014). The excitation–contraction coupling mechanism in skeletal muscle. *Biophysical Reviews*, *6*(1), 133-160. https://doi.org/10.1007/s12551-013-0135-x

Campos, E. Z., Kalva-Filho, C. A., Gobbi, R. B., Barbieri, R. A., Almeida, N. P., & Papoti, M. (2017). Anaerobic contribution determined in swimming distances: relation with performance. *Frontiers in Physiology*, *8*, 755. https://doi.org/10.3389/fphys.2017.00755

Chung, W., Shaw, G., Anderson, M. E., Pyne, D. B., Saunders, P. U., Bishop, D. J., & Burke, L. M. (2012). Effect of 10 week beta-alanine supplementation on competition and training performance in elite swimmers. *Nutrients*, *4*(10), 1441-1453. https://doi.org/https://doi.org/10.3390/nu4101441

Dahlstedt, A. J., Katz, A., & Westerblad, H. (2001). Role of myoplasmic phosphate in contractile function of skeletal muscle: studies on creatine kinase-deficient mice. *The Journal of Physiology*, *533*(2), 379-388. https://doi.org/https://doi.org/10.1111/j.1469-7793.2001.0379a.x

Dahlstedt, A. J., Katz, A., Wieringa, B., & Westerblad, H. (2000). Is creatine kinase responsible for fatigue? Studies of isolated skeletal muscle deficient in creatine kinase. *The FASEB Journal*, *14*(7), 982-990. https://doi.org/https://doi.org/10.1096/fasebj.14.7.982

de Andrade Kratz, C., de Salles Painelli, V., de Andrade Nemezio, K. M., da Silva, R. P., Franchini, E., Zagatto, A. M., Gualano, B., & Artioli, G. G. (2017). Beta-alanine supplementation enhances judo-related performance in highly-trained athletes. *Journal of Science and Medicine in Sport*, *20*(4), 403-408. https://doi.org/https://doi.org/10.1016/j.jsams.2016.08.014

de Salles Painelli, V., Nemezio, K. M., Pinto, A. N. A. J., Franchi, M., Andrade, I., Riani, L. A., Saunders, B., Sale, C., Harris, R. C., Gualano, B., & Artioli, G. G. (2018). High-intensity interval training augments muscle carnosine in the absence of dietary beta-alanine intake. *Medicine & Science in Sports & Exercise*, *50*(11), 2242-2252. https://doi.org/10.1249/MSS.0000000000001697

de Salles Painelli, V., Roschel, H., de Jesus, F., Sale, C., Harris, R. C., Solis, M. Y., Benatti, F. B., Gualano, B., Lancha, A. H., & Artioli, G. G. (2013). The ergogenic effect of beta-alanine combined with sodium bicarbonate on high-intensity swimming performance. *Applied Physiology, Nutrition, and Metabolism*, *38*(5), 525-532. https://doi.org/10.1139/apnm-2012-0286 %M 23668760

de Salles Painelli, V., Saunders, B., Sale, C., Harris, R. C., Solis, M. Y., Roschel, H., Gualano, B., Artioli, G. G., & Lancha Jr, A. H. (2014). Influence of training status on high-intensity intermittent performance in response to β-alanine supplementation. *Amino Acids*, *46*(5), 1207-1215. https://doi.org/10.1007/s00726-014-1678-2

Dieter, B. P., Macias, C. J., Sharpe, T. J., Roberts, B., Wille, M., Young, A., Reisenauer, C., Cantrell, B., & Bayly, W. M. (2021). Transdermal delivery of carnosine into equine skeletal muscle. *Comparative Exercise Physiology*, *17*(5), 429-434. https://doi.org/https://doi.org/10.3920/CEP200077

Ducker, K. J., Dawson, B., & Wallman, K. E. (2013). Effect of beta-alanine supplementation on 2,000-m rowing-ergometer performance. *International Journal of Sport Nutrition and Exercise Metabolism*, *23*(4), 336-343. https://doi.org/10.1123/ijsnem.23.4.336

Duffield, R., Dawson, B., & Goodman, C. (2005). Energy system contribution to 400-metre and 800-metre track running. *Journal of Sports Sciences*, *23*(3), 299-307. https://doi.org/10.1080/02640410410001730043

Dunnett, M., & Harris, R. C. (1999). Influence of oral ß-alanine and L-histidine supplementation on the carnosine content of the gluteus medius. *Equine Veterinary Journal*, *31*(S30), 499-504. https://doi.org/https://doi.org/10.1111/j.2042-3306.1999.tb05273.x

Duthie, G., Pyne, D., & Hooper, S. (2003). Applied physiology and game analysis of rugby union. *Sports Medicine*, *33*, 973-991. https://doi.org/https://doi.org/10.2165/00007256-200333130-00003

