THE EFFECTS OF DIFFERENT CUSTOM-MADE MOUTHGUARD DESIGNS ON RETENTION, COMFORT AND PHYSIOLOGICAL PARAMETERS DURING EXERCISE

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Abstract

There are great variations in design of customised mouthguards and a lack of research assessing possibilities to minimise commonly reported comfort-related issues. The aim of the present thesis was to investigate specific custom-made mouthguard designs and their effects on retention, physiological parameters and comfort within participants.

A novel experimental procedure to examine retention of different mouthguards was proposed. Factors such as design, thickness and conditions of the oral environment were incorporated. It was found that using two EVA blanks to fabricate a mouthguard led to a greater retention. Thus, design plays an importance within retention and potential compliance.

The second part of this thesis examined the influence of three mouthguards, which were found to be most retentive following the retention experimental study, on two groups of participants (rugby players and boxers). Whilst performing newly proposed sport-specific exercise protocols with and without mouthguards, their breath-by-breath gas exchange, heart rate and blood lactate were monitored. Wearing custom-made mouthguards did not have an impact on physiological parameters when compared to wearing no mouthguard. Additionally, participants were asked to assess the level of comfort via a questionnaire, which demonstrated the most favoured design in terms of participants' perception.

Finally, the present thesis evaluated the influence of the two most comfortable devices, whilst performing a boxing protocol under moderate altitude conditions, which has not been assessed previously in mouthguard research. The aim was to compare the effects of mouthguards on physiological parameters under normal (normoxic) and altitude (hypoxic) conditions. The findings of this research reported that custom-made mouthguards did not have any negative influence on the examined parameters, regardless of the testing conditions (e.g. controlled laboratory or simulated moderate altitude).

Publications and Conference Papers Derived from this Thesis

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Overview of Thesis

Chapter 1 summarises the importance of wearing sports mouthguards in decreasing the incidence of injury prevention. It also highlights the characteristics of different mouthguard types, with emphasis on the benefits of using custom-made devices. This chapter is a review of previous published literature that has examined participants' issues with wearing mouth protection and the effects of mouthguards on physiological parameters during exercise and performance. Finally, it outlines the gaps in the field of mouthguard research and the main reasons for the current thesis.

Chapter 2 highlights the importance of mouthguard retention, which often relates to higher comfort. It examines the ability of six different custom-made mouthguard designs to stay in position whilst displacement forces are applied. The aim was to propose a new experimental procedure that would accurately assess the influence of variation in design and thickness of mouthguards on retention. The findings suggested that dental technicians should use specific fabricating techniques in order to limit the dislodgement of custom devices during usage in dynamic sports. For instance, they should ensure the thickness of mouthguards is more than 2 mm, especially in the region over the front teeth.

Chapter 3 includes a study that investigated the influence of three custom mouthguards, which demonstrated high level of retention, on physiological parameters and comfort during exercise. This chapter examined male rugby players, who performed a newly designed sport-specific exercise protocol, reflecting the intensity of a rugby game, without a mouthguard and with the three chosen mouthguard designs. The findings demonstrated that wearing any of the mouthguards did not interfere with participants' oxygen uptake, minute ventilation, heart rate and build-up of blood lactate. These parameters are essential in measuring aerobic and anaerobic metabolic performance and the results showed that breathing was not negatively influenced by the use of custom devices. Finally, the participants rated a mouthguard with thicker posterior region and no palatal extension as most comfortable.

Chapter 4 presents a study that followed the principles described in Chapter 3. However, the participants recruited were male boxers who performed a sport-specific boxing exercise protocol. Similarly, the findings showed that using any of the three selected mouthguards did not have an impact on respiratory flow parameters and blood lactate compared to wearing no device. However, it is recommended that boxers remove their mouthguards between the boxing rounds in order to consume more oxygen. In contrast to the rugby players, the boxing participants preferred a mouthguard device that extends 4 mm in the palate and has an even thickness.

Chapter 5 includes a follow-up study where boxing participants performed with two mouthguards and without a mouthguard in an environmental chamber, under simulated altitude conditions. Nowadays, it is likely to compete at a location where the oxygen concentration in the air is reduced or include simulated altitude conditions into conditioning training. The findings of this study demonstrated that it is essential for athletes to use their mouthguards during such training as the initial increase in respiratory flow parameters and accumulation of blood lactate due to higher altitude conditions was decreased. This beneficial effect of wearing a customised mouthguard should be considered by coaches and athletes in order to increase the use of mouth protection.

Chapter 6 highlights the novel findings and contributions to knowledge derived from the current research. Additionally, it outlines the strengths of the proposed experimental procedures and methodologies, which enhance the published literature. Finally, it recommends further research work within the area of mouthguards, which would lead to an increase in mouthguard usage and hence reduction in dental trauma.

