# The Application of a Topical Carnosine Gel and its Effects on Intermittent High-Intensity Exercise Performance in Olympic-Level Rugby Sevens Players

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# Abstract

Recently, the topical application of a carnosine gel has been investigated as a possible ergogenic aid and may be beneficial for improving high-intensity exercise performance in team sport athletes. However, there is currently no research on the effects of a topical carnosine gel on performance in rugby sevens players. This thesis aims to: (1) review the current literature regarding carnosine, its importance for high-intensity exercise, the supplements used to increase carnosine concentrations, and the topical application of a carnosine gel (Chapter One); (2) investigate the application of a topical carnosine gel and its effects on intermittent high-intensity exercise performance in Olympic-level rugby sevens players (Chapter Two); and (3) summarise the finding of the thesis, discuss the strength and weaknesses and recommend future research directions (Chapter Three).

In Chapter One, the literature on carnosine, its importance for high-intensity exercise, the supplements used to increase carnosine concentrations and the novel topical application of carnosine was reviewed. Carnosine has been reported to increase high-intensity exercise performance by enhancing intramuscular buffering capacity, regulating myosin ATPase, and increasing calcium sensitivity within the muscle. Nutritional supplements containing carnosine or β-alanine have been shown to increase intramuscular carnosine concentrations. However, these supplements often require a loading period of at least two weeks and often fail to substantially elevate plasma carnosine levels. Recently the topical application of a carnosine gel has been investigated as a possible ergogenic aid and may provide a more efficient method for increasing carnosine concentrations and improving high-intensity exercise performance in team sports, such as rugby sevens.

In Chapter Two, eight Olympic-level rugby sevens players completed two performance tests in which they received both treatments, 10 mL of a topical carnosine gel (CAR) or an ultrasound placebo gel (PLA). The performance test was completed on a cycle ergometer and consisted of a warm-up, 12 intermittent sprints with a 2-minute half-time break. Each sprint effort consisted of 24 seconds cycling at 3 W/kg, 6 s at maximal intensity, and a 30 s rest. For peak power output the two-way ANOVA revealed a statistically significant time effect ($p = 2.16 \times 10^{-9}$) and condition effect ($p = 1.44 \times 10^{-7}$). The carnosine condition experienced an increase in peak power output for sprint 2 (+101.3 ± 81.0 Watts (W); $p = 0.048$; Cohen's $d = 0.99$; *large* effect size), sprint 4 (+102.6 ± 82.1 W; $p = 0.043$; Cohen's $d = 0.74$; *moderate* effect size) and sprint 7 (+ 156.0 ± 106.2 W; $p = 0.025$; Cohen's $d = 0.98$; *large* effect size) compared to the placebo.

Of note, all other sprints except for Sprint 12 were substantially greater in the CAR condition ($d$ = 0.49 to 0.91). For mean power output, the two-way ANOVA revealed a statistically significant time effect ($p$ = 5.98 x $10^{-19}$), but no condition effect ($p$ = 0.211). Although non-significant, there was still a substantial increase for mean power in the CAR condition in Sprints 10 (51.3 ± 48.2 W; $p$= 0.079; Cohen's $d$= 0.57; *moderate* effect size) and 11 (44.7 ± 37.0 W; $p$= 0.069; Cohen's $d$= 0.39; *small* effect size) compared to the placebo. These results suggest that the positive effects observed in this study are not limited to the typical intracellular buffering mechanism proposed for carnosine.

In Chapter Three, the findings from Chapter Two were summarised and the conclusions of the thesis were presented. Overall, this thesis provides evidence for the efficacy of a topical carnosine gel for improving performance during repeated bouts of intermittent high-intensity exercise, especially in highly trained Olympic-level rugby sevens players. Further research should be conducted to investigate the possible mechanisms of action associated with the topical application of a carnosine gel in human athletes.

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# List of Abbreviations

ANOVA – Analysis of variance

ANS – Anserine

ATP – Adenosine triphosphate

b·min – Beats per minute

CAR – Carnosine

$Ca^{2+}$ – Calcium ion

CB – Chicken broth

CBEX – Chicken breast extract

CCT – Cycle capacity test

cm – Centimetres

CN1 – Carnosinase-1

fg – Femtogram

g – Grams

$H^{+}$ - Hydrogen ion

HR – Heart rate

ISSN – International Society of Sports Nutrition

J – Joule

kg – Kilogram

kJ – Kilojoule

L – Litre

m – Metre

µg – Microgram

mg – milligram

min – Minute

mL – Millilitre

mmol – Millimole

$Na^+$ – Sodium

ng – Nanogram

MVC – Maximal voluntary isometric contraction

pg – Picogram

PLA – Placebo

$PWC_{FT}$ – Working capacity at fatigue level

RPE – Rate of perceived exertion

RSA – Repeated cycling sprint ability test

s – Seconds

TT – Time trial

TTE – Time to exhaustion

TWD – Total work done

VT – Ventilatory threshold

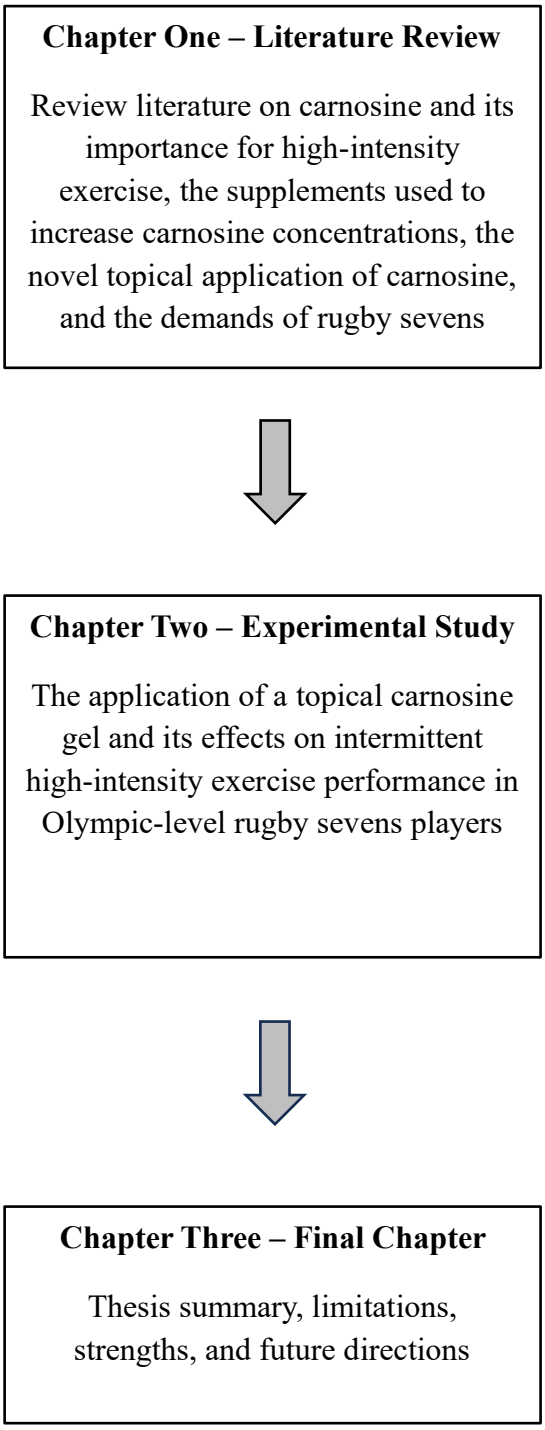
W – Watts

$W_{max}$ – Maximum power output

yd – Yards

y – Years of age

## Thesis Outline



# Background

During high-intensity exercise, there is an accumulation of $H^+$ which decreases intracellular pH and has been reported to impair performance by limiting the resynthesis of high-energy phosphates (Fitts, 1994). To mitigate these effects, the human body contains intracellular and extracellular buffer systems that help with the regulation of $H^+$ and pH levels during high-intensity exercise (Lancha Junior et al., 2015). Carnosine is a strong intracellular buffer found at high concentrations in the cytosol of the muscle and can be used to enhance intramuscular buffering capacity and improve high-intensity exercise performance (Jones, 2014). Carnosine can also be used to improve anaerobic exercise by augmenting the enzyme regulation of myosin ATPase (Parker Jr & Ring, 1970). Carnosine may also increase calcium sensitivity within the human muscle, which should reduce or slow the decline in performance during fatiguing stimulation (Dutka et al., 2012). Thus, ergogenic aids containing carnosine are commonly used during training and competition.

Previously nutritional supplements containing high levels of carnosine or β-alanine, a naturally occurring amino acid that is involved in the formation of carnosine (Culbertson et al., 2010), have been used to increase intramuscular carnosine concentrations and improve high-intensity exercise performance (Lancha Junior et al., 2015). However, these supplements often fail to substantially elevate plasma carnosine levels as the ingestion of carnosine is susceptible to hydrolysis (Yeum et al., 2010). More recently the novel topical application of a carnosine gel has been investigated as a possible ergogenic aid. The topical application of carnosine directly to the muscle may allow carnosine to be absorbed into the bloodstream via a transdermal drug delivery system (Zaid Alkilani et al., 2015), increasing muscle carnosine concentrations, and improving high-intensity exercise performance. Sharpe and Macias (2016) provided evidence for the efficiency of a topical carnosine gel in improving maximal aerobic and anaerobic capacity. However, this is the only study that shows a positive ergogenic benefit following the topical application of a carnosine gel. Due to gaps in the current literature, there is limited evidence to substantiate these claims. Therefore, more research should be conducted to investigate the topical application of a carnosine gel and its effectiveness as an ergogenic aid.

Strategies to increase carnosine concentrations and enhance muscle buffering capacity are likely to be relevant for improved performance in rugby sevens. Due to the short duration of the games and more space on the pitch per player, high locomotor activity and repeated high-

intensity performances are required during a rugby sevens match (Hogarth et al., 2016). Thus, it seems feasible that rugby sevens players will benefit from the possible increase in carnosine concentrations following the topical application of a carnosine gel. However, there is currently no evidence on the topical application of a carnosine gel and its efficiency as an ergogenic aid in rugby sevens.

Therefore, the aim of this thesis was to investigate the application of a topical carnosine gel and its effects on intermittent high-intensity exercise performance in a test designed to mimic the performance demands of rugby sevens in elite rugby sevens players.

# Chapter One – Literature Review

## Review Aim

This literature review aims to introduce carnosine, as an intracellular buffer, and its effects on high-intensity exercise performance. This review will then analyse and critique the literature on the use of existing nutritional supplements and their efficiency in increasing carnosine concentrations and improving high-intensity exercise performance. This will then be compared to the existing literature on the novel topical application of a carnosine gel. Lastly, the combination of all this literature will evaluate the possible benefits of a topical carnosine gel for improving intermittent high-intensity exercise performance in rugby sevens players.

## 1. Buffer Systems in the Human Body

During high-intensity exercise, the buffer systems of the body act to reduce the accumulation of $H^+$ and delay the onset of fatigue (Douroudos et al., 2006). For high-intensity exercise, there is a much greater turnover of nonmitochondrial ATP (McGinley & Bishop, 2016). In the quest for sufficient ATP, there is a rapid accrual of intracellular lactate and $H^+$ that typifies metabolic acidosis (Caruso et al., 2012). The accumulation of $H^+$ decreases intracellular pH, which in turn, has been reported to impair performance by limiting the resynthesis of high-energy phosphates and inhibiting the rate of anaerobic glycolysis (Fitts, 1994). During high-intensity exercise, intramuscular pH may decline from a resting value of about 7.1 to less than 6.5 (Cairns, 2006). This reduction of intracellular pH also hinders the binding of calcium to troponin, disrupting the process of muscle excitation-contraction coupling (Calderón et al., 2014). When contractions proceed at high force outputs during supramaximal exercise, the high $H^+$ levels also impair intramuscular $Ca^{2+}$ release and compromise $Ca^{2+}$ -ATPase activity (Gladden, 2004). These changes impair exercise and recovery rates, which is a concern when repeated bouts of supramaximal activity may occur (Stallknecht et al., 1998). To mitigate these negative effects, the human body contains intracellular and extracellular buffering systems, which help regulate $H^+$ and pH levels during high-intensity exercise (Lancha Junior et al., 2015).

The intracellular buffer system is located within the cell and is mediated primarily by phosphates, proteins, and dipeptides, which exert their buffering action within the cytosol (Lancha Junior et al., 2015). Carnosine is a strong intracellular buffer found at high

concentrations in the cytosol of the muscle (Jones, 2014). Improving intracellular buffering capacity can aid in the regulation of $H^+$ ions within the cell and slow the decline in pH (Stellingwerff, 2020). Extracellular buffers are located outside the cell and include sodium bicarbonate, sodium citrate, and calcium lactate (de Oliveira et al., 2022). Improving extracellular buffering capacity can increase blood bicarbonate concentrations and increase the efflux of $H^+$ from the muscle (Jones, 2014).

## 2. Ergogenic Aids to Enhance Intramuscular Buffering Capacity

To improve intramuscular buffering capacity, and therefore, the regulation of $H^+$ and pH levels, ergogenic aids, such as carnosine, are commonly used during training and competition (Lancha Junior et al., 2015). Improving intramuscular muscle-buffering capacity can help athletes maintain intense muscle contractions for longer periods of time. Greater muscle buffering capacity may improve performance by allowing anaerobic glycolysis to continue during maximal exercise, resulting in higher lactate production without an increase in $H^+$ accumulation (Edge et al., 2006).