Dutka, T. L., & Lamb, G. D. (2004). Effect of carnosine on excitation–contraction coupling in mechanically-skinned rat skeletal muscle. *Journal of Muscle Research & Cell Motility*, *25*(3), 203-213. https://doi.org/10.1023/B:JURE.0000038265.37022.c5

Dutka, T. L., Lamboley, C. R., McKenna, M. J., Murphy, R. M., & Lamb, G. D. (2011). Effects of carnosine on contractile apparatus Ca^{2+} sensitivity and sarcoplasmic reticulum Ca^{2+} release in human skeletal muscle fibers. *Journal of Applied Physiology*, *112*(5), 728-736. https://doi.org/10.1152/japplphysiol.01331.2011

Gee, T. I., French, D. N., Howatson, G., Payton, S. J., Berger, N. J., & Thompson, K. G. (2011). Does a bout of strength training affect 2,000 m rowing ergometer performance and rowing-specific maximal power 24 h later? *European Journal of Applied Physiology*, *111*(11), 2653-2662. https://doi.org/10.1007/s00421-011-1878-3

Glenn, J. M., Gray, M., Stewart, R., Moyen, N. E., Kavouras, S. A., DiBrezzo, R., Turner, R., & Baum, J. (2015). Incremental effects of 28 days of beta-alanine supplementation on high-intensity cycling performance and blood lactate in masters female cyclists. *Amino Acids*, *47*(12), 2593-2600. https://doi.org/10.1007/s00726-015-2050-x

Grgic, J., Pedisic, Z., Saunders, B., Artioli, G. G., Schoenfeld, B. J., McKenna, M. J., Bishop, D. J., Kreider, R. B., Stout, J. R., Kalman, D. S., Arent, S. M., VanDusseldorp, T. A., Lopez, H. L., Ziegenfuss, T. N., Burke, L. M., Antonio, J., & Campbell, B. I. (2021). International Society of Sports Nutrition position stand: sodium bicarbonate and exercise performance. *Journal of the International Society of Sports Nutrition*, *18*(1), Article 61. https://doi.org/10.1186/s12970-021-00458-w

Grgic, J., Rodriguez, R. F., Garofolini, A., Saunders, B., Bishop, D. J., Schoenfeld, B. J., & Pedisic, Z. (2020). Effects of sodium bicarbonate supplementation on muscular strength and endurance: a systematic review and meta-analysis. *Sports Medicine*, *50*(7), 1361-1375. https://doi.org/10.1007/s40279-020-01275-y

Gupta, S., Stanula, A., & Goswami, A. (2021). Peak blood lactate concentration and its arrival time following different track running events in under-20 male track athletes. *International Journal of Sports Physiology and Performance*, *16*(11), 1625-1633. https://doi.org/10.1123/ijspp.2020-0685

Hall, M. M., Rajasekaran, S., Thomsen, T. W., & Peterson, A. R. (2016). Lactate: friend or foe. *PM&R*, *8*(3), 8-15. https://doi.org/https://doi.org/10.1016/j.pmrj.2015.10.018

Hannah, R., Stannard, R. L., Minshull, C., Artioli, G. G., Harris, R. C., & Sale, C. (2015). β-Alanine supplementation enhances human skeletal muscle relaxation speed but not force production capacity. *Journal of Applied Physiology*, *118*(5), 604-612. https://doi.org/10.1152/japplphysiol.00991.2014

Harnish, C., & Miller, B. (2023). Effect of transdermal carnosine on repeated sprint performance in trained cyclists: A randomized controlled cross-over trial. *Asian Exercise and Sport Science Journal*, *7*(2), 15-22. https://doi.org/10.33545/27077012.2023.v4.i2b.193

Haugen, T., Sandbakk, Ø., Enoksen, E., Seiler, S., & Tønnessen, E. (2021). Crossing the golden training divide: the science and practice of training world-class 800- and 1500-m runners. *Sports Medicine*, *51*(9), 1835-1854. https://doi.org/10.1007/s40279-021-01481-2

Hill, C. A., Harris, R. C., Kim, H. J., Harris, B. D., Sale, C., Boobis, L. H., Kim, C. K., & Wise, J. A. (2007). Influence of β-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. *Amino Acids*, *32*(2), 225-233. https://doi.org/10.1007/s00726-006-0364-4

Hipkiss, A. R., Michaelis, J., & Syrris, P. (1995). Non-enzymatic glycosylation of the dipeptide l-carnosine, a potential anti-protein-cross-linking agent. *FEBS Letters*, *371*(1), 81-85. https://doi.org/https://doi.org/10.1016/0014-5793(95)00849-5

Hobson, R. M., Harris, R. C., Martin, D., Smith, P., Macklin, B., Gualano, B., & Sale, C. (2013). Effect of beta-alanine with and without sodium bicarbonate on 2,000-m rowing performance. *International Journal of Sport Nutrition and Exercise Metabolism*, *23*(5), 480-487. https://doi.org/10.1123/ijsnem.23.5.480