Chapter 1 : A Review of Mouthguard Use in Sport

1.1 Prevalence and Prevention of Dental Injuries in Sport

In the past two decades, the need of sports dentistry practitioners has increased due to a greater popularity of high and medium-risk sports that often lead to orofacial injuries (Brionnet et al., 2001; Ranalli, 2002; Deogade et al., 2016). Sports injuries contribute to nearly a third of all dental injuries, 80% of which affect the upper incisors (ADA, 2006). Sustaining a sport-related dental trauma is six times more likely to occur during body collisions and falls compared to work-related accidents (Kumamoto and Maeda, 2004; Galic et al., 2018). Recent studies have reported that lacerations, tooth loss, fracture, subluxation and intrusion were most prevalent (Gould et al., 2016; Piccininni et al., 2017; Galic et al., 2018). The frequency and extent of oral injuries depends mainly on the type of sport and its popularity. In high-risk contact sports, facial or dental trauma is likely to occur whilst playing or interacting with opponents (Kumamoto and Maeda, 2004; Fernandes et al., 2018). For instance, Vidovic-Stesevic et al. (2015) reported that 50.7% of 420 karate participants (240 males and 180 females; mean age 19.5 yrs) from 43 European countries experienced facial trauma and 44 of those fighters had previously experienced a dental injury. Aman et al. (2018) similarly found that 20% of injuries in male ice-hockey were dental. However, medium-risk sports such as basketball and handball have also demonstrated a high percentage of dental trauma, 16.6% and 10.7% respectively (Lang et al., 2002; Perunski et al., 2005; Cohenca et al., 2007). An audit of national sports insurance data (2006-2015) found that although handball was classed as medium-risk sport, the number of facial and dental injuries in females (19 ± 6 yrs) was higher than in ice-hockey where mouth protection was required (facial N = 324 vs N = 27 and dental N = 308 vs N = 78) (Aman et al., 2018). Likewise, there were significantly more injuries in participants (N = 229; 12.9 ± 3.2 yrs of age; 4.8 ± 3.1 yrs of training) playing handball (21.8%) and water polo (18.6%) than in high-risk martial art sport such as taekwondo (3.5%) (Galic et al., 2018). It has been shown that the use of mouthguards has led to a reduction in the number of orofacial injuries (Kumamoto and Maeda, 2004; Tiwari et al., 2014; Gould et al., 2016; Aman et al., 2018; Galic et al., 2018). In their systematic literature review, Knapik et al. (2007) highlighted that the chance of avoiding such traumas with a mouthguard was found to be 1.6 - 1.9 times higher than without a mouthguard. Cohenca et al. (2007) reported that in high-risk sports such as American football, where the use of mouthguards is compulsory, the incidence rate of dental injuries was five times lower than in other sports such as basketball (rate of 10.6 injuries per 100 athletes in males and rate of 5.0 in females). They also conducted a ten-year retrospective audit (1996 – 2005) and demonstrated that since mouthguards were made mandatory in women's basketball teams, the number of injuries decreased from 8.3 incidence rate per 100 athletes to 2.8. Similarly, since New Zealand introduced mouthguards as compulsory in rugby union at all times (1997/ 1998), a 26.0% increase in their usage and a 43.0% reduction in dental trauma claims were reported over a 8-year period (1995 – 2003) (Quarrie et al., 2005). In addition, it was estimated that non-wearers are 4.6 times more likely to make a dental injury claim.

In the UK, the governing bodies of national level rugby union, hockey and martial arts strongly recommend the use of a mouthguard but it is still not obligatory; except when competing in martial arts at international level (Holmes, 2000; England Hockey, 2016; England Rugby, 2017). In 2017, the English Lacrosse Association introduced mouth protection as compulsory (ELA, 2017). Boxing remains the only sport that requires a mouthguard at all times (England Boxing, 2018). Additionally, schools should have protective equipment policies in place for all children participating in contact sports (Parker et al., 2017). The regulations for mouthguard usage vary not only between sports and levels, but also between countries. In the USA, mouthguards are mandatory for boxing, American football, men's lacrosse, ice hockey and women's field hockey (Liew et al., 2014). The ADA and the National Academy for Sports Dentistry recommend well-fitted mouthguards for 29 sports (Table 1-1) (FDI, 1990; ADA, 2004).

Table 1-1: Sports where mouthguard use is recommended (ADA, 2004).

Acrobatics	Field events	Martial Arts	Softball
Basketball	Field hockey	Racquetball	Squash
Bicycling	Football	Rugby	Surfing
Boxing	Gymnastics	Shot-putting	Volleyball
Equestrian events	Handball	Skateboarding	Water polo
Extreme sports	Ice hockey	Skiing	Weightlifting
	Inline skating	Skydiving	Wrestling
	Lacrosse	Soccer	

There is still a great variation in guidelines, regulations and club policies regarding the use of mouthguards within rugby union. Broad and Welbury (2015) evaluated Scottish junior rugby union club policies and reported that 77.7% of the clubs had a mouthguard policy. They found that 91.0% (*N* = 137) of clubs recommended the use of mouthguards during training and 96.0% (*N* = 145) during competitions. However, only 11.0% (*N* = 17) of them did not allow a player to participate in training without wearing a device and 17.0% (*N* = 26) excluded players from match days. Although majority of the clubs (*N* = 118; 78.0%) had a documented policy, only 60 clubs recommended custom-made devices specifically. The authors concluded that in order to decrease the rate of injuries and create a habit of wearing mouth protection since early age, clubs should not allow participants to train and compete without a mouthguard. Although some schools have made mouthguards mandatory, others still only recommend them. Jagger et al. (2010) reported that out of 223 injuries amongst 178 boys playing rugby at school, the most common were dental (25.8%). Similarly, Nicol et al. (2011) found that 87.0% (*N* = 470) of children playing rugby wore mouthguards. However, still almost a third of all recorded injuries were to the head and face. Hence, governing bodies, school policies, coaches and trainers should take more action in the early prevention of such injuries (Jagger et al., 2010; Nicol et al., 2011; Broad and Welbury, 2015; Kroon et al., 2016).

Coaches should take more responsibility in encouraging mouthguard use, especially amongst junior players in order to limit the chance of traumatic dental injuries as well as promote the use of the devices since early age. Whilst there is a greater awareness of mouthguards in developed countries, previous work has suggested that further education and training with emphasis on the prevention of dental injuries is necessary (ADA, 2006; Boffano et al., 2012; Levin and Zadik, 2012; Tiwari et al., 2014; Miller et al., 2016). Although mouthguards are not compulsory in some sports, sporting bodies and dental associations should promote their use in order to prevent long-term traumatic dental problems (Holmes, 2000; Dhillon et al., 2014; Tuna and Ozel, 2014; Parker et al., 2017). Close collaboration between the dental team and schools, clubs, national and international sports teams should be further encouraged (Galic et al., 2018). A study showed that 73.0% out of 196 consultant orthodontists from UK hospital departments routinely advised on the use of mouthguards. However, they are often recommended to buy their mouth protectors from a sports shop or visit a private general dental practitioner (Bussell and Barreto, 2014). In the UK, there are no dental regulatory bodies that provide specific guidelines as to what mouthguard is best for each sport. Consequently, there is great variety of mouthguard types and uncertainty to which ones are ideal in terms of properties.