### 2.1. Carnosine Supplementation

Carnosine (beta-alanyl-L-histidine), a strong intramuscular buffer, is a naturally occurring histidine-containing compound found in animal muscle tissues (Culbertson et al., 2010). Carnosine is formed by bonding histidine and β-alanine in a reaction catalysed by carnosine synthase (Sale et al., 2010). In humans, the mean concentration of carnosine is $17.5 \pm 4.8$ mmol $kg^{-1}$ dm in females and $21.3 \pm 4.2$ mmol $kg^{-1}$ dm in males, (Mannion et al., 1995). Carnosine is a multifunctional dipeptide with many roles including intramuscular pH-buffering, metal-ion chelation, antioxidant capacity, and the ability to protect against the formation of advanced glycation and lipoxidation end-products. For these reasons, the therapeutic potential of carnosine supplementation has been clinically tested in numerous diseases where ischemic or oxidative stress is involved (Boldyrev et al., 2013). Compared to other intracellular buffers the pKa of carnosine is closer to the physiological pH range and therefore, is likely utilised sooner during high-intensity exercise (Sahlin, 1980). There is also a link between carnosine and

enzyme regulation concerning the activation of myosin-ATPase, which is used to help maintain ATP stores (Parker Jr & Ring, 1970). In addition, carnosine has been reported to improve calcium sensitivity within the human muscle as it potentiates force responses by 'sensitising' the contractile apparatus of $Ca^{2+}$, which is expected to reduce or slow the decline in performance during fatiguing stimulation (Dutka et al., 2012).

Previous research has suggested that intramuscular carnosine may act as a diffusible $Ca^{2+}/H^{+}$ exchanger at the sarcomere and thereby can be used to enhance skeletal muscle force production (Swietach et al., 2013). During high-intensity exercise, there is an increase in intracellular acidity which has a direct inhibitory effect on the contractile apparatus, but an up-regulation in $Ca^{2+}$ signalling can correct for this and even produce positive inotropy (Choi et al., 2000). Carnosine may prompt a buffer-mediated rise of resting of $Ca^{2+}$ during acidosis which should provide a physiological compensation for $H^{+}$ interference with $Ca^{2+}$-activated processes that share common $Ca^{2+}/H^{+}$ binding sites. The uphill $Ca^{2+}$ flux evoked by an $H^{+}$ gradient could facilitate $Ca^{2+}$ handling by improving diffusive coupling (Swietach et al., 2014). As a result, these changes may enhance skeletal muscle force production and improve high-intensity muscular performance.

Carnosine is found at high concentrations in the cytosol of the skeletal muscle (Derave et al., 2010) and is dependent on several individual factors including age, gender, diet, muscle fibre type distribution, and training status (Derave et al., 2010). In terms of gender, research has shown that men have approximately 20-25% higher intramuscular carnosine concentrations compared to women (Simoneau & Bouchard, 1989). This difference may be a result of the greater percentage of Type II muscle fibres in men compared to women, with carnosine content in Type II muscle fibres being approximately 30-100% greater than carnosine content in Type I fibres (Kendrick et al., 2009). With carnosine typically being greater in fast-twitch muscle fibres, human athletes involved in anaerobic sports tend to have higher intramuscular carnosine concentrations (Culbertson et al., 2010). Previous research has also suggested that carnosine loading may be more pronounced in trained vs untrained muscles, with training status being a possible determinant of carnosine loading. Specifically, trained individuals experienced a 77.95% increase in carnosine concentrations compared to untrained individuals who experienced only a 42.88% increase in carnosine concentrations (Bex et al., 2014). Thus, the physiological carnosine loading mechanism is possibly more effective in trained individuals. Research has also indicated an inverse curvilinear correlation between muscle carnosine content and the curvature constant of the power-duration relationship (Skiba et al., 2015).

However, Skiba et al. (2015) found that there was no correlation between carnosine and the minimum pH, pH at exhaustion, the recovery of pH between two bouts of exercise, or changes in pH at exhaustion in recreationally trained athletes. Thus, it seems possible that the mechanisms of action concerning fatigue may be through some process unrelated to pH. For example, as previously mentioned, carnosine can improve calcium sensitivity within the human muscle and potentiate force responses, which is expected to slow the decline in performance during fatiguing stimulation (Dutka et al., 2012). In conclusion, Skiba et al. (2015) stated that a more complete investigation involving the role of muscle carnosine in shaping the power-duration relationship should be conducted.

Thus, elevating levels of circulating carnosine could be beneficial for improving high-intensity exercise performance. The issue is that the carnosinase-1 (CN1) enzyme, which is responsible for hydrolysing the carnosine dipeptide, is highly active (Lenney et al., 1985) and even relatively high doses of carnosine are rapidly degraded (Sauerhöfer et al., 2012). Therefore, Baguet et al. (2014) hypothesised that athletes with low CN1 activity may be more responsive to the potential ergogenic effects of acute carnosine supplementation before high-intensity exercise. The results of their study demonstrated that elite athletes who excel in short, high-intensity exercise had lower CN1 activity and protein content compared to untrained athletes. With the genetic basis of serum CN1 content and activity, this finding may have been a result of performance-related genetic selection. They also found that the acute supplementation of 20 mg/kg body weight of pure carnosine did not improve acid-base balance or performance during high-intensity exercise, indicating that carnosine supplementation is ineffective, even in subjects with low CN1 activity. This result may be related to the fact that carnosine is susceptible to hydrolysis due to the action of the CN1 enzyme. However, it seems that anserine is much less prone to hydrolysis, and therefore, a combination of carnosine and anserine may induce a more beneficial ergogenic effect (Yeum et al., 2010). For example, Barbaresi et al. (2021) found that post-exercise plasma carnosine and anserine levels were significantly increased following the supplementation of a chicken meat extract that contained 46.4 mg/kg body weight of both carnosine and anserine.

Anserine is a histidine-containing dipeptide and is one of the methylated analogues of carnosine. Anserine synthesis is catalysed by the carnosine-N-methyltransferase enzyme and therefore depends on the prior synthesis of carnosine (de Souza Gonçalves et al., 2023). Both carnosine and anserine are found in many of the foods we consume, such as chicken meat. Carnosine and anserine are also both effective antioxidants that can influence the generation of

reactive oxygen and nitrogen species and aid in the injury-repair-regeneration cascade in skeletal muscles (Rezzani et al., 2019). In a recent study, Zembron-Lacny et al. (2022) found that high pre-exercise dipeptide intake can increase antioxidant status and regulate the oxi-inflammatory response to intense exercise. Thus, dietary supplements containing carnosine and anserine should have favourable therapeutic potential in modulating oxidative stress and inflammation in physically active individuals following strenuous exercise.

### 2.1.1. Carnosine Supplementation and High-Intensity Exercise Performance

An increase in carnosine concentrations has previously been correlated to improvements in overall exercise performance. For example, when 18 elite rowers were supplemented with 5 g/day of β-alanine for seven weeks muscle carnosine content increased by 45.3% in the soleus and 28.2% in the gastrocnemius (Baguet, Bourgois, et al., 2010). This increase in carnosine content was positively correlated to improvements in 2,000 m rowing performance. Specifically, the β-alanine group which experienced an increase in carnosine content were 4.3 s faster than the placebo group. Suzuki et al. (2002) also found a strong positive correlation between muscle carnosine concentrations and mean power relative to body mass during a 30 s maximal sprint on a cycle ergometer in 11 healthy males. These results further demonstrate the importance of carnosine during high-intensity exercise. Thus, an increase in carnosine concentrations should further enhance high-intensity exercise performance.

The dietary supplementation of carnosine and anserine has been proven to increase intramuscular carnosine concentrations and thereby improve high-intensity exercise performance. Specifically, Suzuki (2004) found that the use of combined carnosine and anserine improved intermittent high-intensity exercise performance. Eight male subjects performed two experimental trials in which they received a chicken breast extract (CBEX) or a placebo. Thirty minutes before each trial subjects received 190 g of an experimental soup that contained either 40 g of CBEX or no CBEX. For each trial, subjects performed 10 intermittent sprints on a cycle ergometer separated by a 20-minute half-time break. Each intermittent sprint consisted of a 5 s maximal intensity effort followed by a 25 s recovery. Anserine was detectable in plasma 30 minutes after CBEX supplementation, however, carnosine was not. Subjects in the CBEX group exhibited a higher power output during the latter half of the intermittent

sprints when compared to the placebo group. Based on these findings Suzuki (2004) stated that pre-exercise CBEX supplementation can improve intermittent exercise performance by possibly restraining the decrease of intracellular pH and thereby delaying the onset of fatigue. However, in a follow-up study, Suzuki et al. (2006) found that CBEX supplementation did not improve intermittent exercise performance. Therefore, even though later studies demonstrated an increase in high-intensity exercise performance following CBEX supplementation, there may be a more effective method for increasing intramuscular carnosine concentrations.

Maemura et al. (2006) subsequently used a CBEX product to investigate the effects of long-term carnosine and anserine supplementation on high-intensity exercise performance. Sixteen healthy male subjects were divided into either a CBEX or placebo group. The CBEX group consumed a 200 mL CBEX drink containing 4 g of carnosine and anserine per day for 30 days. The placebo group consumed a taste-matched CBEX drink that did not contain any carnosine or anserine. Before and after the ingestion period subjects performed three sessions of consecutive endurance exercises on a cycle ergometer. The first session was 30 minutes at 50% $VO_2$ max, the second was 15 minutes at 75% $VO_2$ max, and the third was until exhaustion at 100% $VO_2$ max. Post supplementation the CBEX group experienced a significant increase in exercise duration time at 100% $VO_2$ max and a significant decrease in blood lactate concentrations and ratings of perceived exertion (RPE) at 75% $VO_2$ max. These findings indicate that the chronic supplementation of a combined carnosine and anserine product can improve high-intensity exercise capacity while reducing blood lactate levels during sub-maximal exercise.

More recently Barbaresi et al. (2021) used chicken broth to investigate the effects of pre-exercise carnosine and anserine supplementation on an 8-minute high-intensity cycling time trial (TT) on a cycle ergometer. Fourteen healthy, recreationally trained males were divided into a chicken broth (CB) or placebo group. Forty minutes before performing the TT participants ingested either a CB soup that contained 46.4 $mg{\cdot}kg^{-1}$ body weight of combined carnosine and anserine, or a similar-tasting placebo soup. During the TT there was a significant difference between the CB and placebo conditions, with mean power output being 5.2% higher in participants supplemented with CB compared to placebo (5.0 W/kg vs 4.75 W/kg respectively), indicating that the acute supplementation of carnosine and anserine can improve high-intensity cycling performance. For this study, it was verified that post-exercise plasma anserine levels were significantly increased following CB supplementation. In contrast to the previously mentioned Suzuki (2004) paper, there was also an increase in plasma carnosine

levels following CB supplementation. This increase in plasma carnosine levels may be related to the timing of supplementation as participants in this study were supplemented for an extra 10 minutes before exercise compared to the Suzuki (2004) study where participants were supplemented 30 minutes before exercise.

Blancquaert et al. (2021) then went on to use capsules instead of chicken meat extract to test the effects of combined carnosine and anserine supplementation on maximal power output for two different performance tests. Thirty-five minutes before each performance test, participants were given either a capsule containing 20 $mg \cdot kg^{-1}$ body weight of both carnosine and anserine (CAR + ANS) or a placebo capsule. For the first performance test, 18 trained males performed a single maximal intensity Wingate test following a 6-minute high-intensity cycling effort. For this performance test, mean power output was significantly higher (+6%) in the first 5 seconds of the all-out Wingate test for participants supplemented with CAR + ANS (12.8 W/kg) compared to the placebo (12.1 W/kg). For the second performance test, 12 trained males performed three repeated Wingate tests in which peak power output was 3% higher throughout the three consecutive tests for participants supplemented with CAR + ANS (10.5 W/kg) compared to placebo (10.2 W/kg).

More recently, de Jager et al. (2022) conducted a dose-response study to investigate the effects of combined carnosine and anserine supplementation. Eleven healthy males participated in this study. Participants randomly received either 10, 20 or 30 $mg \cdot kg^{-1}$ of both carnosine and anserine or a placebo. Before supplementation, then 30 and 60 minutes after supplementation participants performed three maximal voluntary isometric contractions (MVC), followed by a 5 x 6 s repeated cycling test designed to assess repeated sprint ability (RSA). Serum samples were also collected to determine carnosinase enzyme activity. The dosage of 30 $mg \cdot kg^{-1}$ ingested 60 minutes before performance yielded the best result and showed an increase of 3% in peak power and 4.5% in peak torque on RSA and MVC. However, there was a negative correlation between carnosinase enzyme activity and improved performance. In a second study de Jager et al. (2022) supplemented 15 males with 30 $mg \cdot kg^{-1}$ of both carnosine and anserine to investigate the possible effects on neuromuscular function. Before supplementation, then 60 minutes after, participants performed three MVCs with femoral nerve electrical stimulation, followed by an RSA test. The results from this second study indicated that there was no effect of carnosine and anserine supplementation on neuromuscular function.

