Introduction

When competing for the medals at the Olympic Games, World Championships and other competitions at the highest level, it is often hundredths of a second or centimetres, that determine who wins the gold, silver and bronze and who must go home empty-handed. In the intense endurance events lasting ~45 seconds to 8 minutes in the 2016 Olympic Games in Rio, the mean difference in speed between the gold and silver medallist was 0.54 % and 0.42 % between bronze and fourth place (Christensen et al. 2017). Thus, any legal method to gain even small improvements is desired. In 2006, Harris et al. showed for the first time that humans could readily increase skeletal muscle carnosine (β-alanyl-L-histidine) levels by chronically supplementing with β-alanine (3-aminopropanoic acid) over 4 weeks (Harris et al. 2006). Carnosine was, as early as 1938, recognised as an important skeletal muscle buffer (Smith 1938), and, in 1953, carnosine was found to have a possible fatigue delaying effect in vitro, when it was added to contracting frog sartorius muscle at the time of fatigue (Boldyrev 2012). In 1992, it was found that the buffer-capacity of muscle carnosine was no more than approximately 7% in samples from human vastus lateralis muscle (Mannion et al. 1992), and thus not as important as previously suggested. However, as the study by Harris and co-workers in 2006 showed, a more than 70% increase in muscle carnosine after ingesting approximately 143 g of β-alanine over a 4-week period, β-alanine rapidly became a supplement that received considerable attention from athletes and researchers.

The possible mechanism behind a performance-enhancing effect of increased muscle carnosine levels is manifold, and is still not fully understood. Carnosine has been ascribed as having a role in the attenuation of skeletal muscle acidosis by acting as a H⁺ buffer (Parkhouse et al. 1985; Smith 1938). However, only a few studies have measured H⁺ buffering capacity, and, as previously mentioned, Mannion and co-workers found that carnosine only contributed to a limited extent to muscle buffering (Mannion et al. 1992), which questioned the importance of carnosine as a skeletal muscle buffer in humans. Gross and co-workers measured, as the only group, skeletal muscle buffer capacity. After a 38-day period with 3.2 g of β-alanine supplementation daily, no difference in muscle buffer capacity was obtained (Gross, Boesch, et al. 2014). The results have been challenged by other researchers, as the technique used by Mannion and Gross might be underestimating the true contribution of carnosine in muscle buffering (Sale et al. 2013). In in vitro studies, the addition of carnosine was found to improve contractile performance due to increased Ca²⁺ sensitivity, and therefore this has also been
suggested as a possible mechanism explaining performance improvements after β-alanine supplementation (Dutka et al. 2012; Dutka & Lamb 2004). This has, however, not been confirmed in the in vivo studies addressing this question (Hannah et al. 2014). Furthermore, carnosine has been shown to act as a scavenger of reactive oxygen species, leading to a potentially greater recovery from strenuous exercise (Boldyrev et al. 2013; Slowinska-Lisowska et al. 2014; Belviranli et al. 2016).

Sale (2011) was the first to investigate the effect of co-ingestion of sodium bicarbonate and β-alanine on high-intensity exercise capacity (Sale et al. 2011). Sodium bicarbonate ingestion leads to pre-exercise alkalosis in the circulation, and thus an increased extracellular buffer capacity and a possible increase in H+ efflux from the muscle. This may then reduce intramuscular pH and might also reduce stimulation of group III and IV muscle afferents that are central in cardiovascular and ventilator reflex responses, and facilitate central fatigue. The reduction in the facilitation of central fatigue results in a attenuation of the drop in central motor output that follows central fatigue (Amann et al. 2015; Siegler et al. 2016). The possible increased skeletal muscle buffer capacity, reduction in reactive oxygen species (ROS) production, and increased Ca²⁺ sensitivity and handling, have been suggested as possible effects of increased muscle carnosine after prolonged β-alanine (BA) supplementation (Derave 2013).