1.2 Types and Characteristics of Mouthguards

In the 1950's the American Dental Association (ADA) introduced the mouthguard as a resilient protective equipment to protect the dentition and surrounding structures in order to reduce the number of injuries by absorbing external impact energy (Hoffmann et al., 1999; Newsome et al., 2001; ADA, 2006). Mouthguards fall into three major groups:

(i) Stock mouthguards

Stock mouthguards are ready-made devices that are produced in different standard sizes and their shape cannot be personalised. This type of mouthguard is the cheapest and it has minimal protective properties (Patrick et al., 2005). In addition, it often causes significant interference with speech and breathing as it requires the user to apply occlusal pressure and hold it in place (ADA, 2006; Gawlak et al., 2015; Parker et al., 2017).

(ii) 'Boil-and-bite' mouthguards

'Boil-and-bite' devices are readily found in sports shops or available to purchase online. Each manufacturer provides their own instructions, however, generally the mouthguards are placed into hot water to soften and then participants mould or self-adapt the device by biting into it until it hardens (Holmes, 2000). 'Boil-and-bite' mouthguards could be useful for patients with fixed orthodontic treatment as the device could be re-shaped when tooth movement occurs (Parker et al., 2017).

(iii) Custom-made mouthguards

Customised mouthguards are superior to stock and 'boil-and-bite' types in regards to functionality, fit and comfort. They provide better protection, retention and adaptability to individual patient requirements (Newsome et al., 2001; Parker et al., 2017). Dental impressions or digital scans of the dentition and oral soft tissues are taken by a dental clinician, and then sent to a dental laboratory for fabrication (ADA, 2006; Morales et al., 2015). Custom devices are usually fabricated via vacuum-forming or pressure-forming machines. It has been suggested that greater adaptation and less reduction in terms of material thickness could be achieved by using heat and pressure forming rather than vacuum (Newsome et al., 2001). Factors such as tooth misalignment, spaced dentition or missing teeth should be considered during fabrication and the custom-made device designed appropriately for the individual (Gialain et al., 2014; Takeda et al., 2014; Chowdhury et al., 2015).

The most popular mouthguards are maxillary, however, people with a protruding lower jaw may be recommended to wear an additional mandibular mouthguard (Takeda et al., 2014). A mouthguard should be comfortable, resilient, tear-resistant, durable and fit properly without impeding breathing and speech (ADA, 2004). Ethylene vinyl acetate (EVA) is the most common material used for custom-made mouthguards, as it is biocompatible and shock resistant with good tear strength (210 – 565 N/cm) (Knapik et al., 2007; Patrick et al., 2005; Parker et al., 2017). Additionally, EVA is a thermoplastic copolymer with viscoelastic properties, which allows the material to be reshaped by heat and then reformed after cooling (Gould et al., 2016; Dias et al., 2018). Due to the viscoelastic responses of EVA, a mouthguard has the ability to protect the dental structures by absorbing energy and dissipating that into heat (Gould et al., 2016). The chance of dental injury is reduced as the mouthguard distributes the impact force over a larger area. The typical amount of vinyl acetate (VA) is around 18-20%. If it was higher, the material would have less crystallized regions, which could lead to higher water absorption and dimensional changes and that will lower the retention (Dias et al., 2018). Although, EVA is relatively cheap and easy to work with, its rebound resilience is not ideal due to a low glass transition temperature (T_g = - 20°C). The optimal material should have a T_g of around 37°C (Gould et al., 2016). Therefore, further research should investigate whether other materials could have applications in mouthguards.

The levels of mouthguard protection and comfort are two key properties, which depend on the type of mouthguard and the quality of fabrication (Patrick et al., 2005; Gawlak et al., 2015; Guerard et al., 2017). Patrick et al. (2005) proposed a 10-graded scale of protection (0 – having no mouthguard and 10 – ultimate aim). The authors suggested that the highest protection is provided by a customised mouthguard, which meets the following criteria: newly made with improved design, extending beyond the second maxillary molars, thickness of 3 mm labially, 2 mm occlusally and 1-10 mm palatally, mounted on an articulator against a mandibular dental model, made of EVA (18.0% vinyl acetate) and passed an effective instrumented test for quality. According to Parker et al. (2017), the thickness of the device should be optimal, so the mouthguard does not feel too bulky causing discomfort and simultaneously not too thin, sacrificing the protective properties. Westerman et al. (2002) used a pendulum impact test to demonstrate the transmitted forces through EVA mouthguard material with thicknesses of 2 mm (15.70 kN), 3 mm (11.40 kN) and 4 mm EVA (4.38 kN). They reported that after 4 mm there was a very little difference in impact absorption and that increasing the thickness to 5 mm or 6 mm could potentially interfere with comfort without being beneficial in terms of energy absorption. These findings were supported by Maeda et al. (2008) who conducted a similar pendulum impact test and also examined the transmitted force in samples of 1-6 mm EVA. They reported force absorption of 50.1% (1 mm), 76.9% (2 mm), 85.6% (3 mm), 88.2% (4 mm), 88.5% (5 mm) and 89.6% (6 mm).

‘Boil-and-bite’ mouthguards are self-adapted around the dental structures and it is often difficult to achieve sufficient and even thickness (Patrick et al., 2005; Gawlak et al., 2015; Guerard et al., 2017; Lee et al., 2013). Hence, the fit and the protective properties could be jeopardized by thinning within areas where impact could occur, which could increase the risk of injury. To minimise the risk of trauma, customised mouthguards should be the first option as they have a superior fit and sufficient thickness for higher protection compared to ‘boil-and-bite’ mouthguards (ADA, 2006; Newsome et al., 2001; Guerard et al., 2017; Tribst et al., 2018). However, not many participants are aware of the advantages and disadvantages of each type of mouthguards and frequently they are not able to choose which device would provide superior functionality (Levin and Zadik, 2012; Gawlak et al., 2014).