**Table 1.** Summary of Carnosine Supplementation and High-Intensity Exercise

| Authors | Participants | Supplementation Protocol | Exercise Protocol | Results |
|---|---|---|---|---|
| Suzuki (2004) | 8 males | 30 mins before each trial participants consumed 190 g of a soup that contained either 40 g of CBEX (CAR + ANS) or no CBEX (placebo) | • 10 sprints on a cycle ergometer separated by a 20 min half-time break<br>• Each sprint was 5 s on 25 s off | • ↑ in power output during latter half of the sprints<br>• 30 mins after supplementation ANS was detected in plasma, but CAR was not |
| Maemura et al. (2006) | 16 healthy males | Participants consumed either a 200 mL CBEX drink containing 4 g of CAR and ANS or a same taste placebo per day for 30 days | Pre- and post-supplementation:<br>• Session 1: 30 min at 50% $VO_2$ max<br>• Session 2: 15 min at 75% $VO_2$ max<br>• Session 3: until exhaustion at 100% $VO_2$ max | • ↑ in exercise duration time at 100% $VO_2$ max<br>• ↓ in blood lactate concentrations and RPE at 75% $VO_2$ max |
| Blancquaert et al. (2021) | 14 healthy, recreationally trained males | 40 mins before exercise participants consumed either a CB soup containing 46.4 mg/kg body weight of combined CAR and ANS or a taste matched placebo | High-intensity 8 min TT on a cycle ergometer | • During the TT there was a 5.2% ↑ in mean power output<br>• Post-exercise plasma CAR and ANS levels were significantly ↑ |
| Blancquaert et al. (2021) | • Test 1: 18 trained males<br>• Test 2: 12 trained males | 35 mins before the performance test participants were given either a capsule containing 20 mg/kg body weight of both CAR + ANS or a placebo capsule | • Test 1: single all-out Wingate test following a 6 min high-intensity cycling effort<br>• Test 2: 3 repeated Wingate tests | • Test 1: mean power output was 6.1% ↑ in the first 5 s of the Wingate test<br>• Test 2: peak power output was 2.5% ↑ in the 3 consecutive Wingate tests |
| de Jager et al. (2022) | • Study 1: 11 males<br>• Study 2: 15 males | • Study 1: participants randomly received 10, 20, or 30 $mg/kg^{-1}$ of CAR + ANS or a placebo<br>• Study 2: participants received 30 $mg/kg^{-1}$ of CAR + ANS | • Study 1: before, then 30 and 60 minutes after supplementation participants performed 3x MVC followed by a 5 x 6s RSA<br>• Study 2: 3x MVCs with femoral nerve electrical stimulation, followed by an RSA test. | • Study 1: 30 $mg.kg^{-1}$ led to a 3% ↑ in peak power and 4.5% ↑ in peak torque on RSA and MVC<br>• Study 2: There was no correlation between carnosinase enzyme activity and performance. |

PerformanceAbbreviations: Carnosine, (CAR), Anserine (ANS), Chicken breast extract (CBEX), Chicken broth (CB), Rate of perceived exertion (RPE), Time trial (TT), Maximal voluntary isometric contractions (MVC), Repeated cycling sprint ability test (RSA).

The results from the studies of carnosine supplementation and high-intensity exercise performance are summarised in Table 1. Specifically, these studies indicate that the ingestion of combined carnosine and anserine as a pre-exercise supplement may increase carnosine concentrations and improve peak power by 3% during a repeated cycling sprint ability test (de Jager et al., 2022), mean power output by 6.1% in the first 5 seconds of a maximal intensity Wingate test (Blancquaert et al., 2021), and time to exhaustion at 100% $VO_2$ max (Maemura et al., 2006). These results may be more pronounced in elite-level athletes who excel in short, high-intensity exercise as they have a lower activity of the CN1 enzyme and protein content compared to untrained athletes (Baguet et al., 2014), meaning they may benefit more from the ergogenic effects associated with the ingestion of carnosine. The increase in performance associated with the supplementation of combined carnosine and anserine tends to be more pronounced in high-intensity exercise ranging between 5 s (Suzuki, 2004) to 15 minutes (Maemura et al., 2006). However, Suzuki et al. (2006) also found that the supplementation of combined carnosine and anserine does not improve intermittent high-intensity exercise performance. Thus, there may be a more effective method for increasing carnosine concentrations and improving high-intensity exercise performance, especially since carnosine is susceptible to hydrolysis due to the action of the CN1 enzyme (Yeum et al., 2010).

### 2.1.2. Precautions of Carnosine Supplementation

As noted, carnosine is susceptible to hydrolysis, due to the action of the CN1 enzyme, meaning it often fails to substantially elevate plasma carnosine levels (Yeum et al., 2010). It seems possible that carnosine is absorbed as an entire molecule and the subsequent intracellular hydrolysis is relatively slow, which is the rate-limiting step in the total absorption process (Asatoor et al., 1970). After the supplementation of 60 $mg \cdot kg^{-1}$ body weight of carnosine, Gardner et al. (1991) found that up to 14% of ingested carnosine was lost in urine. In a later study, Everaert et al. (2012) noted that only 8 out of 25 participants (responders) displayed a measurable increase in plasma carnosine levels following the supplementation of 60 $mg \cdot kg^{-1}$ body weight of pure carnosine. Anserine is much less prone to hydrolysis by carnosinase, and therefore, to avoid this issue, a combination of carnosine and anserine may induce a more beneficial ergogenic effect (Yeum et al., 2010). Carnosine supplementation may also lead to a

slight increase in ammonia, which could negatively impact the level of several amino acids, such as alanine, glutamine, and glutamate (Holeček, 2022).

## 2.2. Beta-Alanine Supplementation

β-alanine is a naturally occurring amino acid which is involved in the formation of carnosine and may provide a more effective method for increasing intramuscular carnosine concentrations as it may bypass the hydrolysis associated with the previously mentioned CN1 enzyme (Culbertson et al., 2010). The synthesis of β-alanine occurs via three main biosynthetic pathways: a product of L-aspartate decarboxylation produced by gut microbes; a by-product of the interchangeable reaction of pyruvate to L-alanine; and a product of deamination and carboxylation of the pyrimidine uracil (Tiedje et al., 2010). Once synthesised β-alanine is transported to muscle cells where it crosses the sarcolemma via a $Na^{+}$- and $C1^{-}$ -dependent process (Artioli et al., 2010). β-alanine has several functions within the nervous system, such as the ability to act as a neurotransmitter and having hippocampal binding sites and glycine receptors that can aid in the learning of new information (Tiedje et al., 2010). β-alanine supplementation also has the potential to directly benefit athletes engaged in intense training regimes and allows researchers to explore the role of muscle carnosine in detoxifying aldehydes in diseases characterised by abnormal oxidative stress (Carvalho et al., 2018). β-alanine can be found in many of the foods we consume, such as beef, chicken, and turkey. When ingested as part of a meal it is normally in the form of a histidine-containing dipeptide such as anserine and carnosine. After ingestion, the hydrolysis of these dipeptides yields β-alanine, which gets absorbed into the skeletal tissue for the resynthesis of carnosine (Hoffman et al., 2012). Thus, β-alanine supplementation can significantly increase intramuscular carnosine concentrations (Stout et al., 2007).

Specifically, four weeks of oral β-alanine supplementation at a rate of 4-6 $g{\cdot}day^{-1}$ has been proven to raise intramuscular carnosine levels by about 60% (Harris, 2005). β-alanine supplementation for 5-6 weeks (4.8 $g{\cdot}day^{-1}$) has also been shown to increase muscle carnosine content in the soleus by 39%, tibialis anterior by 27%, and the medial head of the gastrocnemius by 23% (Baguet et al., 2009). In a later study, β-alanine supplementation increased muscle carnosine concentrations by 143 ± 151% in the gastrocnemius and 161 ± 56% in the soleus, although, this did not affect 1-h time-trial performance (Chung et al., 2014). More recently

Perim et al. (2022) reported that 28 days of β-alanine supplementation (6 $g \cdot day^{-1}$) increased muscle carnosine content (+9.4 ± 4.0 $mmol \cdot kg^{-1}dm$) but did not improve performance during a simulated road cycling race. More specifically, the increase in carnosine content did not affect performance in a prolonged intermittent cycling protocol lasting 125 minutes, with a 10 s sprint every 20 minutes. However, the increase in muscle carnosine concentrations and the associated enhancement of intramuscular buffering capacity should improve high-intensity exercise performance in events lasting between 1-6 minutes (Jones, 2014).

To investigate different β-alanine dosing protocols and their effects on carnosine synthesis, Stellingwerff et al. (2012) conducted a dose-response study in which 31 young males were randomised into three different dosing groups. The first group (high-low), were supplemented with 3.2 g β-alanine/day for 4 weeks, followed by 1.6 g β-alanine/day for another four weeks. The second group (low-low) were supplemented with 1.6 g β-alanine/day for eight weeks. Then the third group were supplemented with a placebo. During the first four weeks, the increase in muscle carnosine for the high-low group was about twofold greater than the low-low group. This dose-response showed a carnosine increase of 2.01 mmol/kg per 100 g of consumed β-alanine. This increase depended upon the total amount of accumulated β-alanine consumed within a daily intake range of 1.6 – 2.3 g β-alanine/day. Thus, the authors concluded that the absolute increase in muscle carnosine content depends only on the total amount of β-alanine consumed and not on baseline muscle carnosine, the muscle type, or the daily amount of supplemented β-alanine.

Baguet et al. (2009) investigated the supplementation-induced amplitude of carnosine synthesis and its subsequent elimination on cessation of supplementation (washout) following the ingestion of β-alanine for 5-6 weeks and found that carnosine declines at a rate of 2-4% per week. High responders experienced a washout period of 15 weeks while low responders experienced a washout period of six weeks, indicating that the carnosine washout period may take months rather than weeks depending on the individual. In both slow-twitch and fast-twist muscle fibres, the increase in carnosine concentrations appears to be stable, which is supported by the slow and linear washout profile following cessation of beta-alanine supplementation.

With the ability to enhance intramuscular carnosine content, β-alanine supplementation has also been shown to increase high-intensity exercise performance. Since carnosine is a physiologically relevant pH buffer an increase in carnosine content is theorised to reduce the acidosis associated with high-intensity exercise. To investigate whether β-alanine

supplementation impacted acidosis during high-intensity cycling, 14 male physical education students were supplemented for four weeks with 4.8 g/day of β-alanine or a placebo (Baguet, Koppo, et al., 2010). Before and after supplementation, participants performed a 6-minute cycling exercise bout at an intensity that was 50% between the ventilatory threshold and $VO_2$ max. Following four weeks of β-alanine supplementation the pH levels from baseline to the end of high-intensity cycling decreased by 0.015 units in the β-alanine group and increased by 0.012 units in the placebo group. These results indicate that β-alanine supplementation can significantly reduce exercise-induced acidosis. The time delay for the fast component of the oxygen uptake kinetics was also significantly reduced following β-alanine supplementation compared to the placebo, however, this did not reduce the calculated oxygen deficit. As for the slow components, they did not differ between the supplementation groups. These results indicate that β-alanine supplementation can attenuate the fall in blood pH and reduce acidosis during high-intensity exercise.

### 2.2.1. Beta-Alanine Position Stand

The International Society of Sports Nutrition (ISSN) has provided an objective and critical review of the mechanisms and use of β-alanine supplementation (Trexler et al., 2015). Based on the available literature in 2015, the conclusions of the ISSN were: 1) Four weeks of β-alanine supplementation, at a dose of 4-6 g daily, significantly increases muscle carnosine concentrations, thereby acting as an intracellular pH buffer; 2) β-alanine supplementation appears to be safe in healthy populations when recommended doses are consumed; 3) The only reported side effect is paraesthesia, but this can be attenuated by using divided lower doses or a sustained-release formula; 4) Daily supplementation with 4-6 g of β-alanine for at least 2-4 weeks has been shown to improve exercise performance, with more substantial benefits in open end-point tasks/time trials lasting 1-4 minutes in duration; 5) β-alanine attenuates neuromuscular fatigue, especially in older subjects, with preliminary evidence indicating that β-alanine may improve tactical performance; 6) The combined use of β-alanine with other single or multi-ingredient supplements may be advantageous when supplementation of β-alanine is high enough (4-6 g daily) and long enough (minimum of four weeks); 7: More research is needed to determine the effects of β-alanine supplementation on strength, endurance

performance lasting longer than 25 minutes, and other health-related benefits associated with carnosine.

Saunders et al. (2016) later conducted a systematic review and meta-analysis on β-alanine supplementation, reporting on 40 papers and 65 different exercise protocols. In their review, they reported that exercise lasting longer than 4 minutes was improved with β-alanine supplementation and had a greater effect size than exercise 1-4 minutes in duration (0.233 vs 0.210, respectively). This is an interesting finding as the ISSN position stand previously stated that β-alanine supplementation led to more substantial benefits in exercise lasting 1-4 minutes in duration. However, it was speculated that the reported findings in the Saunders et al. (2016) review may be related to shorter duration exercise protocols (6-7 min). It was also reported that effect sizes for moderate-duration exercise (5-10 min) were significant ($d$ = 0.224) and that short-duration exercise (≤0.5 min) was not affected ($d$ = 0.040) following β-alanine supplementation. Together these reviews suggest that β-alanine supplementation is a safe way to increase carnosine concentrations and would likely improve high-intensity exercise performance in moderate duration exercise (0.5-10 min).

### 2.2.2. Beta-Alanine Supplementation and High-Intensity Exercise Performance

β-alanine supplementation has been associated with many desirable training adaptations. Following eight weeks of β-alanine supplementation at a dose of 4 g per day, previously trained collegiate athletes experienced desirable results in a timed 300 yd shuttle, 90° flexed arm hang, and body composition compared to those who took a placebo. Performance improvements were greatest in football players with a 1.1 s decrease in 300 yd shuttle time and a 3.0 s increase in the flexed arm hang, where wrestlers lost weight and increased lean body mass compared to the placebo group that lost lean body mass (Kern & Robinson, 2011). These results indicate that β-alanine supplementation and the probable increase in carnosine concentrations can improve both sprint and endurance performance and stimulate an increase in lean body mass in trained athletes. Thus, the use of β-alanine as a nutritional supplement should be an effective ergogenic aid for improving high-intensity exercise performance.