Hobson, R. M., Saunders, B., Ball, G., Harris, R. C., & Sale, C. (2012). Effects of β-alanine supplementation on exercise performance: a meta-analysis. *Amino Acids*, *43*(1), 25-37. https://doi.org/10.1007/s00726-011-1200-z

Hopkins, W. G., Marshall, S. W., Batterham, A. M., & Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. *Medicine & Science in Sports & Exercise*, *41*(1), 3-13. https://doi.org/10.1249/MSS.0b013e31818cb278

Inzlicht, M., & Marcora, S. M. (2016). The central governor model of exercise regulation teaches us precious little about the nature of mental fatigue and self-control failure. *Frontiers in Psychology*, *7*, 656. https://doi.org/10.3389/fpsyg.2016.00656

Jagim, A. R., Wright, G. A., Brice, A. G., & Doberstein, S. T. (2013). Effects of beta-alanine supplementation on sprint endurance. *The Journal of Strength & Conditioning Research*, *27*(2), 526-532. https://doi.org/10.1519/JSC.0b013e318256bedc

Jaques, M., Glick, D., Greco-Henderson, D., & Ives, S. J. (2019). The effects of short term beta-alanine supplementation on performance in division III male and female rowers. *Journal of Exercise and Nutrition*, *2*(3), Article 15. Retrieved from https://www.journalofexerciseandnutrition.com/index.php/JEN/article/view/49

Jones, R. L., Barnett, C. T., Davidson, J., Maritza, B., Fraser, W. D., Harris, R., & Sale, C. (2017). β-alanine supplementation improves in-vivo fresh and fatigued skeletal muscle relaxation speed. *European Journal of Applied Physiology*, *117*(5), 867-879. https://doi.org/10.1007/s00421-017-3569-1

Jukić, I., Kolobarić, N., Stupin, A., Matić, A., Kozina, N., Mihaljević, Z., Mihalj, M., Šušnjara, P., Stupin, M., Ćurić Ž, B., Selthofer-Relatić, K., Kibel, A., Lukinac, A., Kolar, L., Kralik, G., Kralik, Z., Széchenyi, A., Jozanović, M., Galović, O.,…Drenjančević, I. (2021). Carnosine, small but mighty—prospect of use as functional ingredient for functional food formulation. *Antioxidants*, *10*(7), 1037. https://doi.org/10.3390/antiox10071037

Keenan, K. G., Senefeld, J. W., & Hunter, S. K. (2018). Girls in the boat: Sex differences in rowing performance and participation. *PLoS One*, *13*(1), e0191504. https://doi.org/10.1371/journal.pone.0191504

Kendrick, I. P., Kim, H. J., Harris, R. C., Kim, C. K., Dang, V. H., Lam, T. Q., Bui, T. T., & Wise, J. A. (2009). The effect of 4 weeks β-alanine supplementation and isokinetic training on carnosine concentrations in type I and II human skeletal muscle fibres. *European Journal of Applied Physiology*, *106*(1), 131-138. https://doi.org/10.1007/s00421-009-0998-5

Keyser, R. E. (2010). Peripheral fatigue: high-energy phosphates and hydrogen ions. *PM&R*, *2*(5), 347-358. https://doi.org/https://doi.org/10.1016/j.pmrj.2010.04.009

LactiGo. (n.d.-a). *FAQ: How To Use*. https://www.lactigo.com/pages/faqs

LactiGo. (n.d.-b). *Go further. Go faster*. https://www.lactigo.com/

Lamont, C., & Miller, D. J. (1992). Calcium sensitizing action of carnosine and other endogenous imidazoles in chemically skinned striated muscle. *Journal of Physiology*, *454*, 421-434. https://doi.org/10.1113/jphysiol.1992.sp019271

Lievens, E., Klass, M., Bex, T., & Derave, W. (2020). Muscle fiber typology substantially influences time to recover from high-intensity exercise. *Journal of Applied Physiology*, *128*(3), 648-659. https://doi.org/10.1152/japplphysiol.00636.2019

Macias, C. (2016). *Home* [LinkedIn Profile]. LinkedIn. https://www.linkedin.com/in/chad-macias-099218113/

Maté-Muñoz, J. L., Lougedo, J. H., Garnacho-Castaño, M. V., Veiga-Herreros, P., Lozano-Estevan, M. d. C., García-Fernández, P., de Jesús, F., Guodemar-Pérez, J., San Juan, A. F., & Domínguez, R. (2018). Effects of β-alanine supplementation during a 5-week strength training program: a randomized, controlled study. *Journal of the International Society of Sports Nutrition*, *15*(1), Article 19. https://doi.org/10.1186/s12970-018-0224-0

Matthews, J. J., Artioli, G. G., Turner, M. D., & Sale, C. (2019). The physiological roles of carnosine and β-alanine in exercising human skeletal muscle. *Medicine & Science in Sports & Exercise*, *51*(10), 2098–2108. https://doi.org/10.1249/MSS.0000000000002033