The first meta-analysis on the effect of β-alanine supplementation on exercise performance was published in 2012. This showed that there was no effect of β-alanine supplementation on exercise performance, but, on test to exhaustion, referred to as exercise capacity, this effect was most significant when the test to exhaustion lasted between 60 and 240 seconds (Hobson et al. 2012). The meta-analysis also revealed that the majority of studies are performed in sedentary or recreationally active young men, and not in elite-level athletes. This is also the case in a recent meta-analysis (Saunders et al. 2017). The importance of using elite athletes when studying the effect of a possible ergogenic supplement has been previously demonstrated. In a meta-analysis from 2012, it was found that the overall effect size on performance for supplementing with sodium bicarbonate in trained individuals was low to moderate on 0.13 compared to a moderate effect size on 0.36 for untrained ones (Peart et al. 2012). Moreover, other studies where supplementation has been used as, for instance, nitrate/beetroot juice supplementation, have shown it to be more effective in less trained subjects (Porcelli et al. 2015; Christensen et al. 2013). Therefore, one aim of the present thesis was to gain a deeper insight into the effect of β-alanine supplementation on exercise performance and capacity in elite athletes. A challenge in
the existing literature when this thesis was initiated was the lack of knowledge about mechanisms’ underlying performance and capacity improvements after prolonged supplementation with β-alanine. Most studies only measured performance and a few blood parameters. A few studies included muscle carnosine measurement using the biopsy technique, and other studies used Magnetic Resonance (MR) scan technique. However, studies investigating the effect of β-alanine supplementation in elite athletes and at the same time measuring physiological parameters related to the effect of β-alanine supplementation were limited. It was then, and still is, relevant to look into the effects after prolonged β-alanine supplementation on muscle pH, muscle buffer capacity, muscle lactate concentration, muscle contractile properties that could relate to changes in Ca\(^{2+}\) sensitivity and handling and changes in enzymes and proteins involved in the transport, synthesis, deamination and degradation of carnosine and β-alanine. It is most valuable if studies are carried out in athletes, as it is known that, for example, muscle buffer capacity is increased after a training period with high-intensity training (Gross, Boesch, et al. 2014).

Therefore, the purpose of the present thesis’ work was to obtain an in-depth insight into physiological mechanisms underlying the possible ergogenic effects of prolonged β-alanine supplementation and the potential additive or synergistic effect of supplementing with both β-alanine and sodium bicarbonate on exercise performance and capacity in elite- and well-trained athletes. In addition, the aim was to study the effect of increased muscle carnosine on muscle excitability and contraction in fatigue development.

The questions were:
Did β-alanine supplementation have an effect on exercise performance in elite athletes, and, more interestingly, would an increased muscle carnosine content lead to increased muscle buffer capacity and decrease muscle pH drop in well-trained athletes? Would a potential increase in muscle buffer capacity translate into a lesser decrement in muscle pH after a 6-minute work bout on a fixed work load? And was this followed by a greater exercise capacity in a time to exhaustion test? Additionally, did a co-ingestion of β-alanine and sodium bicarbonate give an additive or perhaps synergistic effect on exercise capacity? And did the co-supplementation influence the muscle pH during the 6-minute work bout or the time to exhaustion test?
To answer these questions, athletes were included. A combination of continuous, intermittent, isometric high-intensity exercises and submaximal exercise tests were applied. Different methods including surface electromyography, electrical stimulation, and magnetic resonance scan, together with muscle biopsies and blood sampling, were employed.

This thesis comprises:
An overview of the current knowledge of the metabolism of carnosine and β-alanine and the role of carnosine in relation to exercise performance and capacity.

Two papers:
Signe Refsgaard Bech¹, Ian Henry Lambert², Nikolai Nordsborg¹, Erik A. Richter¹, Bente Kiens¹

*Muscle buffer capacity is improved by β-alanine supplementation and exercise capacity is improved by co-ingestion of β-alanine and sodium bicarbonate*

*Manuscript in preparation*

Signe Refsgaard Bech¹, Tobias Schmidt Nielsen¹, Martin Hald¹, Jarl Pors Jakobsen¹, Bente Kiens¹, Nikolai Bastrup Nordsborg¹

*Performance and muscle function is unaffected by β-alanine supplementation in trained kayakers.*

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Furthermore, the overview includes some unpublished data.