As previously mentioned, β-alanine supplementation has been proven to increase muscle carnosine concentrations and as a result, has the potential to improve high-intensity exercise

performance. Hill et al. (2007) studied the influence of beta-alanine supplementation on skeletal muscle carnosine concentrations and high-intensity cycling capacity. Twenty-five physically active males were supplemented with either β-alanine or a matching placebo. During the first week participants started by taking 4.0 g per day of β-alanine or a maltodextrin placebo, this was then increased by 0.8 g a week until the start of the fourth week when participants were taking 6.4 g per day. Participants either stopped supplementation after four weeks or continued for another six weeks. Pre-supplementation, each participant's maximum power output ($W_{max}$) was determined using a cycle ergometer ramp test until volitional exhaustion. To measure high-intensity cycling capacity, participants completed a cycle capacity test at 110% $W_{max}$ until volitional exhaustion. After four weeks of β-alanine supplementation participants experienced a 13.0% increase in total work done and a 10.2 $mmol \cdot kg^{-1}$ increase in muscle carnosine concentrations compared to baseline. After 10 weeks there was a further 3.2% increase in total work done and a 4.6% $mmol \cdot kg^{-1}$ increase in carnosine concentrations. These results indicated that β-alanine supplementation for at least four weeks can increase muscle carnosine concentrations and improve high-intensity cycling capacity.

Stout et al. (2007) examined the effects of β-alanine supplementation on neuromuscular fatigue and ventilatory threshold. Twenty-two females participated in this study and were supplemented with either β-alanine or a placebo for 28 days. For the first week participants started by taking 3.2 g per day of β-alanine or maltodextrin (placebo), this was then increased to 6.4 g per day for the remaining three weeks of the study. Pre- and post-supplementation participants performed a continuous, incremental cycle ergometer test to exhaustion. Participants started cycling at 40 Watts which was increased at a rate of 20 Watts every 3 minutes until the participant could no longer maintain the power output at a pedalling rate of 70 rpm or until volitional exhaustion due to fatigue. The performance test was used to determine the physical working capacity at fatigue threshold ($PWC_{FT}$), ventilatory threshold (VT), maximal oxygen consumption ($VO_2$ max) and time-to-exhaustion (TTE). After supplementation, the β-alanine group experienced a 13.9% increase in VT, a 12.6% increase in $PWC_{FT}$, and a 2.5% increase in TTE, with no changes in the placebo group. Neither group experienced any change in $VO_2$ max. These results indicate that β-alanine supplementation can be used to improve high-intensity cycling performance by delaying the onset of neuromuscular fatigue and improving ventilatory threshold and time-to-exhaustion.

Saunders et al. (2012) investigated the effects of β-alanine supplementation on intermittent exercise performance. For this study, 17 amateur football players were supplemented with either 3.2 g·day$^{-1}$ of β-alanine or a maltodextrin-based placebo. Players were supplemented for 12 weeks from early to mid-season or mid- to the end of the season. Pre and post-supplementation players performed a Level 2 YoYo Intermittent Recovery Test. Following supplementation, the β-alanine group experienced a significant improvement in YoYo performance (+34.3%) compared to the placebo group which showed no improvement (-7.3%). In conclusion, 12 weeks of β-alanine supplementation can improve intermittent exercise performance in amateur football players and not only halt the decline in fitness levels shown during a competitive season but improve them above typical levels.

de Salles Painelli et al. (2014) later went on to examine the effects of training status on β-alanine supplementation and high-intensity intermittent performance. Forty young males participated in this study and were divided into a trained or non-trained group before being randomly allocated with beta-alanine or a dextrose-based placebo. Athletes in the trained group were cyclists involved in structured training programmes that were competing in national and state-level competitions. The non-trained participants were recreationally active individuals and were involved in a variety of physical activities 1-3 times per week. Participants were supplemented with either 6.4 g·day$^{-1}$ of β-alanine or a dextrose-based placebo for four weeks. Pre- and post-supplementation participants completed a performance test which involved four repeated bouts of a 30-second all-out Wingate protocol. Following β-alanine supplementation there was a significant increase in total work done for both the trained (+ 1,978 J) and non-trained (+1,349 J) groups. Beta-alanine supplementation increased mean power output in Bout 4 for the non-trained group and in Bouts 1, 2 and 4 for the trained group. Both trained and non-trained individuals experienced a significant improvement in repeated high-intensity cycling performance, however, these results were more pronounced in the more trained individuals.

Bellinger and Minahan (2016) conducted a study to investigate the effects of β-alanine supplementation on the resultant blood acidosis, lactate accumulation and energy provision during high-intensity cycling, as well as the aerobic and anaerobic contribution to power during a 4000 m cycling TT. Seventeen trained cyclists were supplemented with 6.4 g/day of β-alanine or placebo for 28 days. Pre- and post-supplementation participants performed a supramaximal cycling test to exhaustion. Participants also performed a 4000 m TT on a cycle ergometer before and after supplementation while aerobic and anaerobic contributions to power output were

quantified. β-alanine supplementation increased time to exhaustion by 12.8 ± 8.2 s and anaerobic capacity by 1.1 ± 0.7 kJ in the post-test compared with the pre-test. For the 4000 m TT β-alanine supplementation reduced performance time by 6.3 ± 4.5 s and the mean anaerobic power output was increased by 6.2 ± 4.5 W. These results suggest that β-alanine supplementation can improve time to exhaustion by increasing anaerobic capacity during supramaximal cycling, which is mirrored by a rise in the anaerobic contribution to power output during a TT. In conclusion, 28 days of β-alanine supplementation can improve anaerobic work capacity and increase overall high-intensity exercise performance.

In a more recent study, Kim et al. (2018) studied the effects of 10 weeks of β-alanine supplementation on peak power output and lactate responses in 19 male Korean national team boxers. Participants were divided into two groups and were supplemented with either 4.9-5.4 g/day of β-alanine or a placebo during a 10-week training block. Pre- and post-supplementation participants completed a 30 s Wingate anaerobic test using both an arm ergometer and a cycle ergometer. Blood lactate responses were also investigated during 3-rounds of a 3 min sparring session interspersed with 1 min rest periods. Following supplementation participants in the β-alanine group experienced a significant improvement in lower body peak power (+6.06%) compared to the placebo group. There were no significant differences detected between the two groups for changes in blood lactate responses following supplementation. This study supports the effects of long-term β-alanine supplementation for increasing lower body peak power output in national level athletes.

**Table 2.** Summary of β-Alanine Supplementation and High-Intensity Exercise Performance.

| Authors | Participants | Supplementation Protocol | Exercise Protocol | Results |
|---|---|---|---|---|
| Hill et al. (2007) | 25 physically active males | β-alanine or a matching placebo for 4 or 10 weeks<br>• Week 1: 4 g per day<br>• Week 2-4: increase of 0.8 g a week until week 4<br>• Stopped after week 4 or continued until week 10 | • Participants completed a CCT at a 110% $W_{max}$ until volitional exhaustion<br>• Was completed pre-supplementation, then at week 4 and week 10 | • After 4 weeks participants experienced a 13% ↑ in TWD<br>• Further 3.2% ↑ in TWD after 10 weeks<br>• The ↑ in TWD was correlated to an ↑ in muscle carnosine concentrations |

| | | | | |
|---|---|---|---|---|
| Stout et al. (2007) | 22 females | β-alanine or a maltodextrin placebo for 28 days<br>• Week 1: 3.2 g per day<br>• Remaining 3 weeks: 6.4 g per day | Pre- and post-supplementation participants performed a continuous, incremental cycle test to exhaustion | • 13.9% ↑ in VT, a 12.6% ↑ in $PWC_{FT}$, and a 2.5% ↑ in TTE.<br>• Delayed the onset of neuromuscular fatigue and improved VT |
| Van Thienen et al. (2009) | 21 healthy young male cyclists | 2-4 g $day^{-1}$ of β-alanine or a matched placebo for 8 weeks | Pre- and post-supplementation participants completed a 110 min simulated cycling race before performing a 10 min TT and a 30 s isokinetic sprint | During the final sprint peak power ↑ by 11.4% and mean power output ↑ by 5.0% |
| de Salles Painelli et al. (2014) | 40 trained and non-trained young males | 6.4 g $day^{-1}$ of β-alanine or a dextrose-based placebo for 4 weeks | Pre- and post-supplementation participants completed a performance test which involved 4 repeated bouts of a 30s all-out Wingate protocol | • ↑ TWD for both trained and non-trained groups<br>• ↑ in mean power output in bout 4 for the non-trained group and in bouts 1, 2 and 4 for the trained group |
| Bellinger and Minahan (2016) | 17 trained cyclists | 6.4 g $day^{-1}$ of β-alanine or a placebo for 28 days | • Pre- and post supplementation participants performed a supramaximal cycle ergometer test<br>• Participants also performed a 4000 m TT | • ↑ in TTE by 12.8 s and anaerobic capacity by 1.1 kJ<br>• For 4000 m TT performance time was ↓ by 6.3 s and anaerobic power output ↑ by 6.2 W |
| Kim et al. (2018) | 19 male Korean national team boxers | 4.9-5.4 g/day of β-alanine or a placebo during a 10-week training block | • Pre- and post-supplementation participants completed a 30 s Wingate test using both an arm and cycle ergometer<br>• Blood lactate responses were investigated during a 3-round 3 min sparring session | • The β-alanine group experienced a significant 6.06% ↑ in lower body peak power output<br>• No significant difference in blood lactate responses |

Abbreviations: Time trial (TT), Cycle capacity test (CCT), Total work done (TWD), Working capacity at fatigue threshold ($PWC_{FT}$), Ventilatory threshold (VT), Maximal oxygen consumption ($VO_2$ max) and Time-to-exhaustion (TTE)

The results from the studies of β-alanine supplementation and high-intensity exercise performance are summarised in Table 2. These studies indicate that supplementing β-alanine for at least four weeks can increase muscle carnosine concentrations by 10.2 mmol·kg$^{-1}$ (Hill et al., 2007), thereby, improving high-intensity exercise performance by delaying the onset of neuromuscular fatigue. Specifically, β-alanine supplementation led to a 12.6% increase in physical working capacity at the fatigue threshold (Stout et al., 2007) and improved mean anaerobic power output by 6.2 W during supramaximal cycling (Bellinger & Minahan, 2016), YoYo Intermittent Recovery Test performance by 34.3% (Saunders et al., 2012), and peak power output by 6.06% (Kim et al., 2018). Both trained and non-trained individuals have shown improvements in performance following β-alanine supplementation, however, these results seem to be slightly more pronounced in higher-trained individuals (de Salles Painelli et al., 2014). It has also been noted that the efficacy of β-alanine is not affected by exercise mode and that both intermittent and continuous exercise are equally likely to benefit from β-alanine supplementation (Saunders et al., 2016). Lastly, β-alanine has been shown to improve high-intensity exercise performance in both males and females (Stout et al., 2007), further demonstrating its efficiency as an ergogenic aid. However, with a loading period of at least 2-4 weeks, β-alanine supplementation may not be the most efficient way to increase muscle carnosine concentrations.

### 2.2.3. Precautions of Beta-Alanine Supplementation

One of the most common side effects of β-alanine supplementation that needs to be considered is paraesthesia, which is characterised by the heightened sensitivity of the nociceptive neurons that transmit neuropathic pain, leading to flushing and prickly sensations on the skin (Harris et al., 2006). The severity of paraesthesia episodes is dose-dependent but generally starts about 10 minutes after ingestion and can last up to 1 hour (Lancha Junior et al., 2015). Harris et al. (2006) found that the acute ingestion of β-alanine evoked mild flushing at a dose of 10 mg·kg$^{-1}$ body mass, while significant paraesthesia occurred at a dose of 20 mg·kg$^{-1}$ body mass. When β-alanine was consumed as part of a meal at a dose of 40 mg·kg$^{-1}$ body mass, no side effects occurred, yet when given as an oral dietary supplement participants were much more likely to experience paraesthesia. To limit the risk of paraesthesia it has been suggested that individual β-alanine doses mimic those incurred from a normal diet (Harris et al., 2006). Another issue of

β-alanine supplementation is its poor conversion rate to carnosine. Stegen et al. (2013) calculated β-alanine efficiency by dividing the molar increase in muscle carnosine by the total molar amount of β-alanine. They reported that just 2.80% of ingested β-alanine was incorporated into muscle carnosine, with the rest being metabolized by non-carnosine-directed pathways. Again, this suggests that β-alanine supplementation may not be the most efficient method for increasing muscle carnosine concentrations.

## 3. Novel Topical Application of Carnosine

Recently the novel topical application of carnosine has been investigated as a possible ergogenic aid and may provide an effective method for increasing intramuscular carnosine concentrations compared to the previously mentioned nutritional supplements. Topical gels transport molecules across the skin via a transdermal drug delivery system (Prausnitz & Langer, 2008). Transdermal drug delivery is a painless method of delivering drugs by applying a drug formulation to the skin. The drug initially penetrates the stratum corneum before passing through the epidermis and dermis. When the drug reaches the dermal layer, it then becomes available for systemic absorption via dermal microcirculation, which allows the drug to be absorbed into the bloodstream (Zaid Alkilani et al., 2015). Peptides, such as carnosine, have been used in topical formulation for collagen stimulation, wound healing, wrinkle soothing and other antioxidative effects (Dissette et al., 2018). Specifically, the topical application of carnosine in a water solution has been proven to reduce ultraviolet B radiation erythema in human skin (Schagen, 2017).