Maughan, R. J., Burke, L. M., Dvorak, J., Larson-Meyer, D. E., Peeling, P., Phillips, S. M., Rawson, E. S., Walsh, N. P., Garthe, I., Geyer, H., Meeusen, R., van Loon, L., Shirreffs, S. M., Spriet, L. L., Stuart, M., Vernec, A., Currell, K., Ali, V. M., Budgett, R. G. M.,…Engebretsen, L. (2018). IOC consensus statement: dietary supplements and the high-performance athlete. *International Journal of Sport Nutrition and Exercise Metabolism*, *28*(2), 104-125. https://doi.org/10.1123/ijsnem.2018-0020

Mense, S. (2008). Muscle pain: mechanisms and clinical significance. *Dtsch Arztebl International*, *105*(12), 214-219. https://doi.org/10.3238/artzebl.2008.0214

Metzger, J. M., & Moss, R. L. (1987). Greater hydrogen ion-induced depression of tension and velocity in skinned single fibres of rat fast than slow muscles. *The Journal of Physiology*, *393*(1), 727-742. https://doi.org/https://doi.org/10.1113/jphysiol.1987.sp016850

Metzger, J. M., & Moss, R. L. (1990). Effects of tension and stiffness due to reduced pH in mammalian fast- and slow-twitch skinned skeletal muscle fibres. *The Journal of Physiology*, *428*(1), 737-750. https://doi.org/https://doi.org/10.1113/jphysiol.1990.sp018238

Murphy, M. J., Rushing, B. R., Sumner, S. J., & Hackney, A. C. (2022). Dietary supplements for athletic performance in women: beta-alanine, caffeine, and nitrate. *International Journal of Sport Nutrition and Exercise Metabolism*, *32*(4), 311-323. https://doi.org/10.1123/ijsnem.2021-0176

Nakagawa, Y., & Hattori, M. (2002). Relationship between muscle buffering capacity and fiber type during anaerobic exercise in human. *Journal of Physiological Anthropology and Applied Human Science*, *21*, 129-131. https://doi.org/10.2114/jpa.21.129

Nielson, B. H. (1999). pH after competitive rowing: the lower physiological range? *Acta Physiologica Scandinavica*, *165*(1), 113-114. https://doi.org/https://doi.org/10.1046/j.1365-201x.1999.00485.x

Noakes, T. (2011). The central governor model and fatigue during exercise. *Regulation of Fatigue in Exercise*, 1-26. Retrieved from https://www.novapublishers.com/wp-content/uploads/2019/06/978-1-61209-334-5_ch1.pdf

Nuzzo, J. (2023). Sex differences in skeletal muscle fiber types: A meta-analysis. *Clinical Anatomy, 37*(1), 81–91. https://doi.org/10.1002/ca.24091

Oronova-Hristova, D. (2024). Development of the women's participation in world rowing championships and Olympic Games for 10-year period. T. *Research in Physical Education, Sport & Health*, *13*(2), 45. https://doi.org/10.46733/pesh2413245oh

Pardi, D. (2020). Carnosine and LactiGo - Dr Brad Dieter. [Audio Podcast] In *HumanOS Radio*. https://soundcloud.com/humanosradio/087-carnosine-and-lactigo-dr-brad-dieter?si=71facb6670fc40028f16fc76b93ae4de&utm_source=clipboard&utm_medium=text&utm_campaign=social_sharing

Parkhouse, W. S., McKenzie, D. C., Hochachka, P. W., & Ovalle, W. K. (1985). Buffering capacity of deproteinized human vastus lateralis muscle. *Journal of Applied Physiology*, *58*(1), 14-17. https://doi.org/10.1152/jappl.1985.58.1.14

Pergolizzi Jr, J. V., Taylor Jr, R., LeQuang, J.-A., Raffa, R. B., & Group, t. N. R. (2018). The role and mechanism of action of menthol in topical analgesic products. *Journal of Clinical Pharmacy and Therapeutics*, *43*(3), 313-319. https://doi.org/https://doi.org/10.1111/jcpt.12679

Price, M. J., & Singh, M. (2008). Time course of blood bicarbonate and pH three hours after sodium bicarbonate ingestion. *International Journal of Sports Physiology and Performance*, *3*(2), 240-242. https://doi.org/10.1123/ijspp.3.2.240

Rezende, N. S., Swinton, P., de Oliveira, L. F., da Silva, R. P., da Eira Silva, V., Nemezio, K., Yamaguchi, G., Artioli, G. G., Gualano, B., Saunders, B., & Dolan, E. (2020). The muscle carnosine response to beta-alanine supplementation: a systematic review with Bayesian individual and aggregate data e-max model and meta-analysis. *Frontiers in Physiology*, *11*, 913. https://doi.org/10.3389/fphys.2020.00913

Rodríguez, F. A., & Mader, A. (2011). Energy systems in swimming. In L. Seifert, D. Chollet, & I. Mujika (Eds.), *World Book of Swimming: From Science to Performance* (Vol. 11, pp. 225-240). Nova Science Publishers, Inc. https://doi.org/10.13140/2.1.3260.5128.