In addition, currently there is a lack of standard guidelines or regulations proposing the optimal characteristics/ dimensional parameters of custom devices and therefore numerous variations of designs exist. The design features of a mouthguard often define whether a participant would wear it or not (e.g. too bulky or stimulates a gag reflex). Although certain parameters in terms of thickness and design are required for sufficient protection, it is important to maximise participants’ comfort to improve compliance (Tuna and Ozel, 2014). Yamanaka et al. (2002) used a modal analysis system over the maxillary dentition of a dry skull to examine four custom mouthguards. The distal extension of the devices vary up to the second premolar, first molar, second molar and third molar. The authors proposed that mouthguards should cover at least the second molar in order to disperse impact forces and prevent potential dental injury. By contrast, in the report from the First International Sports Dentistry Workshop (2016) it was agreed that mouthguards should extend up to the first molar (Lloyd et al., 2017). The extension of the palatal flange has also been investigated as to whether it could relate to level of protection, retainability and interference with comfort, communication and breathing (Maeda et al., 2006; Yamada, 2006; Maeda et al., 2009; Gebauer et al., 2011; Nozaki et al., 2013; Gomez-Gimeno et al., 2019; Otani et al., 2018). According to the British Orthodontic Society, the palatal flange should not extend more than 10 mm from the gingival margin and the labial flange should be within 2 mm of the vestibular reflection (BOS, 2012). In terms of retention, Maeda et al. (2009) conducted a pull test study and found that it was more difficult to displace a vacuum-formed mouthguard with a 4 mm palatal extension (Figure 1-1) than a mouthguard without palatal flange (with a dry dental cast – 116 ± 27 gf vs 86 ± 15 gf and with a wet dental cast – 58 ± 17 gf vs 36 ± 7 gf, respectively; 1 gf = 0.00981 N).

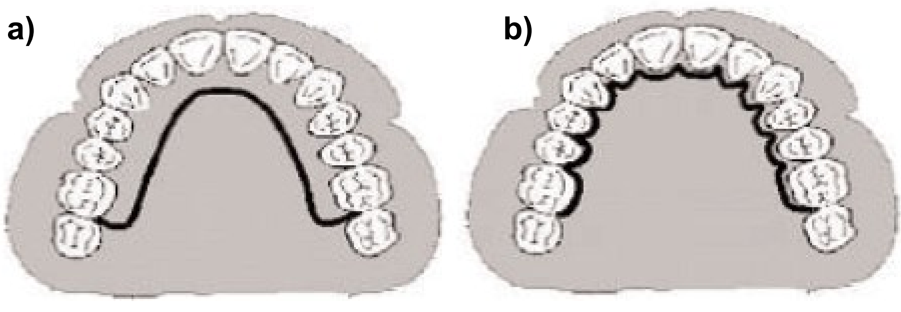

Figure 1-1: (a) Mouthguard design with 4 mm palatal extension and (b) Design with no palatal extension; both mouthguard designs have a full labial extension (adapted from Maeda et al. (2009)).

Currently, there is a lack of literature addressing the retention of mouthguards, which has been found to relate to comfort (Maeda et al., 2009). Maeda et al.

(2006) reported that trimming the palatal flange up to the cervical margin could lead to significant improvements in comfort without influencing participants' perceived level of retention. Additionally, Gebauer et al. (2011) identified that a device made from an EVA blank (4mm) with 4 mm palatal extension was rated less comfortable than a mouthguard without palatal extension (N = 27, 23.5 ± 3.8 yrs). Gomez-Gimeno et al. (2019) also demonstrated a similar outcome with a cohort of elite water polo players (23.7 yrs) who preferred the use of a mouthguard with shorter palatal extension, 2 mm from the cervical line, compared to the conventional 6 mm. The players expressed significantly less issues with communication, breathing, swallowing and athletic performance without affecting the perception of protection. Therefore, future studies should consider the importance of mouthguard design and its role to limit the level of discomfort, speech impedances and airway obstruction (Brionnet et al., 2001; Duarte-Pereira et al., 2008; Maeda et al., 2009; Gebauer et al., 2011; Nozaki et al., 2013; Gomez-Gimeno et al., 2019). Sports participants are more likely to wear a mouthguard, if the device meets their individual preferences in relation not only to protection but also to shape, colour, comfort and minimal interferences with communication and breathing.

1.3 Awareness and Attitudes towards Mouthguards

Despite the rules and regulations in some sports, many players are still reluctant to wear mouthguards because of poor fit or lack of awareness as to which types are best (Patrick et al., 2005; Boffano et al., 2012; O'Malley et al., 2012; Emerich and Nadolska-Gazda, 2013). Parker et al. (2016) conducted an audit at the Eastman Dental Hospital and stated that the main reason for mouthguards not to be used during contact sport is due to participants being unaware of the need. Similarly, Galic et al. (2018) reported that 37.0% of their participants (N = 229; 12.9 ± 3.2 yrs) considered wearing a mouthguard unnecessary. Studies have also highlighted that over the years a large proportion of athletes or recreational participants in sport mainly use 'boil-and-bite' mouthguards due to easy accessibility and low cost compared to custom-made devices (Holmes, 2000; Boffano et al., 2012; Bussell and Barreto, 2014; Dhillon et al., 2014; Ilia et al., 2014; Kroon et al., 2016).

There is also an underlying belief amongst some sports participants that wearing a mouthguard causes discomfort (Gebauer et al., 2011; Lee et al., 2013). This is possibly due to the large popularity of 'over-the-counter' devices, which usually have poorer fit and low retention that causes participants to apply occlusal forces to prevent dislodgment (Lee et al., 2013; Liew et al., 2014; Parker et al., 2017). Boffano et al. (2012) indicated that the superior function, comfort and fit of custom mouthguards was considered by only 18.5% of their participants (*N* = 10 out of 54 wore the device; males; 22.15 ± 5.66 yrs), whereas 50.0% used 'boil-and-bite' mouthguards. Likewise, Liew et al. (2014) reported via a self-administrated questionnaire that 21.1% of rugby players within their study (males; 22.73 ± 3.98 yrs) wore 'boil-and-bite' and only 1.8% had customised mouthguards; with the participants overall usage of 31.1% (*N* = 456). They also found that 17.1% expressed general discomfort issues, which was the main reason to discontinue using a mouthguard. Two other studies reported that athletes, who were asked to wear three different 'boil-and-bite' and five custom mouthguards during training over a period of six weeks, preferred the usability characteristics, comfort and protection of any custom device to the 'boil-and-bite' types (Gawlak et al., 2014, 2015). However, custom-made devices have also been rated negatively in terms of comfort. For example, Berry et al. (2005) reported that although 82.0% of their participants (158 ice-hockey players; 20.99 ± 1.67 yrs) wore custom-made mouthguards and a much smaller proportion used 'boil-and-bite' devices (6.3%), 74.7% still agreed that mouthguards felt uncomfortable.