Dieter et al. (2021) examined the efficacy of a transdermal topical system for delivering carnosine into the skeletal muscle of horses. Carnosine plus a proprietary transdermal delivery agent or a placebo were applied to the middle gluteal muscles of 10 Thoroughbred racehorses, with muscle biopsies being taken before and 30, 60 and 120 minutes after application. Following the application of a transdermal delivery agent containing carnosine, mean carnosine concentrations were greater by about 35% at 30 minutes and by about 46% after 60 minutes, but not at 120 minutes when compared to concentrations following the application of the placebo. These results suggest that the application of carnosine via a transdermal delivery system can increase intramuscular carnosine concentrations within 60 minutes which could have important implications for the horses and their capacity to perform and recover from

physical activity. These results suggest that the topical application of carnosine is an efficient method for increasing intramuscular carnosine concentration and thus, could be an effective ergogenic aid for human athletes.

In an early study Nagai et al. (2019) investigated the topical application of carnosine to the right femoral muscle on the nerve activity of the left femoral muscle in urethane-anesthetised rats. Specifically, 100 fg, 1 pg, 10 pg, 100 pg, 1 ng, 10 ng, 100 ng, or 1 μg of carnosine was topically applied to the right femoral muscle. Skeletal muscle sympathetic nerve activity and blood flow of the left leg were then recorded at 5-minute intervals over a 60-minute test period. In rats, the topical application of 10 pg of carnosine to the right femoral muscle increased skeletal muscle sympathetic nerve activity and blood flow of the left femoral muscle. The excitation of sympathetic nerve activity due to the topical application of carnosine might mimic the effect of exercise on the excitation of the skeletal muscle sympathetic nerve activity seen in humans (Mark et al., 1985). These studies suggest that the application of a topical carnosine gel may be an effective ergogenic aid for enhancing intramuscular carnosine concentrations and improving high-intensity exercise performance in human athletes. However, little is known about the physiological mechanisms of action and benefits to human athletic performance. Topical carnosine gels are applied directly to the muscle area and should allow carnosine to be absorbed into the bloodstream after being transported across the skin via a transdermal drug delivery system. The topical application of a carnosine gel could bypass the hydrolysis associated with ingesting carnosine and therefore provide a more efficient method to increase muscle carnosine concentrations (Sharpe & Macias, 2016). Topical carnosine gels may also avoid the adverse side effects associated with the ingestion of carnosine and β-alanine, such as paraesthesia. Topical carnosine gels may also be faster acting and may not require a loading period as seen with the supplementation of β-alanine.

There are currently only two studies that examine the topical application of carnosine and its efficiency as an ergogenic aid for human performance. Sharpe and Macias (2016) investigated the effects of a topical carnosine gel on aerobic and anaerobic performance in elite male soccer players. It should be noted that Sharpe serves as the director of clinical research for Outplay Inc., which is the company that created the LactiGo carnosine gel which was used in this study. Eleven healthy elite male soccer players participated in this study. Participants were divided into three groups: a no-cream group, a warm-up cream group, or a warm-up cream + LactiGo group. Participants then completed a Level 1 Yo-Yo Intermittent Recovery Test and a maximal

intensity 3 x 1000 m Run Series, separated by a three-day washout period. Forty-five minutes before each respective performance test, the warm-up cream or warm-up cream + 10 mL of the LactiGo carnosine gel was topically applied to the musculature of the arms, legs, and torso. The group that received LactiGo experienced a 5.41 ± 4.80% improvement in the Yo-Yo test when compared to the no-cream group. In contrast, the average time to complete the 1000 m running tests improved by 4.13 ± 0.68% for the LactiGo group when compared to the no-cream group.

More recently Harnish and Miller (2023) studied the effects of a topical carnosine gel on repeated Wingate performance in trained male cyclists. Fifteen trained male cyclists randomly applied 10 mL of the LactiGo carnosine gel or a placebo to the legs at least 60 minutes before each cycling session. Cycling sessions were completed on a cycle ergometer and included a 15 s sprint to estimate glycolytic capacity followed by five Wingate sprints separated by 4-5 minutes of active recovery. In this crossover study, cyclists completed three sessions, the first was a familiarisation trial and the second and third utilised the application of the treatments. Following the topical application of a carnosine gel there were no statistically significant improvements in glycolytic capacity or Wingate sprint performance. This data suggests that a single recommended dose of a carnosine gel does not improve repeated Wingate sprint performance in trained cyclists. This is an interesting finding as it shows that the topical application of a carnosine gel does not improve high-intensity exercise performance, unlike the Sharpe and Macias (2016) paper that provided evidence for the efficacy of a topical carnosine gel in improving maximal aerobic and anaerobic capacity. These contrasting findings may be related to the training status of the participants. Specifically, Sharpe and Macias (2016) conducted their study on elite-level athletes, who speculatively may have had lower CN1 activity and therefore benefited more from the application of a topical carnosine gel compared to the less-trained athletes in the Harnish and Miller (2023) study. However, due to gaps in the current literature, there is only limited evidence to substantiate claims regarding the effectiveness of a topical carnosine gel for enhancing athletic performance. Thus, more research should be conducted to investigate the topical application of a carnosine gel and its effectiveness as an ergogenic aid.

# 4. Topical Application of Carnosine and Performance in Rugby Sevens

## 4.1. Physical Demands of Rugby Sevens

Rugby Sevens is played on a conventional rugby pitch (94-100 m long x 68-70 m wide) and is essentially played under the same laws as 15-a-side rugby union. Rugby sevens teams consist of seven players rather than fifteen and matches are 14 minutes (two 7-minute halves with a 2-minute half-time break). Competitions are often scheduled across 3 days and may involve a total of 2-3 matches per day (James et al., 2023). Despite similar technical and tactical requirements, sevens players face very different physical demands compared to rugby union players. As a result of shorter games and more space on the pitch per player greater locomotor activity and high-intensity repeated performances are required (Hogarth et al., 2016). Rugby sevens players require highly developed aerobic and anaerobic fitness to tolerate the demands of training and competition (Lamont, 2019).

Suarez-Arrones et al. (2012) conducted a study to investigate the running demands of a rugby sevens match. They found that during a rugby sevens match a male player covers 1,580.8 ± 146.3 m at various speeds, with an average speed of 6.4 $km \cdot h^{-1}$. Players also spent 13.7% of the time in high-intensity running activities (18-20 $km \cdot h^{-1}$) or sprinting (>20.1 $km \cdot h^{-1}$), which is slightly higher than the 10.5% reported in rugby union players. Lastly, they reported that the average number of sprints (>20.1 $km \cdot h^{-1}$) was reported to be approximately 7 per match and corresponded to an average distance of ~18 m (Suarez-Arrones et al., 2012). Overall, the running demands of rugby sevens are much higher compared to 15-a-side rugby union.

More recently James et al. (2023) investigated the blood lactate responses of players across an international rugby sevens tournament (5 matches over 2 days). Before warm-up, immediately after matches, and 30 minutes post-match, earlobe blood samples were taken from 25 professional players competing in an international tournament. Post-match blood lactate for males was 10.3 $mmol \cdot L^{-1}$ and for females was 9.1 $mmol \cdot L^{-1}$, with about 43% of samples exceeding 10 $mmol \cdot L^{-1}$, and 2 exceeding 19 $mmol \cdot L^{-1}$. Thirty minutes post-match, about 20% of values remained above 4 $mmol \cdot L^{-1}$. Together, these results build upon previous research that the glycolytic energy system plays an important role in the performance of rugby sevens players (Granatelli et al., 2014) and that considerable metabolic stress occurs during a rugby sevens

match. Thus, strategies to enhance muscle buffering capacity may be relevant for improved performance in rugby sevens.

## 4.2 Topical Carnosine Gel as a Potential Ergogenic Aid in Rugby Sevens

Based on previous research, the topical application of a carnosine gel may enhance muscle buffering capacity in rugby sevens players, which would improve high-intensity exercise performance by allowing anaerobic glycolysis to continue during high-intensity exercise, resulting in a higher lactate production without the accumulation of $H^+$ (Edge et al., 2006). The topical application of a carnosine gel may also enhance enzyme regulation of myosin ATPase which would help maintain ATP stores during anaerobic exercise (Parker Jr & Ring, 1970). Topical carnosine gels may also reduce or slow the decline in performance during fatiguing stimulation by improving calcium sensitivity within the human muscle (Dutka et al., 2012). As previously mentioned, a topical carnosine gel may be a more efficient method for improving muscle buffering capacity as it may bypass the carnosinase-mediated hydrolysis associated with the ingestion of carnosine (Sharpe & Macias, 2016). The topical application of a carnosine gel may also lower levels of perceived effort (Glenn et al., 2015), meaning rugby sevens players can compete at higher intensities for longer periods before feeling fatigued. As a result, rugby sevens players may be able to better manage the high-intensity running demands associated with rugby sevens competition with enhanced muscular performance. Thus, it seems feasible that the topical application of a carnosine gel may be an effective ergogenic aid to improve performance and lower levels of fatigue in rugby sevens players. However, there is currently no evidence of a topical carnosine gel and its efficiency as an ergogenic aid in improving performance for rugby sevens players involved in repeated, high-intensity efforts.

## Conclusion

Previous research has shown that increases in carnosine concentrations following the supplementation of combined carnosine and anserine or β-alanine can enhance intramuscular buffering capacity and thereby improve high-intensity exercise performance. A rise in carnosine concentrations has also been shown to improve force production and increase peak power output and peak torque on a repeated sprint ability test (de Jager et al., 2022). This

increase in muscle buffering capacity may enhance performance by allowing anaerobic glycolysis to continue during maximal exercise, resulting in higher lactate production without an increase in $H^+$ (Edge et al., 2006). An increase in carnosine concentrations has also been shown to improve anaerobic exercise by augmenting the enzyme regulation of myosin ATPase (Parker Jr & Ring, 1970). Carnosine may also increase calcium sensitivity within the human muscle, which should reduce or slow the decline in performance during fatiguing stimulation (Dutka et al., 2012). It has previously been suggested that elite-level athletes who excel in short, high-intensity exercise have lower CN1 activity and therefore may be more responsive to the potential ergogenic benefits associated with the supplementation of combined carnosine and anserine or β-alanine (Baguet et al., 2014).

Recently the novel topical application of carnosine has been investigated as a possible ergogenic aid and could provide a more efficient method to increase muscle carnosine concentrations. Sharpe and Macias (2016) provided evidence for the efficiency of a topical carnosine gel in improving maximal aerobic and anaerobic capacity. However, this is the only study that shows a positive ergogenic benefit following the topical application of a carnosine gel, and there is conflicting evidence, therefore more research needs to be conducted. Based on the available literature it seems feasible that the topical application of a carnosine gel may be an effective ergogenic aid for improving high-intensity exercise performance in rugby sevens players. However, no studies have investigated the topical application of a carnosine gel and its effects on performance in rugby sevens. Therefore, the aim of Chapter Two is to investigate the application of a topical carnosine gel and its effects on intermittent high-intensity exercise performance in elite rugby sevens players.

# Chapter Two – Experimental Study

The Application of a Topical Carnosine Gel and its Effects on Intermittent High-Intensity Exercise Performance in Olympic-Level Rugby Sevens Players

## Abstract

***Background:*** Previous research has suggested that the topical application of a carnosine gel may increase intramuscular carnosine concentrations and thereby improve high-intensity exercise performance. This study aimed to investigate the application of a topical carnosine gel and its effects on intermittent high-intensity exercise performance in rugby sevens players. ***Methods:*** Eight Olympic-level rugby sevens players (age: 30.25 ± 3.65 y, height: 190 ± 4.41 cm, body mass: 97.45 ± 4.09 kg) completed two performance tests in which they received both treatments, 10 mL of a topical carnosine gel (CAR) or an ultrasound placebo gel (PLA). The performance test was completed on a cycle ergometer and consisted of a warm-up, 12 intermittent sprints and a 2-min half-time break. Each sprint effort consisted of 24 s cycling at 3 W/kg, 6 s maximal intensity, and a 30 s rest. RPE was recorded at the end of Sprint 6 and Sprint 12. Average and peak power and HR were recorded for every 6 s sprint. ***Results:*** For peak power output the two-way ANOVA revealed a statistically significant time effect ($p$ = 2.16 x $10^{-9}$) and condition effect ($p$ = 1.44 x $10^{-7}$). The CAR condition experienced an increase in peak power output for sprint 2 (+101.3 ± 62.0 W; $p$ = 0.048; Cohen's $d$ = 0.99; *large* effect size), sprint 4 (+102.6 ± 82.1 W; $p$ = 0.043; Cohen's $d$ = 0.74; *moderate* effect size) and sprint 7 (+ 156.0 ± 106.2 W; $p$ = 0.025; Cohen's $d$ = 0.98; *large* effect size) compared to the PLA condition. For mean power output, the two-way ANOVA revealed a statistically significant time effect ($p$ = 5.98 x $10^{-19}$), but no condition effect ($p$ = 0.211). Although non-significant, there was still a substantial increase in mean power for the CAR condition in Sprints 10 (51.3 ± 48.2 W; $p$= 0.079; Cohen's $d$= 0.57; *moderate* effect size) and 11 (44.7 ± 37.0 W; $p$= 0.069; Cohen's $d$= 0.39; *small* effect size) compared to the PLA condition. ***Conclusion:*** Overall, the application of a topical carnosine gel can significantly improve power production in Olympic-level rugby sevens players. The topical application of a carnosine gel may increase intramuscular carnosine concentrations and as a result, improve anaerobic alactic performance in Olympic-level athletes.