Sahlin, K. (2014). Muscle energetics during explosive activities and potential effects of nutrition and training. *Sports Medicine*, *44*(2), 167-173. https://doi.org/10.1007/s40279-014-0256-9

Sahlin, K., & Ren, J. M. (1989). Relationship of contraction capacity to metabolic changes during recovery from a fatiguing contraction. *Journal of Applied Physiology*, *67*(2), 648-654. https://doi.org/10.1152/jappl.1989.67.2.648

Sahlin, K., Tonkonogi, M., & Söderlund, K. (1998). Energy supply and muscle fatigue in humans. *Acta Physiologica Scandinavica*, *162*(3), 261-266. https://doi.org/https://doi.org/10.1046/j.1365-201X.1998.0298f.x

Sale, C., Saunders, B., & Harris, R. C. (2010). Effect of beta-alanine supplementation on muscle carnosine concentrations and exercise performance. *Amino Acids*, *39*(2), 321-333. https://doi.org/10.1007/s00726-009-0443-4

Santana, J. O., de Freitas, M. C., dos Santos, D. M., Rossi, F. E., Lira, F. S., Rosa-Neto, J. C., & Caperuto, E. C. (2018). Beta-Alanine supplementation improved 10-km running time trial in physically active adults. *Frontiers in Physiology*, *9*. https://doi.org/10.3389/fphys.2018.01105

Saunders, B., de Salles Painelli, V., de Oliveira, L. F., Da Eira Silva, V., Da Silva, R. P., Riani, L., Franchi, M., Gonçalves, L. D. S., Harris, R. C., Roschel, H., Artioli, G. G., Sale, C., & Gualano, B. (2017). Twenty-four weeks of β-alanine supplementation on carnosine content, related genes, and exercise. *Medicine & Science in Sports & Exercise*, *49*(5), 896-906. https://doi.org/10.1249/MSS.0000000000001173

Saunders, B., Elliott-Sale, K., Artioli, G. G., Swinton, P. A., Dolan, E., Roschel, H., Sale, C., & Gualano, B. (2017). β-alanine supplementation to improve exercise capacity and performance: a systematic review and meta-analysis. *British Journal of Sports Medicine*, *51*(8), 658-669. https://doi.org/https://doi.org/10.1136/bjsports-2016-096396

Saunders, B., Sunderland, C., Harris, R. C., & Sale, C. (2012). β-alanine supplementation improves YoYo intermittent recovery test performance. *Journal of the International Society of Sports Nutrition*, *9*(1), 39. https://doi.org/10.1186/1550-2783-9-39

Schabort, E. J., Hawley, J. A., Hopkins, W. G., & Blum, H. (2010). High reliability of performance of well-trained rowers on a rowing ergometer. *Journal of Sport Sciences*, *17*(8), 627-632. https://doi.org/10.1080/026404199365650

Sharpe, T., & Macias, C. (2016). Evaluation of the efficacy of Lactigo™ topical gel as an ergogenic aid. *Journal of Exercise Physiology Online*, *19*, 15-23. https://www.researchgate.net/publication/303708657_Evaluation_of_the_Efficacy_of_Lactigo_Topical_Gel_as_an_Ergogenic_Aid

Smith, T. B., & Hopkins, W. G. (2012). Measures of rowing performance. *Sports Medicine*, *42*(4), 343-358. https://doi.org/10.2165/11597230-000000000-00000

Smith-Ryan, A. E., Fukuda, D. H., Stout, J. R., & Kendall, K. L. (2012). High-velocity intermittent running: effects of beta-alanine supplementation. *The Journal of Strength*

& Conditioning Research, *26*(10), 2798-2805. https://doi.org/10.1519/JSC.0b013e318267922b

Soper, C., & Hume, P. A. (2004). Reliability of power output during rowing changes with ergometer type and race distance. *Sports Biomechanics*, *3*(2), 237-248. https://doi.org/10.1080/14763140408522843

Spieth, P. M., Sophie, K. A., Isabel, P. A., Min-Woo, I. B., Kristian, B., & and Siepmann, T. (2016). Randomized controlled trials – a matter of design. *Neuropsychiatric Disease and Treatment*, *12*, 1341-1349. https://doi.org/10.2147/NDT.S101938

Spriet, L. L., Söderlund, K., Bergström, M., & Hultman, E. (1987). Skeletal muscle glycogenolysis, glycolysis, and pH during electrical stimulation in men. *Journal of Applied Physiology*, *62*(2), 616-621. https://doi.org/10.1152/jappl.1987.62.2.616

Stegen, S., Blancquaert, L., Everaert, I., Bex, T., Taes, Y., Calders, P., Achten, E., & Derave, W. I. M. (2013). Meal and beta-alanine coingestion enhances muscle carnosine loading. *Medicine & Science in Sports & Exercise*, *45*(8). https://doi.org/https://doi.org/10.1249/MSS.0b013e31828ab073