Participants have not only highlighted comfort related issues as one of the main reasons for non-compliance but also breathing and speech problems that may affect performance and communication with team mates (Boffano et al., 2012; Emerich and Nadolska-Gazda, 2013; Lee et al., 2013; Queiroz et al., 2013; Tiwari et al., 2014; Miller et al., 2016). Tiwari et al. (2014) assessed 213 males and 107 females (12-22 yrs of age) and reported that 28.9% of the contact sport athletes did not wear a mouthguard due to their perceived negative influence on performance. Lee et al. (2013) identified that 73.0% of the examined taekwondo participants either ‘agreed’ or ‘strongly agreed’ that breathing was obstructed and 72.0% expressed the same views for communication impedances whilst having a mouthguard. Half of the respondents confirmed that they would increase their compliance if the current issues with the devices were addressed. Queiroz et al. (2013) reported that the perception of impendences is highly dependent on the type of mouthguard used. They examined 25 female footballers who used three main types of device (stock, ‘boil-and-bite’ and custom-made) during physical testing and reported that none of the athletes experienced difficulties in breathing with a custom mouthguard, whereas 64.0% agreed that breathing was obstructed when using the stock and ‘boil-and-bite’ types. This could be explained by the poor fit of stock and ‘boil-an-bite’ devices causing participants to close their mouth in order to keep them in position. Previous studies have attempted to use physical tests to examine the effect of various mouthguards on breathing (Kececi et al., 2005; Bourdin et al., 2006; Duarte-Pereira et al., 2008; Cetin et al., 2009; Gebauer et al., 2011; El-Ashker and El-Ashker, 2015; Morales et al., 2015; Caneppele et al., 2017; Ferreira et al., 2018). However, the published literature has shown variations in terms of investigated mouthguard type and designs, number of participants, participants’ sport and protocols used, which creates difficulties to compare the findings.

1.4 Mouthguard Effects on Physiological Parameters and Performance

Previous work has investigated the changes of respiratory flow in athletes who performed exercise with different types of mouthguard (Table 1-2). The studies presented in Table 1-2 have all examined customised devices and compared their effects on physiological parameters and power output to the use of other types of mouthguards. Although the majority of studies assessed similar aerobic and anaerobic parameters, it could be challenging to compare the findings due to differences in participants’ sport and the performed exercise tests. Nevertheless, some of the published literature has agreed that wearing a custom-made mouthguard has shown no significant interferences with airflow in comparison to having no mouthguard or other device type (e.g. stock or ‘boil-and-bite’) (Kececi et al., 2005; Bourdin et al., 2006; Duarte-Pereira et al., 2008; Cetin et al., 2009; Gebauer et al., 2011; Collares et al., 2014; El-Ashker and El-Ashker, 2015; Piero et al., 2015). For instance, El-Ashker and El-Ashker (2015) reported that when boxing participants were running at 14 km/h over a period of 10 min, the recorded oxygen uptake (VO_2) with a stock mouthguard (40.54 ± 5.68 ml/kg/min) was significantly lower than when a customised device (46.48 ± 3.65 ml/kg/min) or no device (47.37 ± 5.34 ml/kg/min) was used. However, one of the limitations of this study was the lack of sport specificity in the designed physiological protocol (e.g. running on a treadmill rather than boxing). Gebauer et al. (2011) investigated the effects of two customised mouthguards (single 4 mm EVA blank with 4mm palatal extension and without palatal extension) and reported no statistical differences in VO_2, minute ventilation (VE L/min) and VO_2 peak when male athletes (23.5 ± 3.8 yrs) ran on a treadmill with and without the two mouthguards. It was demonstrated that wearing either of the two customised devices has led to an increase in respiratory flow and oxygen consumption. Additionally, the findings demonstrated that change in the palatal extension did not affect gas exchange.

Table 1-2: Summary of the studies examining the effects of mouthguards (MG) on physiological parameters.

Author	MG Type	Participants	Test Protocol
El-Ashker and El-Ashker (2015)	Stock Custom-made (4 mm EVA blank, vacuum formed)	18 elite amateur boxers - males (regional & national level) 19.4 ± 2.01 yrs 3.8 ± 1.77 yrs of training 74.5 ± 5.1 kg mass 1.74 ± 7.9 m height	3 blindly randomized sessions – No MG, Stock MG, Custom-made MG Running on a treadmill: 1st phase - 2 min incremental run at 8 km/h - 12 km/h, 5 min run at 12 km/h, 3 min recovery 2nd phase - 2 min incremental run at 10 km/h - 14 km/h, 5 min run at 14 km/h, 3 min recovery Measurements: HR, VE, VO_2, BF and TV*
Morales et al. (2015)	Custom-made (CleverBite®)	28 males (soccer, field hockey, basketball & handball at regional level) 24.50 ± 3.32 yrs 78.14 ± 8.12 kg mass 181.34 ± 7.4 cm height	Random counterbalance order of sessions 30-sec Wingate Anaerobic test – cycle ergometer & spirometer test Breathing at force & non-force pace at 3 conditions: open mouth without a MG, jaw clenching without a MG and jaw clenching with MG. Measurements: max expiratory vol, mean power (W/kg), peak power (W/kg), time to peak (s), rate to fatigue (W/s) & lactate production 3 min after exercise, rate of perceived exertion
Piero et al. (2015)	Neuromuscular Custom-made	10 amateur road cyclists 34 ± 6 yrs 21 ± 8 yrs of competition	2 randomised testing session with and without MG Incremental ramp exercise until exhaustion on a cycle ergometer; terminated the test when 70 rpm was not maintained