***Keywords:*** carnosine, topical application, rugby sevens, high-intensity exercise

## Introduction

During high-intensity exercise, the accumulation of $H^+$ ions decreases intracellular pH, which has been reported to impair performance by limiting the resynthesis of high-energy phosphates and inhibiting the rate of anaerobic glycolysis (Fitts, 1994). To mitigate these effects the human body contains intracellular and extracellular buffer systems that act to reduce the accumulation of $H^+$ ions and delay the onset of fatigue (Douroudos et al., 2006). Carnosine (beta-alanyl-L-histidine) is a naturally occurring histidine-containing molecule and is a strong intracellular buffer found at high concentrations in the cytosol of the muscle (Jones, 2014). Thus, increasing carnosine concentrations can enhance pH buffering and improve high-intensity exercise performance (Begum et al., 2005). An increase in carnosine concentrations has also been shown to enhance $Ca^{2+}$ sensitivity, which could alleviate the decline in performance during fatiguing stimulations by counteracting factors that may cause reduced calcium sensitivity (Dutka et al., 2012). An increase in carnosine concentrations may also improve myosin-ATPase activity and increase the maximal amount of adenosine triphosphate re-synthesised via anaerobic metabolism (Parker Jr & Ring, 1970).

As a result, nutritional supplements containing high levels of carnosine are commonly used to increase muscle carnosine concentrations and enhance high-intensity exercise performance. However, due to the highly active serum carnosinase enzyme, the ingestion of carnosine is susceptible to hydrolysis and even relatively high doses of carnosine are often rapidly degraded (Sauerhöfer et al., 2012). Therefore, other methods of enhancing muscle carnosine concentrations have been investigated, such as β-alanine supplementation. β-alanine is a naturally occurring amino acid which is involved in the formation of carnosine (Culbertson et al., 2010). Four weeks of oral β-alanine supplementation at a rate of 4-6 $g \cdot day^{-1}$ has been shown to raise intramuscular carnosine levels by about 60% (Harris, 2005) and improve high-intensity exercise performance in events lasting between 1-6 minutes (Jones, 2014). However, since β-alanine supplementation has a loading period of at least 2-4 weeks (Trexler et al., 2015) there may be a more efficient method to increase muscle carnosine concentrations.

More recently the novel topical application of a carnosine gel has been investigated as a possible ergogenic aid, but little is known about the physiological mechanisms of action and the effects on human performance. The topical application of carnosine directly to the muscle may allow carnosine to be absorbed into the bloodstream via a transdermal drug delivery

system (Zaid Alkilani et al., 2015), increasing muscle carnosine concentrations, and improving high-intensity exercise performance. Sharpe and Macias (2016) found that the topical application of a carnosine gel improved Yo-Yo test performance by 5.41% and time to complete a 1000 m time trial by 4.13%. However, in a more recent study, Harnish and Miller (2023) found that following the topical application of carnosine there were no statistically significant improvements in glycolytic capacity or Wingate sprint performance. Due to conflicting results and a lack of literature investigating the effect of topical carnosine in an elite athletic population, more research should be conducted to investigate the topical application of a carnosine gel and its effectiveness as an ergogenic aid.

Strategies to increase carnosine concentrations and enhance muscle buffering capacity may also be relevant for improved performance in rugby sevens. Due to the short duration of games and more space on the pitch per player, greater locomotor activity and high-intensity repeated performances are required during a rugby sevens match (Hogarth et al., 2016). Players spend 13.7% of the time in high-intensity running activities (18-20 $km \cdot h^{-1}$) or sprinting (>20.1 $km \cdot h^{-1}$), which is slightly higher than the 10.5% reported in rugby union players (Suarez-Arrones et al., 2012). The anaerobic requirements of rugby sevens are highlighted by recent research that demonstrated that about 43% of blood lactate samples exceeded 10 $mmol \cdot L^{-1}$, and two samples exceeded 19 $mmol \cdot L^{-1}$ following international matches (James et al., 2023). These results suggest that the glycolytic energy system plays an important role in rugby sevens performance and that considerable metabolic stress occurs during a rugby sevens match (Granatelli et al., 2014). Thus, it seems feasible that rugby sevens players will benefit from the possible increase in muscle carnosine concentrations and improved intramuscular buffering capacity that may be associated with the topical application of a carnosine gel.

However, there is currently no evidence on the topical application of a carnosine gel and its efficiency as an ergogenic aid in rugby sevens. Therefore, the aim of this current study was to investigate the application of a topical carnosine gel and its effects on intermittent high-intensity exercise performance in elite rugby sevens players. Based on the available literature, it was hypothesised that the application of a topical carnosine gel would increase peak power output and repeated sprint performance during repeated bouts of high-intensity exercise in rugby sevens players.

## Methods

### Participants

Twelve elite, male rugby sevens players provided written informed consent to participate in this study. An elite player was defined as an individual currently involved in international competition. The study was conducted following the principles of Research Ethics in Exercise, Health, and Sports Sciences (McNamee et al., 2006) and was approved by the University of Waikato Human Research Ethics Committee. Before participating in the study participants were familiarised with the study and experimental protocol. Out of the 12 participants, 4 had to withdraw from the study due to injury unrelated to the experimental protocol, as well as other training and competition demands. Of the 8 participants who completed the study age, height and body mass were 30.25 ± 3.65 y, 190 ± 4.41 cm, and 97.45 ± 4.09 kg, respectively.

### Research Design

This was a double-blind, placebo-controlled, and counterbalanced crossover study. To ensure the treatment and placebo were double-blinded, an independent researcher who was not involved in the data collection separated the two products into unlabelled yellow and red bottles. One bottle contained the carnosine gel (CAR: LactiGo™, Outplay Inc, Las Vegas, NV) and the other contained the placebo gel, which was a clear ultrasound gel (PLA: Aquasonic 100, Parker Laboratories Inc, Fairfield, NJ). During the first testing day, and in a counterbalanced design, participants randomly received the CAR or PLA gel. Seven days later the treatments were switched, and the experimental protocol was repeated. Four participants received CAR on testing day one, and four received CAR on testing day two. After the two testing days, the contents of each bottle were disclosed to the participants.

### Testing Day Protocol

Forty-five minutes before the performance test participants manually applied 10 mL of either the CAR or PLA gel to each leg (thigh and calves). The quantity and timing of the gel application were based on the manufacturer's recommendation for the LactiGo™ gel. The LactiGo™ carnosine gel comprises water, glycerin, magnesium sulphate, 1.25% menthol, and a proprietary carnosine complex. The PLA gel had 1.25% menthol added to mimic the odour and stated active ingredient of the CAR gel. The performance test was completed between 1:00 PM and 4:00 PM after the team's gym session. During the first week, before the start of the test, each participant's height (cm) and body mass (kg) were recorded. Body mass was then

used to calculate target zones for 2, 2.5, and 3 $W \cdot kg^{-1}$ for the performance test. The Wattbike, seat and handlebar position were recorded and standardised across testing sessions.

**Performance Test Protocol**

The performance test was completed on a calibrated cycle ergometer (Wattbike Ltd, Nottingham UK) and was designed to simulate the work-to-rest ratio of a rugby sevens match. The performance test protocol used in this study was based on the protocol used by Fenemor et al. (2023), which was also used to test performance in elite rugby sevens players. During the performance test heart rate (HR) was measured using Polar H10 chest strap monitors. Ratings of perceived exertion (RPE) were recorded using the Borg Rating of Perceived Exertion Scale (Borg, 1982). The protocol consisted of a warm-up, 12 intermittent sprints and a 2-minute half-time break. The warm-up took the following structure: 2 min cycling at 2 $W \cdot kg^{-1}$, 2 min cycling at 2.5 $W \cdot kg^{-1}$ and 30 s cycling at 3 $W \cdot kg^{-1}$, this was followed by a 2 min rest before the start of the intermittent sprint protocol. The RPE, HR and average power were recorded after each stage of the warm-up. The intermittent sprint section of the protocol consisted of 12 repeated efforts of 24 s cycling at 3 W/kg, 6 s maximal intensity, and a 30 s rest. These efforts were broken up by a 2-minute half-time break after Sprint 6. The RPE was recorded at the end of Sprint 6 and Sprint 12. Average and peak power and HR were recorded for every 6 s sprint.

**Statistical Analysis**

Data was initially grouped as CAR or PLA and results were analysed for HR, RPE, average 6 s sprint power (MP) and peak 6 s sprint power (PP). Half-time recovery for average and peak power was calculated as the change in power between Sprints 7 and 6. Data was analysed using a two-way ANOVA (Time: Sprint number x Condition: PLA and CAR). When a condition effect was identified a *post-hoc* t-test was used to determine the difference between the two conditions (CAR and PLA). The difference (CAR – PLA), ± standard deviation, percentage change, and effect size were calculated for every data point. To determine if the difference was statistically significant *p*-values were analysed on log-transformed data. The statistical significance threshold level was set at an alpha level of 0.05. To interpret the effect size between the two conditions (CAR and PLA) the magnitude of the effect was determined and expressed as mean differences ± standard deviation and Cohen's *d* was calculated with a 90% confidence level. The thresholds used to describe effect sizes were *trivial* (< 0.20), *small* (0.20 – 0.49), *moderate* (0.50 – 0.79), and *large* (≥ 0.80) (Cohen, 2013).

# Results

## Peak Sprint Power

The two-way ANOVA revealed a statistically significant time effect ($p$ = 2.16 x $10^{-9}$) and condition effect ($p$ = 1.44 x $10^{-7}$). Overall, peak power decreased from Sprint 1 to Sprint 12 by 261.9 ± 123.2 W ($p$ = 2.39 x $10^{-6}$). Post-hoc analyses showed that in Sprints 2, 4 and 7 the difference between the two conditions was statistically significant (Table 3; $p < 0.05$). Specifically, the CAR condition experienced an increase of 101.3 ± 81.0 W for Sprint 2 ($p$ = 0.048; Cohen's $d$ = 0.99; *large* effect size), 102.6 ± 82.1 W for Sprint 4 ($p$ = 0.043; Cohen's $d$ = 0.74; *moderate* effect size) and 156.0 ± 106.2 W for Sprint 7 ($p$ = 0.025; Cohen's $d$ = 0.98; *large* effect size) compared to the PLA condition. Although non-significant, there was still a noticeable increase in peak power for the CAR condition in Sprints 3, 8, 9, and 10 (101.1 to 145.6 W; Cohen's $d$ = 0.81 to 0.91; *large* effect size), Sprints 5 and 11 (97.3 and 113.3 W; Cohen's $d$ = 0.79 and 0.67; *moderate* effect size), and Sprints 1 and 6 (58.9 and 74.4 W; Cohen's $d$ = 0.49 and 0.49; *small* effect size) compared to the PLA condition. For sprint 12 there was no clear or statistically significant difference in peak power between the two conditions. The half-time recovery of peak power was 144.1 ± 97.6 W for the CAR condition and 62.6 ± 81.9 W for the PLA condition, although this was non-significant ($p$ = 0.193; Cohen's $d$ = 0.71; *moderate* effect size).

**Table 3.** The Difference in 6 s Peak Power Output for The Topical Carnosine Gel Compared to the Ultrasound Placebo Gel for All 12 Sprints

| | Sprint 1 | Sprint 2 * | Sprint 3 | Sprint 4 * | Sprint 5 | Sprint 6 |
|---|---|---|---|---|---|---|
| Difference | + 58.9 ± 62.0 | + 101.3 ± 81.0 | + 101.1 ± 94.9 | + 102.6 ± 82.1 | + 97.3 ± 80.7 | + 74.4 ± 102.2 |
| % Change | 4.1% | 7.0% | 7.8% | 7.8% | 7.3% | 6.0% |
| *P* Value | 0.114 | 0.048* | 0.075 | 0.043* | 0.055 | 0.188 |
| Cohen's *d* | 0.49 | 0.99 | 0.91 | 0.74 | 0.79 | 0.49 |
| | **Sprint 7 *** | **Sprint 8** | **Sprint 9** | **Sprint 10** | **Sprint 11** | **Sprint 12** |
| Difference | + 156.0 ± 106.2 | + 145.6 ± 139.7 | + 139.3 ± 121.8 | + 138.6 ± 155.5 | + 113.3 ± 126.5 | + 84.6 ± 125.9 |
| % Change | 11.2% | 11.0% | 10.8% | 11.4% | 9.1% | 7.1% |
| *P* Value | 0.025* | 0.086 | 0.057 | 0.117 | 0.118 | 0.219 |
| Cohen's *d* | 0.98 | 0.87 | 0.87 | 0.81 | 0.67 | 0.57 |

Values are mean ± SD and are recorded as watts (W).
*$P < 0.05$, results are statistically significant.