Steinacker, J. M. (1993). Physiological aspects of training in rowing. *International Journal of Sports Medicine*, *14*, 3-10. Retrieved from https://pubmed.ncbi.nlm.nih.gov/8262704/

Street, D., Bangsbo, J., & Juel, C. (2001). Interstitial pH in human skeletal muscle during and after dynamic graded exercise. *The Journal of Physiology*, *537*(Pt 3), 993-998. https://doi.org/10.1111/j.1469-7793.2001.00993.x

Suszter, L., Ihász, F., Szakály, Z., Nagy, D., Alföldi, Z., Bálint, M. V., & Mák, E. (2020). Effect of five-week beta-alanine supplementation on the performance, cardio-respiratory system, and blood lactate level in well-trainied rowing athletes: A double-blind randomized pre-post pilor study. *Journal of Physical Education and Sport*, *20*(5), 2501-2507. https://doi.org/10.7752/jpes.2020.05341

Suzuki, Y., Ito, O., Takahashi, H., & Takamatsu, K. (2004). The effect of sprint training on skeletal muscle carnosine in humans. *International Journal of Sport and Health Science*, *2*, 105-110. https://doi.org/10.5432/ijshs.2.105

Tobias, G., Benatti, F. B., de Salles Painelli, V., Roschel, H., Gualano, B., Sale, C., Harris, R. C., Lancha, A. H., & Artioli, G. G. (2013). Additive effects of beta-alanine and sodium bicarbonate on upper-body intermittent performance. *Amino Acids*, *45*(2), 309-317. https://doi.org/10.1007/s00726-013-1495-z

Turnes, T., Cruz, R. S. O., Caputo, F., & De Aguiar, R. A. (2019). The impact of preconditioning strategies designed to improve 2000-m rowing ergometer performance in trained rowers: A systematic review and meta-analysis. *International Journal of Sports Physiology and Performance*, *14*(7), 871-879. https://doi.org/10.1123/ijspp.2019-0247

Vigh-Larsen, J. F., Ørtenblad, N., Spriet, L. L., Overgaard, K., & Mohr, M. (2021). Muscle glycogen metabolism and high-intensity exercise performance: a narrative review. *Sports Medicine*, *51*(9), 1855-1874. https://doi.org/10.1007/s40279-021-01475-0

Volianitis, S., & Secher, N. H. (2009). Rowing, the ultimate challenge to the human body – implications for physiological variables. *Clinical Physiology and Functional Imaging*, *29*(4), 241-244. https://doi.org/https://doi.org/10.1111/j.1475-097X.2009.00867.x

Volianitis, S., Yoshiga, C. C., & Secher, N. H. (2020). The physiology of rowing with perspective on training and health. *European Journal of Applied Physiology*, *120*(9), 1943-1963. https://doi.org/10.1007/s00421-020-04429-y

Walsh, B., Tiivel, T., Tonkonogi, M., & Sahlin, K. (2002). Increased concentrations of P_i and lactic acid reduce creatine-stimulated respiration in muscle fibers. *Journal of Applied Physiology*, *92*(6), 2273-2276. https://doi.org/10.1152/japplphysiol.01132.2001

Westerblad, H., Allen, D. G., & Lännergren, J. (2002). Muscle fatigue: lactic acid or inorganic phosphate the major cause? *Physiology*, *17*(1), 17-21. https://doi.org/10.1152/physiologyonline.2002.17.1.17

Westerblad, H., Bruton, J. D., & Katz, A. (2010). Skeletal muscle: Energy metabolism, fiber types, fatigue and adaptability. *Experimental Cell Research*, *316*(18), 3093-3099. https://doi.org/https://doi.org/10.1016/j.yexcr.2010.05.019

Westerblad, H., Lännergren, J., & Allen, D. G. (1997). Slowed relaxation in fatigued skeletal muscle fibers of xenopus and mouse: Contribution of Ca^{2+} and cross-bridges. *Journal of General Physiology*, *109*(3), 385-399. https://doi.org/10.1085/jgp.109.3.385

Weston, A. R., Myburgh, K. H., Lindsay, F. H., Dennis, S. C., Noakes, T. D., & Hawley, J. A. (1996). Skeletal muscle buffering capacity and endurance performance after high-intensity interval training by well-trained cyclists. *European Journal of Applied Physiology and Occupational Physiology*, *75*(1), 7-13. https://doi.org/10.1007/s004210050119

World Rowing. (2018). *2018 FISA NF Survey Olympic Solidarity*. https://worldrowing.com/wp-content/uploads/2020/12/2018-FISANFSURVEY-FACTSHEET_Neutral.pdf

Appendix A - Ethics Application Approval

The University of Waikato
Private Bag 3105
Gate 1, Knighton Road
Hamilton, New Zealand

Human Research Ethics Committee
Roger Moltzen
Telephone: +64021658119
Email:humanethics@waikato.ac.nz

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

5 August 2024

James Rountree
Division of Health
By email: jr207@students.waikato.ac.nz

Dear James

HREC(Health)2024#29 : Investigating the effect of topical carnosine on rowing performance

Thank you for your responses to the Committee feedback.