Author	MG Type	Participants	Test Protocol
		70 ± 10 kg mass 178 ± 7 cm height	Measurements: Work rate, cycling economy, HR, VO_2, VCO_2, VE at three points: lactate threshold, respiratory compensation and maximal exertion*
Collares et al. (2014)	Custom-made (3 mm single EVA blank)	40 boys (3 Brazilian clubs of soccer & futsal) 16.20 ± 0.55 yrs	20 m shuttle run test Measurements: total distance covered & VO_{2max} VAS questionnaire – rating issues with breathing, speech, oral dryness, stability (before & after 2 weeks of use)
Queiroz et al. (2013)	Stock 'Boil-and-bite' Custom-made	25 female soccer players (club) 18-22 yrs	12-min Cooper test (VO_{2max} & physical fitness) Shuttle run test with ball Measurements: VO_{2max}, time & distance during shuttle run Questionnaire to assess discomfort, nausea, breathing, speaking, pain, removal, distraction
Duddy et al. (2012)	'Boil-and-bite' Custom-made (3 mm double EVA blanks)	18 college athletes 19-23 yrs	3-stroke max power ergometer test; 1 min ergometer test & 1 600 m run Measurements: max power output (W), power output per min (W/min), time of running Questionnaire to evaluate the overall satisfaction with MGs
Gebauer et al. (2011)	2 Custom-made (4 mm EVA blank, vacuum-formed)	19 hockey and 8 water polo players – males (1st division, state / national level)	3 sessions in randomized counterbalanced order– No MG, MG with 4 mm palatal flange, MG with no palatal flange Running on a treadmill: 1 min at 9 km/h 1st phase – 5 min at 10 km/h

Author	MG Type	Participants	Test Protocol
	1st MG – 4 mm palatal flange 2nd MG – no palatal flange	23.5 ± 3.8 yrs 81.7 ± 8.6 kg mass 1.82 ± 0.08 m height	2nd phase – 5 min at 12 km/h Measurements: HR, VE, VO_2 & VO_2 peak Assessment of attitudes towards MGs & comfort, scale 1 – 10
Cetin et al. (2009)	Custom-made	11 males & 10 females taekwondo athletes 17.0 ± 1.34 yrs 7.07 ± 2.84 yrs of training	2 randomized conditions: with or without MG 20 m sprint time, isokinetic strength tests, jumping tests Measurements: anaerobic power and capacity, isokinetic measurements, handgrip strength, isometric leg strength and back strength
Duarte-Pereira et al. (2008)	'Boil-and-bite' Custom-made (EVA, pressure-formed)	10 rugby players - males 21-23 yrs 84 kg mass 175 cm height Min 3 yrs of training; < 7 h per week of training	3 randomized cross-over sessions – No MG, 'Boil-and-bite' MG, Custom-made MG Test - changes of direction for 5 s, max 10 m short race, aerobic work for 6 s; CMJ & RB Jump for 15 s, using contact platform Measurements: RB jump - height, number of repetition & mean power; CMJ; FEV1, PEF and FVC prior and after test** VAS questionnaire assessing: comfort, adaptability, stability, tiredness, thirst, oral dryness, nausea, speech, breathing, drinking
Bourdin et al. (2006)	'Boil-and-bite' Custom-made (Methylmet acrylate Resin; raised occlusion from canine to	16 rugby, 2 handball and 1 ice hockey players - males 27 ± 4.8 yrs 91.4 ± 18.6 kg mass 180 ± 8.7 cm height	3 randomized sessions – No MG, 'Boil-and-bite' MG, Custom-made MG Cycle ergometer test: 5 min warm-up; 3 max cycling sprints for 6 s, 4 min rest between each (frictional loads – 0.25, 0.50 and 0.75N/kg body mass) 20 min rest

Author	MG Type	Participants	Test Protocol
	second molar; 2.0 ± 0.5 mm anterior gap)		VO_{2max} test- incremental cycling (start at 100-150 W & increase with 35 W every 4 min). Measurements: FVC test, FIV1, FEV1, PIF and PEF; *f* , P_{max}, V_{opt} & F_{opt}; Expired gas collected at the last 30 seconds of each stage – VO_2 & VE; HR, ECG, Bla post-exercise, RPE on a Borg's scale***
Kececi et al. (2005)	Custom-made (3.8 mm EVA, pressure-formed)	22 elite taekwondo athletes - 11 males & 11 females (junior national team) 16 ± 1.11 yrs 6.77 ± 2.53 yrs of training; 9-10 h per week of training	2 sessions – No MG and Custom-made MG 20 m shuttle run test – run back and forth, starting speed 8.5 km/h, sound signal frequency increased 0.5 km/h after each minute. Measurements with portable gas analysis: HR, VO_2, VCO_2, VE, TV and RER****

*HR - heart rate (bpm), VE – minute ventilation (L/min), VO_2 – oxygen uptake (ml/kg/min), BF – breathing frequency, TV – tidal volume (L), VCO_2 – carbon dioxide production (L/min); **FEV 1 - forced expiratory air volume at 1 s, PEF - expiratory flow rates peak, FVC - forced vital capacity, RB jump - rebound jump, CMJ - counter-movement jump; VAS – Visual Analogue Scal;. ***FIV 1- forced inspiratory air volume at 1 s, PIF – inspiratory flow rates peak, P_{max} – maximum power (W), power-velocity relationship, V_{opt} – optimal pedalling rate at which P_{max} occurred, F_{opt} – optimal force at which P_{max} occurred, *f* – respiratory frequency, ECG – electrocardiograph, RPE – rating of perceived exertion; ****RER – respiratory exchange ratio.