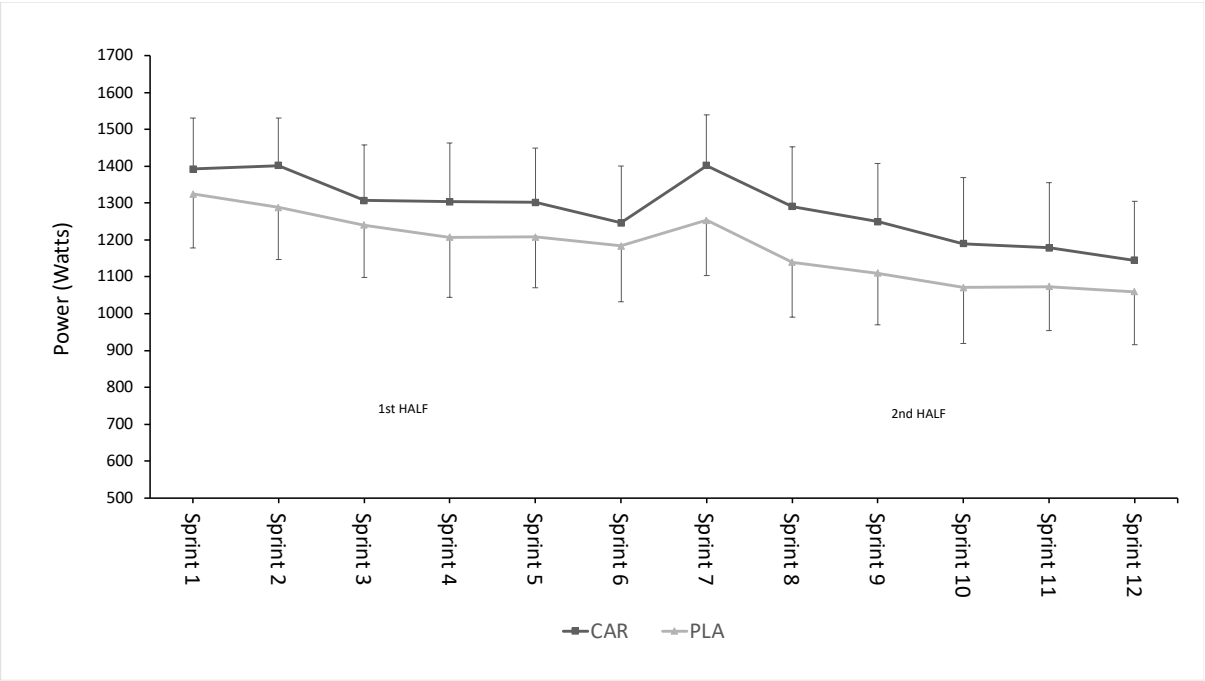



**Figure 1.** Peak 6 s Sprint Power Output Following the Topical Application of the Carnosine Gel (CAR) and the Ultrasound Placebo Gel (PLA)

**Mean Sprint Power**

The two-way ANOVA revealed a statistically significant time effect ($p$ = 5.98 x $10^{-19}$), but no condition effect ($p$ = 0.211). Overall, mean power decreased from Sprint 1 to Sprint 12 by 284.9 ± 113.9 W ($p$ = 3.86 x $10^{-7}$). Despite, no significant differences between the two conditions, mean power was substantially higher in the CAR condition in Sprints 10 (51.3 ± 48.2 W; $p$= 0.079; Cohen's $d$= 0.57; *moderate* effect size) and 11 (44.7 ± 37.0 W; $p$= 0.069; Cohen's $d$= 0.39; *small* effect size) compared to the PLA condition. For the remaining sprints as well as half-time recovery, there was no clear or statistically significant difference between the two conditions for mean power.

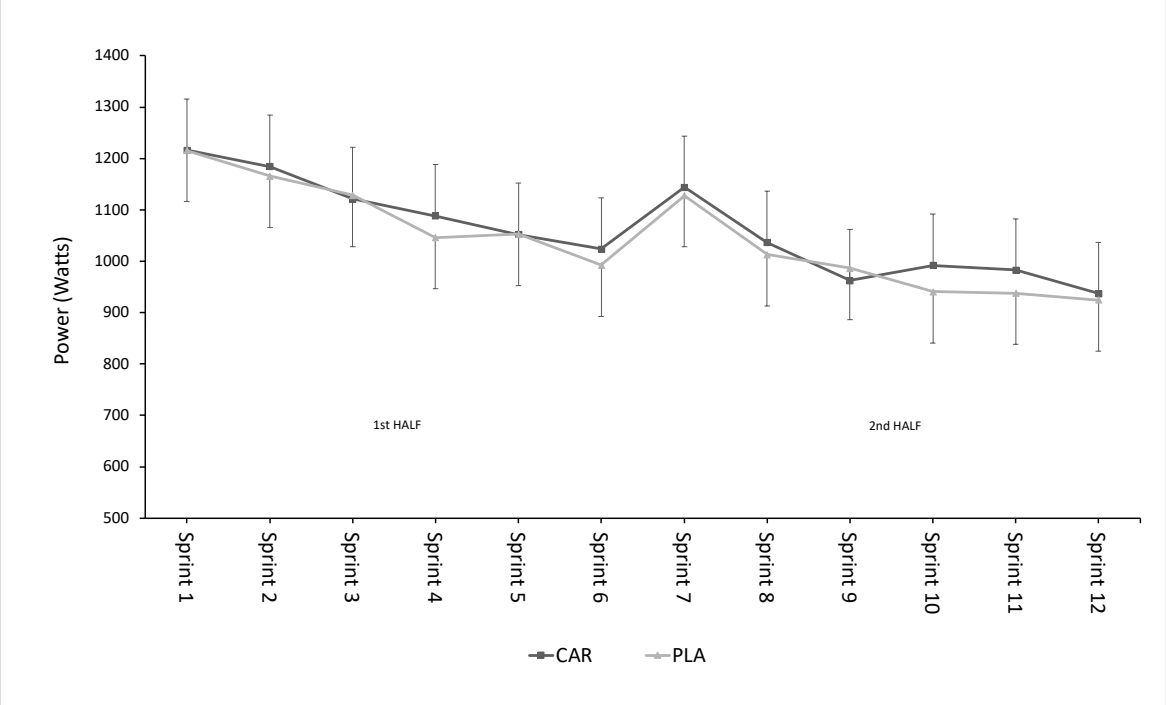



**Figure 2**. Mean 6 s Sprint Power Output Following the Topical Application of the Carnosine Gel (CAR) and the Ultrasound Placebo Gel (PLA).

**Heart Rate**

The two-way ANOVA revealed a statistically significant time effect ($p = 3.12 \times 10^{-15}$) but no condition effect ($p = 0.228$). Overall, HR increased from Sprint 1 to Sprint 12 by 20.8 ± 9.5 bpm ($p = 4.26 \times 10^{-7}$) with an average of 164.5 ± 7.5 bpm after Sprint 12.

**Ratings of Perceived Exertion**

The two-way ANOVA revealed a statistically significant time effect ($p = 9.88 \times 10^{-17}$) but no condition effect ($p = 0.847$). Overall, RPE increased from post-warm-up to Sprint 12 by 7.1 ± 1.9 units ($p = 1.41 \times 10^{-10}$) with an average of 18.7 ± 0.9 units after Sprint 12

## Discussion

Previous research has indicated that a topical carnosine gel may be an effective ergogenic aid to improve high-intensity exercise performance. Therefore, this study aimed to investigate the topical application of a carnosine gel and its effects on intermittent high-intensity exercise performance in rugby sevens players. Based on the available literature, it was hypothesised that a topical carnosine gel would increase peak power output during repeated bouts of high-intensity exercise in rugby sevens players. The results from this current study have shown for the first time that the topical application of a carnosine gel can significantly improve peak power output during high-intensity intermittent exercise performance in Olympic-level athletes and the positive effects appear to be independent of the known physiological buffering effects of carnosine.

The application of a topical carnosine gel transports carnosine across the skin via a transdermal drug delivery system, thereby allowing carnosine to be absorbed into the bloodstream and increasing carnosine concentrations (Prausnitz & Langer, 2008). This possible rise in carnosine concentrations may be responsible for the acute increases in peak power output observed in our elite athletic cohort as previous research has indicated that there is a link between carnosine concentrations and peak power output (Hill et al., 2007). In this current study, peak power output increased by 4.1% in Sprint 1, with the largest increase being 11.4% in Sprint 10. This increase observed in Sprint 10 is significantly higher than the previously seen 3% increase in peak power output following the supplementation of combined carnosine and anserine (Blancquaert et al., 2021) and the 6.06% increase following β-alanine supplementation (Kim et al., 2018). Thus, the application of a topical carnosine gel may be more effective at raising intramuscular carnosine concentrations and thereby lead to a larger increase in peak power output compared to the pre-existing nutritional supplements.

In a previous study conducted by Harnish and Miller (2023) it was concluded that a topical carnosine gel does not improve repeated high-intensity exercise performance in trained cyclists. However, the results of this current study suggest that a topical carnosine gel can improve repeated high-intensity exercise performance. The increase in performance observed in this current study may be related to training status. Compared to cyclists, Olympic-level rugby sevens players are typically more involved in repeated, high-intensity exercise and may be impacted differently by the use of a topical carnosine gel. Previous research has suggested

that athletes who excel in short, repeated, high-intensity exercise tend to have lower serum carnosinase activity and may be more responsive to the potential ergogenic benefits associated with increased carnosine concentrations (Baguet et al., 2014). Therefore, it is possible that rugby sevens players have lower serum carnosinase activity and thus they may benefit more from the topical application of a carnosine gel compared to cyclists involved in more endurance-based training.

An increase in muscle buffering capacity associated with the possible rise in carnosine concentrations could explain why there was an increase in performance for repeated high-intensity sprints. The increase in muscle buffering capacity would aid in the regulation of $H^+$ and pH levels, which would improve high-intensity exercise performance by allowing anaerobic glycolysis to continue during maximal exercise, resulting in higher lactate production without an increase in $H^+$ (Edge et al., 2006). The glycolytic energy system plays an important role in the performance of rugby sevens players (James et al., 2023), therefore a higher lactate production without the accumulation of $H^+$ would be beneficial to improving performance in rugby sevens players. As previously demonstrated by Suzuki (2004), who used a similar performance protocol, muscle buffers tend to only impact performance in the latter half of a series of sprints. However, with an increase in performance during the first half, the results from this current study suggest that an increase in performance following the topical application of a carnosine gel is unlikely to be solely attributable to muscle buffering capacity and is more likely related to the possible increase in anaerobic capacity or $Ca^{2+}$ sensitivity.

Similar to the Harnish and Miller (2023) paper our data showed a very minimal increase in anaerobic lactic capacity (mean power output). But, in contrast, there was a significant increase in the anaerobic alactic capacity, as indicated by a rise in peak power output (Gastin, 2001), which was apparent throughout the performance test. These immediate and acute effects suggest that, similar to the Sharpe and Macias (2016) paper, there was likely an increase in anaerobic work capacity following the topical application of the carnosine gel. Previous research has shown that a rise in carnosine concentrations may enhance anaerobic work capacity by improving myosin-ATPase activity and increasing the maximal amount of adenosine triphosphate re-synthesised via anaerobic metabolism during a specific mode of short, high-intensity exercise (Parker Jr & Ring, 1970). Increased intramuscular carnosine concentrations has also been reported to facilitate $Ca^{2+}$ handling and improve diffusive coupling (Swietach et al., 2014), which would enhance skeletal muscle force production and

improve high-intensity exercise performance. Thus, a rise in carnosine concentrations following the topical application of a carnosine gel may improve anaerobic work capacity and increase alactic capacity during repeated bouts of high-intensity exercise, which would be beneficial for improving performance during a range of sports that rely on anaerobic energy pathways, including rugby sevens (Lamont, 2019).

The results from this current study also suggest that a topical carnosine gel acts rapidly, thus eliminating the need for the loading period typically needed to increase carnosine concentrations (Harris, 2005). A topical carnosine gel would likely be absorbed into the interstitial fluid before being taken up by the muscle (Ramadon et al., 2021), bypassing the potential for breakdown via the highly active serum carnosinase enzyme (Sauerhöfer et al., 2012). Thus, the topical application of carnosine may provide a more efficient method to increase intramuscular carnosine concentrations compared to pre-existing methods, such as nutritional supplements. While these results are promising, the current study cannot conclude whether the topical application of a carnosine gel is more effective at increasing carnosine concentrations than the previously used oral dosing as these results were not compared.

Thus, the main limitation of this study is that there is no evidence to suggest that a topical carnosine gel is more effective at raising circulating carnosine concentrations compared to the oral ingestion of carnosine as no measures of serum, interstitial fluid or muscle carnosine levels were analysed. Another limitation is that the performance tests were completed straight after the gym session, meaning fatigue levels may have impacted the results. Also, the players' diets and baseline muscle carnosine levels were not monitored before the performance test and it can not be concluded whether circulating levels of carnosine or other dietary interventions impacted these results. Lastly, this study was only conducted on elite male rugby players, therefore, it remains to be established how the topical application of a carnosine gel may improve high-intensity exercise performance in women or non-elite individuals. Previous research has suggested that women tend to have lower levels of carnosine so may see a larger increase in intramuscular carnosine concentrations (Simoneau & Bouchard, 1989).

Nonetheless, the results from this current study provide compelling evidence for the efficiency of a topical carnosine gel for improving performance during repeated bouts of intermittent, high-intensity exercise. Since this was a double-blind, crossover study there should be minimal bias or placebo effect associated with data collection. To improve the validity of the results players used the same cycle ergometer and heart rate monitor for each performance test. The

practical implications of this study relate to the novel topical application of a carnosine gel and its efficiency as an ergogenic aid for a range of other sports that have a high anaerobic component as well as the need for repeated bouts of intermittent high-intensity exercise, such as 15-a-side rugby, football, ice hockey and basketball.