We are now pleased to provide formal approval for your project.

Please contact the Committee by email (humanethics@waikato.ac.nz) if you wish to make changes to your project as it unfolds, quoting your application number with your future correspondence. Any minor changes or additions to the approved research activities can be handled outside the monthly application cycle.

We wish you all the best with your research.

Regards,

Emeritus Professor Roger Moltzen MNZM
Chairperson
University of Waikato Human Research Ethics Committee

Appendix B - Participant Information Sheet

Participant Information Sheet

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

Title: Investigating the effect of topical carnosine on rowing performance

Research Team: James Rountree (Principal Investigator), Dr Martyn Beaven (Chief Supervisor), Steven Finlayson (co-researcher).

Research Type: Masters research, led by James Rountree (Principal Investigator).

Invitation: You are invited to participate in a study investigating the effect of topical carnosine gel on rowing performance. Please take time to read this participant information sheet. This sheet contains why the research is being done and what will be involved. Please ask questions if you are unsure of any aspect of the study.

Background: Rowing at maximal effort causes a build-up of acid in the working muscles, affecting performance and causing pain. The most common strategies used to slow the build-up of acid are oral bicarbonate and/or beta-alanine supplementation. Prior research shows that using one or both of these strategies can improve rowing performance. Recently, commercially available products have been developed that claim to deliver the same benefits as bicarbonate and beta-alanine supplementation, simply by applying a gel to the skin.

Purpose: This study aims to assess the effectiveness of topical carnosine application on rowing performance.

Gel Information: The gel that will be tested in this study is a product named Lactigo™.

Lactigo Ingredients: Menthol 1.25%, Ethoxdiglycol, Glycerine, L-Carnosine, Magnesium Sulphate, Phenoxyethanol, Water, Xanthan Gum
Banned Substance Tested: To ensure Lactigo contains no banned substances that would result in a doping violation, Lactigo is tested by Informed Sport. Informed Sport is a third-party testing organisation that tests every batch of Lactigo using ISO 17025 accredited methods.
Storage: The risk of inadvertent contamination of the gel is low. When not used for this study, the gel will be safely stored in a clean, dry, secure location. There will be no unsupervised access to the gel between tests.

More information about the product can be found here:
https://www.lactigo.com/

What will we ask you to do?: If you decide to participate, you will be asked to sign an informed consent form, complete a baseline questionnaire, and participate in two testing sessions, separated by one week. You will be asked to refrain from high-intensity exercise (heavy breathing, > 80% max heart rate) for 24 hours before each time trial. The test is a 1,500 m rowing ergometer time trial (maximal effort). You will be asked to complete one time trial per session (two in total). Each testing session will start with the application of the topical gel. The researcher will give you instructions on how to apply the gel to your own leg and arm muscles. The researcher (male) will apply the gel to your back. For more information on gel application, see the *Topical Gel Application Information* sheet. Gel application will take place 45 minutes before the time trial begins. During this time, you can adjust the rowing ergometer to suit your comfort and be given a 25-minute warm-up opportunity. Each session will take approximately 60 minutes, but you can stay for longer if you would like an extended cool-down.

What are the possible risks?:
Exercise risks: All possible precautions have been taken to minimise risk, however, as with all forms of exercise there is an element of risk involved. The risks involved with participating in this study are no greater than those involved in a standard, high-intensity rowing training or testing session.
Allergic reaction risk: When using a topical product, there is a risk of an allergic or skin reaction. All ingredients in the gel are low-risk, but please check the ingredient list above (gel information) to assess whether you have reacted to one of these ingredients in the past. If so, you will be unable to participate in this study. To check for immediate reaction, the gel will be applied to a small area of skin at least five minutes before the gel is applied to back and limb muscles.
If you are harmed: If harm occurs at any point during the session, the research team will offer immediate first aid and seek professional assistance if necessary.

What are the possible benefits of participating? You will be sent a report that summarises the findings of this study. You will also receive an individualised report showing the effect of the gel on your rowing performance. This information will give you insight into whether the gel improves rowing performance, from which you can either rule out or consider it as a strategy to improve performance.

Do I have to take part? Taking part in this study is 100% **voluntary**, you are not required to take part in this research. It is your choice whether you want to participate or not. If you wish to participate, you can ask the researchers any questions regarding the study before you give official consent. You have the right to bring a support person to both testing sessions. You have the right to ask questions, refuse to answer questions, and withdraw at any point during the study. No explanation of withdrawal is needed and there will be no consequences for doing so. The withdrawal period lasts for two weeks after data collection has taken place, by contacting the principle investigator (James Rountree). After this two-week period, you are unable to withdraw your data.