Some studies have also proposed that wearing a mouthguard could be beneficial for performance (Cetin et al., 2009; Duddy et al., 2012; Queiroz et al., 2013; Garner and McDivitt, 2015; Morales et al., 2015; Piero et al., 2015). Morales et al. (2015) examined 28 physically active males (24.50 ± 3.32 yrs) who performed a maximum effort 30-sec Wingate Anaerobic Test with a custom-made mouthguard and without a mouthguard. The authors found significant improvements in mean power, peak power, time to peak and rate to fatigue whilst the mouthguard was worn during exercises. In addition, positive effects on post-exercise blood lactate accumulation (BLa mmol/L) was reported when a mouthguard was used (11.01 mmol/L, SEM = 0.35 with mouthguard and 11.91 mmol/L, SEM = 0.34 without mouthguard). Similarly, Piero et al. (2015) proposed that the use of a neuromuscular customised mouthguard in amateur cyclists could lead to significant increase of work rate, 4.0% at maximum effort and 7.0% at respiratory compensation point, and no effect on respiratory parameters. Garner and McDivitt (2015) also highlighted that BLa significantly improved by 23.0%. The authors investigated 21 physically active males and 3 females (20.84 ± 1.62 yrs) that were provided with a 'boil-and-bite' mouthguard. The participants performed a 30-min treadmill exercise and BLa concentration was 4.01 mmol/L whilst wearing a mouthguard, whereas a significantly higher BLa of 4.92 mmol/L was recorded without a mouthguard. Lower BLa was explained by improvements in airflow due to wearing a repositioning mouthguard that brings the mandible forward. Hence, the reported improvements in respiratory rates. A decrease in BLa could be a motivation for players to wear the device as it reduces the level of muscle fatigue.

However, none of the studies have assessed how much of a change to the physiological variables is meaningful to the athletes during sport and whether changes in one or more variables are associated with performance enhancement; which may differ between different levels of ability.

Although previous literature has used valid exercise protocols to examine any changes in physiological parameters and power output, the majority of studies used either treadmill or cycle ergometer and standard exercise protocols rather than addressing the specific demands of the participants' sports. This issue will be further addressed in Chapters 3 and 4 of this thesis.

1.5 The Effect of Environmental Changes on Physiological Performance

Often athletes compete in locations where the altitude and environmental conditions such as temperature and humidity differ to where they train. It is known that at high altitudes the air pressure is reduced (hypoxia) and this may influence any aerobic performance and activities (Vogt and Hoppeler, 2010). Nassis (2013) analysed the performance of 109 teams playing at 2010 FIFA World Cup in South Africa. The author reported that there was a 3.1% decline in the total distance covered by players during the games at 1200-1400 m and 1401-1753 m altitude compared with sea level. Since the 1968 Olympics in Mexico City (altitude 2300 m; oxygen concentration 16.15%), where impaired performances of male and female middle-distance runners and swimming athletes were recorded, training at hypoxic environment has become more popular (Péronnet et al., 1991). For example, the Cuban boxing team, which is a leader in the World Amateur Boxing Championships, has used this technique and trained under natural altitude conditions in Quito, Ecuador (altitude 2850 m) (BoxingScience, 2008). Acclimatisation causes the body to secrete more erythropoietin that stimulates the production of red blood cells. Hence, oxygen transportation and utilisation could improve due to greater availability and benefit athlete's performance (Bunn, 2013; McArdle et al., 2015). However, it is not always possible to train under natural conditions, so training at simulated altitudes is another strategy for acclimation (Billaut and Aughey, 2013). Galvin et al. (2013) examined hypoxic training of academy rugby union and rugby league players (*N* = 30, 18.4 ± 1.5 yrs, males). Following a 4-week training programme with a portable hypoxic generator (13.0% oxygen concentration), participants' VO_2 during exercise increased by approximately 7.0% compared to VO_2 recorded post-training at normal environment (-0.3%). Previous work, including literature reviews and laboratory experiments, have evaluated the importance of athletes' conditioning prior to competing at moderate/ high altitudes (Gore et al., 2008; Hamlin et al., 2008; Billaut and Aughey, 2013; Singh et al., 2014). However, to the authors' knowledge of this thesis, no information that addresses the additional impact of wearing a mouthguard during such conditions has been published. Due to previous research highlighting that mouthguards can affect respiratory parameters and muscle fatigue, coaches should also consider recommending the use of protective equipment during exercise conditioning at hypoxic conditions. This area will be evaluated in Chapter 5 of this thesis where participants will complete a sport-specific physical protocol under both normal and simulated moderate altitude conditions.

1.6 Conclusions

The main purpose of wearing a mouthguard is to protect the dental and surrounding oral structures. Previous literature has identified that custom-made devices provide superior fit, protection and retention compared to other commercially available types. However, players are frequently reluctant to use mouth protection due to issues with comfort and perceived interferences with breathing and communication. There is a lack of standardised guidelines to identify the optimal features of a customised mouthguard in terms of design, material type, thickness, protection and retention. Consequently, this leads to variations in mouthguard designs, which are fabricated most frequently according to the technicians' personal experience. Although quite extensive research has investigated thickness of different devices, this has mainly been associated with transmitting impact forces. Chapter 2 of this thesis will propose a novel technique to examine the retention capabilities of various custom-made mouthguards as well as the correlation between level of retention and thickness. The following Chapters 3 and 4 will address the influence of customised devices on respiratory flow, oxygen uptake, blood lactate and comfort whilst two groups of participants perform sport-specific exercise protocols. Furthermore, a novel aspect of the current research will evaluate the same physiological measurements when participants perform the same exercise with and without mouthguards under simulated altitude conditions

(Chapter 5). This will examine whether using a custom-made mouthguard whilst training at moderate altitudes could affect physiological parameters.

The following chapter of this thesis will propose a new method to measure retention of mouthguards, which could influence design and users' compliance. Additionally, it will investigate changes in the level of retention due to alterations in design and thickness of selected custom-made devices.