## Conclusion

In conclusion, the application of a topical carnosine gel significantly improved intermittent, high-intensity exercise performance in Olympic-level rugby sevens players. The topical application of a carnosine gel can improve high-intensity exercise by possibly increasing intramuscular carnosine concentrations and improving anaerobic alactic performance in Olympic-level athletes. The results from this current study suggest that the increase in performance is unlikely related to muscle buffering capacity and more likely related to an increase in anaerobic work capacity or calcium sensitivity. Compared to pre-existing methods the topical application of a carnosine gel may provide a more efficient method for increasing intramuscular carnosine concentrations. More research, particularly looking at the mechanisms of action, needs to be conducted to better understand the topical application of a carnosine gel and how different timing and dosing protocols may affect the results seen in this current study.

# Chapter Three – Summary and Future Directions

## Summary

Recently the novel topical application of a carnosine gel has been investigated as a possible ergogenic aid for improving high-intensity exercise performance. Previous research has suggested that a topical carnosine gel may increase intramuscular carnosine concentrations after being transported across the skin via a transdermal drug delivery system (Zaid Alkilani et al., 2015). This possible increase in carnosine concentrations may enhance muscle buffering capacity and improve performance by regulating $H^+$ and pH levels (Lancha Junior et al., 2015). However, it seems more likely that the topical application of a carnosine gel may improve performance by enhancing myosin ATPase activity and increasing the maximal amount of adenosine triphosphate re-synthesised via anaerobic metabolism during a specific mode of short, high-intensity exercise (Parker Jr & Ring, 1970) or by improving calcium sensitivity within the muscle (Dutka et al., 2012).

Sharpe and Macias (2016) had previously provided evidence for the efficiency of a topical carnosine gel in improving high-intensity exercise performance. They found that the topical application of a carnosine gel improved Yo-Yo test performance by 5.41% and the time to complete a 1000 m running test by 4.13%. These results suggest that a topical carnosine gel can improve performance in tests of maximal aerobic and anaerobic capacity and are similar to the magnitude of the effects observed in this current study which saw an increase in performance of 4-11%.

Previous research had suggested that rugby sevens players may benefit from the possible increase in muscle carnosine concentrations and improved intramuscular buffering capacity that may be associated with the topical application of the carnosine gel due to the heavy reliance on glycolytic energy systems. Hence, this study aimed to investigate the application of a topical carnosine gel and its effects on intermittent high-intensity exercise performance in rugby sevens players.

The results from this current study suggest that the topical application of a carnosine gel can significantly improve intermittent, high-intensity exercise performance in Olympic-level rugby sevens players. The topical application of a carnosine gel likely improves high-intensity exercise performance by increasing intramuscular carnosine concentrations and enhancing anaerobic alactic performance, with evidence for an effect on anaerobic performance being less clear. The rapid action of the topical application of a carnosine gel may provide a more efficient

method for increasing intramuscular carnosine concentrations compared to other pre-existing oral dosing methods. These results provide compelling evidence for the efficiency of a topical carnosine gel for improving performance during repeated bouts of intermittent, high-intensity exercise.

## Limitations

The main limitation of this study is that no measures of serum, interstitial fluid or muscle carnosine levels were analysed. Thus, it cannot be concluded at what rate carnosine may have been absorbed into the skin or whether a topical carnosine gel is more effective at raising circulating carnosine concentrations compared to the oral ingestion of carnosine, which has been shown to significantly increase intramuscular carnosine concentrations Barbaresi et al. (2021). Additionally, lactate levels were not assessed.

Another limitation is that players' diets and baseline muscle carnosine levels were not monitored before the performance test. Therefore, it could not be concluded whether circulating levels of carnosine or other dietary interventions impacted the results. For example, interventions such as caffeine, protein, essential amino acids and manipulated carbohydrates may enhance high-intensity interval training (Forbes et al., 2020). Previous research has also suggested that circulating carnosine may impact the uptake of carnosine (Lenney et al., 1985).

Lastly, this study was only conducted on elite male rugby players. Thus, it remains to be established how the topical application of a carnosine gel may improve high-intensity exercise performance in women or non-elite individuals. Previous research has suggested that women tend to have lower levels of carnosine so may see a larger increase in intramuscular carnosine concentrations (Simoneau & Bouchard, 1989) and therefore, could potentially be affected differently by the topical application of a carnosine gel.

## Strengths

Since this was a double-blind, crossover study there should have been minimal bias or placebo effect associated with this study. The double blinding of the placebo and the topical carnosine

gel is important as it minimises the bias and maximises the validity of the results (Karanicolas et al., 2010). With this being a crossover study it should have removed player variation across the two performance tests (Sibbald & Roberts, 1998).

Players used the same cycle ergometer and heart rate monitor for each performance test, which should have improved the validity of the results. This ensures accuracy and increases confidence in the practical and scientific application of the final results (Heale & Twycross, 2015).

## Future Directions

Along with the Sharpe and Macias (2016) paper, this study provides compelling evidence for the efficiency of a topical carnosine gel for improving performance during repeated bouts of high-intensity exercise. Therefore, future research should look at the application of a topical carnosine gel for improving performance in other sports where intermittent high-intensity efforts are required, such as 15-a-side rugby, football, hockey and basketball.

Future research should also investigate the effects of a topical carnosine gel on performance in female athletes. Research has suggested that women tend to have lower levels of carnosine (Simoneau & Bouchard, 1989) and therefore may be affected differently by the topical application of carnosine gel.

Lastly, more research needs to be conducted looking at the possible mechanisms of action associated with the topical application of a carnosine gel in human athletes as this may provide more information about how different timing and dosing protocols could affect the results seen in this study. Previous research has suggested that the application of a transdermal delivery agent can increase mean carnosine concentrations in Thoroughbred horses by about 35% after 30 minutes and 46% after 60 minutes (Dieter et al., 2021), suggesting that it may also be effective at increasing carnosine concentrations in human athletes. Therefore, to investigate the rate of absorption and effectiveness in increasing intramuscular carnosine concentrations, future studies should measure serum, interstitial fluid and muscle carnosine levels following the topical application of a carnosine gel to investigate the rate of absorption. This information would assist in optimising the dosage and timing of the topical application of carnosine gels.

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# Appendix A – Ethics application approval

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17 April 2019

Dr Martyn Beaven
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Dear Martyn

**HREC(Health)2019#23 : Effects of topical gel application on high-intensity exercise efforts**

Thank you for submitting your amended application HREC(Health)#2019#23 for ethical approval.

We are now pleased to provide formal approval for your project, where you will assess athlete performance in 1 km cycle ergometer trials under four conditions (no gel application, placebo, and two commercially available gels).

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,

**Julie Barbour PhD**
**Chairperson**
**University of Waikato Human Research Ethics Committee**

# Appendix B – Information sheet for participants

**Title –** Effects of topical gel application on high-intensity exercise efforts

**Aim –**To determine whether a gel applied to the skin can positively influence power and repeated sprint efforts on an exercise bike.

**Background –** When acid builds up in the muscles it can affect performance. It is known that ingestion of certain substances that slow the build-up of acid (e.g. sodium bicarbonate, or baking soda) can improve performance in high-intensity exercise. New products are now available that claim that applying a gel to the skin can also enhance performance by slowing the build-up of acid.

**Overview –** Should you agree to participate, you will be asked to sign an informed consent form and complete a baseline questionnaire. You will be required to attend a total of 4 sessions at the University of Waikato sport science laboratories.

At each session, you will apply one of three gels to your legs, or exercise without any gel applied. Two of the gels are commercially available products and one will contain no active ingredients. After a warm-up, you will perform repeated 6 s sprints on an exercise bike with 24 s to recover between each effort. When you finish exercising a small finger-prick blood sample will be taken to check the acid levels in the blood.

More information on the two commercially available gels can be found at:

https://www.lactigo.com/

https://amphumanperformance.com/

Each session should last about 20 minutes, and will be separated by at least 48 h.

**What are the potential risks –** The risks associated with participating in this study are no greater than those associated with performing typical high intensity training (e.g. spin class). If any harm does occur during study participation, the research team will offer immediate first aid and support you in accessing medical attention as required. If an injury does happen during testing, costs are likely to be covered – at least in part – by Accident Compensation Corporation.

**What will happen to the information collected –** The information collected will be used by the research team to write research reports, give scientific presentations, and help in educating students at the University of Waikato and the wider community. The information could be used in postgraduate student projects and thesis dissertations. Only the research team, their research associates, and students under their supervision will have direct access to the notes, documents, and recordings. 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, after which they will be destroyed. All data will be treated with the strictest confidentiality. No participants will be named in the publications and every effort will be made to disguise their identity. All data used in teaching will be de-identified (i.e., will not contain your personal information) to protect your identity and confidentiality.

**Declaration to participants –** If you take part in the study, you have the right to:

- Ask any further questions about the study that occurs to you during your participation;
- A summary of findings from the study when it is concluded;
- Have a support person (family, whanau, and/or friend) present during your participation;
- Refuse to answer any particular question, and to withdraw from the study at any time;
- Withdraw any information provided up to two weeks after participating in the research activities by contacting the principal investigator.
- Receive an individualised report.

**Who is responsible –** If you have any questions about the project, please feel free to contact:

Dr Martyn Beaven **(Primary Investigator)**

The University of Waikato, Adams Centre for High Performance

52 Miro Street, Mount Maunganui 3116

martyn.beaven@waikato.ac.nz

**Human Research Ethics Committee –** This research project has been approved by the Human Research Ethics Committee (Health) of the University of Waikato under *HREC(Health)**#2019-XX.***

Any questions about the ethical conduct of this research may be addressed to the Secretary of the Committee, email humanethics@waikato.ac.nz, postal address, University of Waikato, Te Whare Wananga o Waikato, Private Bag 3105, Hamilton 3240.

# Appendix C – Informed consent form

**Title –** Effects of topical gel application on high-intensity exercise efforts

*I have read the **Participant 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 free to withdraw from the study at any time or to decline to answer any particular questions.*
- *I can withdraw any information I have provided up to two weeks after participating in the research activities by contacting the principal investigator.*
- *Any data or answers will remain confidential in regards to my identity through a coding system.*
- *The data might be published, so every effort will be made to ensure confidentiality and anonymity. However, anonymity cannot be guaranteed.*

***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:** | _____ | _____ |

*Additional Consent (Optional)*

***I agree to my images and videos being used in their original (unaltered) form for publication, scientific presentation, and/or education purposes.***

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

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*Te Whare Wānanga o Waikato*

# Appendix D - Baseline Questionnaire

**Effects of topical gel application on high-intensity exercise efforts**

**Baseline Questionnaire**

UoW HREC(Health)#2019XX

Name: ……

Date of Birth: ……

Height: …… Weight: ……

Sex: ……

# Appendix E – Physically activity readiness questionnaire

# Physical Activity Readiness Questionnaire (PAR-Q) and You

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly:

| YES | NO | | |
|---|---|---|---|
| ☐ | ☐ | 1. | Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor? |
| ☐ | ☐ | 2. | Do you feel pain in your chest when you do physical activity? |
| ☐ | ☐ | 3. | In the past month, have you had chest pain when you were not doing physical activity? |
| ☐ | ☐ | 4. | Do you lose your balance because of dizziness or do you ever lose consciousness? |
| ☐ | ☐ | 5. | Do you have a bone or joint problem that could be made worse by a change in your physical activity? |
| ☐ | ☐ | 6. | Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition? |
| ☐ | ☐ | 7. | Do you know of any other reason why you should not do physical activity? |

**If you answered:**

## YES to one or more questions

Talk to your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want – as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

## NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- Start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- Take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

**Delay becoming much more active:**

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed use of the PAR-Q: Reprinted from ACSM's Health/Fitness Facility Standards and Guidelines, 1997 by American College of Sports Medicine

# Appendix F – Performance test data collection sheet

***AB7's Performance Test***

NAME: …….  Date: 22/9/2023  Condition: YELLOW / RED

HR MONITOR: ……..  BIKE: …….  FAN RESISTANCE: ……..

| WARM-UP | | | |
|---|---|---|---|
| | **2 W/kg target:** | **HEART RATE:** **RPE:** | **Handle Vert:** **Horiz:** |
| | **2.5 W/kg target:** | **HEART RATE:** **RPE:** | **Seat Vert:** **Horiz:** |
| | **3 W/kg target:** | **HEART RATE:** **RPE:** | |

| | **HR** | **RPE** | **Av. Sprint Power** | **Peak Sprint Power** | **Av. 24s Power** | | **HR** | **RPE** | **Av. Sprint Power** | **Peak Sprint Power** | **Av. 24s Power** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sprint 1** | | | | | | **Sprint 7** | | | | | |
| **Sprint 2** | | | | | | **Sprint 8** | | | | | |
| **Sprint 3** | | | | | | **Sprint 9** | | | | | |
| **Sprint 4** | | | | | | **Sprint 10** | | | | | |
| **Sprint 5** | | | | | | **Sprint 11** | | | | | |
| **Sprint 6** | | | | | | **Sprint 12** | | | | | |

| **0:00** WU 2 min | **2:00** WU 2 min | **4:00** WU 30 s | **4:30** Rest 3 90 s | **6:00** Interval 1 | **7:00** INT 2 | **8:00** INT 3 | **9:00** INT 4 | **10:00** INT 5 | **11:00** INT 6 | **12:00** H/T | **14:00** INT 7 | **15:00** INT 8 | **16:00** INT 9 | **17:00** INT 10 | **18:00** INT 11 | **19:00** INT 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2W/kg | 2.5W/kg | 3W/kg | **Prep** | **START** | | | | | **Measure** | 2 min | 2nd Half | | | | | **END** |