Exclusion Criteria: You will be excluded from this study if you have used beta-alanine supplementation within 30-days of your first time trial. You will be excluded if you have previously reacted (skin or allergic reaction) to any of the ingredients contained in Lactigo.

What will happen to the information collected? The anticipated research outputs from the data collected by the research team are; Master's thesis, conference papers, journal articles, book chapters, media releases, training and learning materials. Only the research team will have access to the questionnaire and time trial results. At the end of the project, any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five years (on OneDrive on supervisors computer), after which they will be destroyed.

Confidentiality
The research team will take every possible step to ensure the published data is unidentifiable to protect your identity and confidentiality. All data handling will take place using unidentifiable code letters that have no relation to your name. Published data will not contain any of your personal information. No participant's name will be published.

Contact details: If you have any questions or concerns about this project, please feel free to contact:

James Rountree
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The University of Waikato Adams Centre for High Performance
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Dr Martyn Beaven

Senior Research Fellow – Exercise Physiology and Endocrinology
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martyn.beaven@waikato.ac.nz

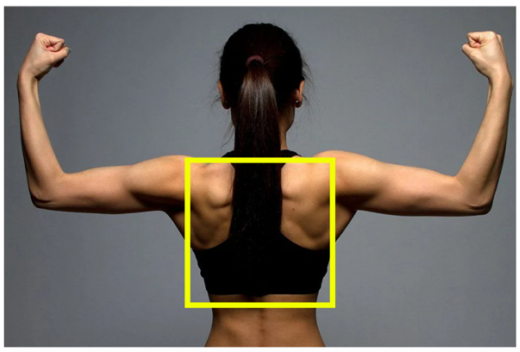
Human Research Ethics Committee:
This research project has been approved by the Human Research Ethics Committee (Health) at the University of Waikato as HREC(Health)2024#29. Any questions or concerns about the ethical conduct of this research may be sent to the Secretary of the Committee, email humanethics@waikato.ac.nz

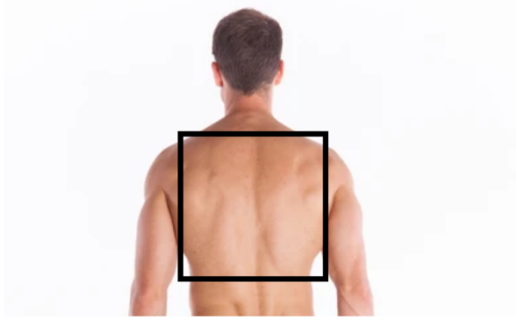
Topical Gel Application Information

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

Topical Gel Application Process

The topical gel is intended to cover as many working muscles (whilst rowing) as reasonably practical. With your consent, a male researcher will apply the topical gel to your back muscles (shown inside of the squares), similar to putting sunscreen on some elses back. The researcher will be wearing gloves. You will given instructions of how to apply the topical gel to your own arms and legs.





Target muscles:
Latisimuss Dorsi (Lats), Rhomboids, Quadriceps, Hamstrings, Calf Muscles, Biceps, Deltoids.

Appendix C – Informed Consent Form

Consent Form for Participants

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

UoW HREC(Health)2024#29

Title: Investigating the effect of topical carnosine on rowing performance

*I have read the **Participant Information Sheet** and **Topical Gel Application Information** sheet for this study and have had the details of the study explained to me. My questions about the study have been answered to my satisfaction, and I understand that I may ask further questions at any time.*

I also understand that:

- *I am participating of my own free will and have not been coerced in any way*
- *I understand I have the right to not answer any question or perform any activity*
- *I understand I am free to withdraw from the study at any time up to 2 weeks without reason and all data connected to me will also be withdrawn*
- *I understand that all personal data will be destroyed at the conclusion of the study, but de-identified study data will be retained for 5 years in accordance with the University of Waikato research policy. The study data will be used for a master's thesis and scientific articles, scientific presentations, and help educate students and sports practitioners at The University of Waikato and the wider community. All personal data will remain confidential and I will not be identifiable in any of the study outputs*
- *I know who to contact if I have any questions or concerns about the study*
- *I consent to participate in this study*

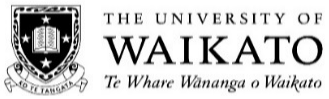
I agree to provide information to the researchers under the conditions of confidentiality set out on the** Participant Information Sheet**.

Consent to Participate

I agree to participate in this study under the conditions set out in the** Participant Information Sheet**.

	Participant:	**Researcher:**
Signature:	_____	_____
Name:	_____	_____
Date:	_____	_____

Appendix D – Baseline Questionnaire



Investigating the effect of topical carnosine on rowing performance
Baseline Questionnaire

UoW HREC(Health)2024#29.

Name: ……

Date of Birth: ……

Height: …… Weight: ……

Sex: ……