Chapter 2 : The Effects of Custom-Made Mouthguard Design on Retention

2.1 Introduction

Chapter one of this thesis has already highlighted the importance of wearing sports mouthguards in order to reduce the incidence rates of dental trauma, especially within contact sports such as boxing, martial arts, rugby and hockey (Muller-Bolla et al., 2003; Hendrick et al., 2008; Schildknecht et al., 2012; Emerich and Nadolska-Gazda, 2013; Al-Arfaj et al., 2016; Vucic et al., 2016). However, participants in high-risk sport activities frequently refuse to wear mouth protection due to experience of comfort issues and impedances with communication and breathing (Boffano et al., 2012; Emerich and Nadolska-Gazda, 2013; Lee et al., 2013; Dhillon et al., 2014; Miller et al., 2016). As mentioned previously, cost is a defining factor in the choice of mouthguard type and therefore 'over-the-counter' devices have been reported to be the most popular type used. These commercially provided mouthguards often lack of precise fit and have lower levels of retention compared to custom-made devices, especially if the participant does not self-adapt the device correctly (Lee et al., 2013).

Retention of mouthguards relates to the ability of the device to stay in position during dynamic sports and it is one of the key factors to improve mouthguard usage. The level of retention may also vary within different custom-made mouthguards due to variations in fabricating techniques and materials used. Currently, there is not enough supporting evidence identifying the characteristics of customised devices that lead to improvements in retention. Lee et al. (2013) reported that about a quarter of a cohort of taekwondo players identified poor retention as a reason for not wearing any mouth protection. In addition, distraction and interruption of the game due to a loose mouthguard is common and it could potentially be reduced by providing well-fitted and retentive devices. In order to improve players' expectations by limiting discomfort, manufacturers should consider fabricating a mouthguard design that would stay in position during sport activities. Higher mouthguard retention could increase the athletes' motivation to wear the device as it could lead to improvements in physical comfort and less interference during performance (Lee et al., 2013).

Thus far, suggested improvements to increase comfort by altering the mouthguard design have been related to reducing the palatal flange up to the gingival margin (Maeda et al., 2006; Gebauer et al., 2011). However, it could be argued that such alterations may affect the retention of the device. Only two notable studies have examined mouthguard retention, both of which have conducted a pull test to examine the displacement of different custom-made devices (Del Rossi et al., 2008; Maeda et al., 2009). Del Rossi et al. (2008) investigated the effect of mouthguard colour on fit and adaptation by attaching a strain gauge to the palatal aspect of the central incisors to record the force required to remove the mouthguards from the model. Significantly more force was required to remove blue, black and green coloured mouthguards than a clear guard due to pigmentation affecting thermal properties during the fabrication process. Maeda et al. (2009) examined the accuracy of fit using a chain mechanism that was attached to the first upper left molar. The authors fabricated three different custom-made mouthguard designs; all made of 3.5 mm clear ethylene vinyl acetate (EVA) blanks. One of the designs had a 4 mm palatal extension, whereas another one was finished at the gingival margins, and the third had an extended buccal outline. No statistical difference between the retention forces of the three mouthguards was found. However, it was demonstrated that pressure-formed mouthguards over well-dried casts showed better fit and retention than those that were vacuum-formed on dry (133 ± 31 gf > 116 ± 27 gf) and wet model casts (133 ± 31 gf > 58 ± 17 gf). Although Maeda et al. (2009) assessed retention of customised devices, the authors outlined certain limitations of their retention test procedures. For instance, thickness and characteristics of the mouthguard blank as well as the size and shape of the dental arch and the teeth were not considered. Additionally, the consistency of saliva should have been taken into account.

Finally, the experimental method investigated retention levels at only one region of the mouthguard.

The aim of the present study was to examine the retentive properties of selected customised mouthguards in relation to design on a dry model and in the presence of artificial saliva to mimic the oral environment. This study will also investigate the correlation between mouthguard thickness and retention to propose further considerations on how to improve potential comfort factors when fabricating custom mouthguards. It is hypothesised that the retention of custom-made mouthguards could be altered by changing the design and thickness of the device. In addition, testing with the presence of artificial saliva may result in recording higher mouthguard retention compared to testing on a dry model due to higher cohesive forces.

2.2 Methods

2.2.1 Mouthguard Fabrication

Prior to any experimental data collection ethical approval was obtained from the School of Healthcare Science, Manchester Metropolitan University (Ethics Number: SE151657C).

A fully dentate maxillary anatomical teaching model was fabricated from a polyurethane polymer Nano – Rock liquid die stone (WHW, Hull, UK), which has high polarity causing the polymer surface to be hydrophilic. This leads to surface features similar to the oral soft and hard tissues. The model had arch dimensions of 32 mm length, 36.5 mm inter-canine width and 50.4 mm inter-molar width; similar to the mean arch dimensions of a cohort with normal occlusion (Uysal et al., 2005). Six different custom-made mouthguard designs were thermoformed following standard technical procedures as described by Padilla (2005) (Table 2-1). All mouthguards had a full labial extension. Mouthguard designs MG1, MG2, MG4 and MG5 were fabricated following previously published studies examining the effects of the devices on comfort and performance (Garner et al., 2011; Gebauer et al., 2011; Gage et al., 2015); with MG1 being commonly used in dental laboratories. Design MG3 has also been used in some dental practices and MG6 was reproduced from Takeda et al. (2014) for a rugby player with mal-alignment.

All mouthguards were pressure – formed on a Drufomat – Te machine (Dreve Dentamid GmbH, Germany) with round, clear 2 mm, 4 mm and 5 mm EVA blanks, 120 mm Ø (diameter) (Bracon Dental Laboratory Products, East Sussex, UK). In order to minimise the thinning of the EVA blanks during thermoforming, the blanks were pressure-formed onto a dry model embedded into metal pellets.

Table 2-1: Types of mouthguard designs and material dimensions.

MG Design	Weight (g)	Layers of EVA	Thickness of EVA (mm)	Palatal Flange*
MG1 Control	8.7 g	Single	5 mm	5 mm 
MG2 No Palatal Flange	6.3 g	Single	4 mm	0 – 1 mm 
MG3 Thicker Anterior Region	9 g	Double Anterior Region Single Posterior Region	2 mm 1st layer 4 mm 2nd layer	0 – 1 mm 
MG4 Thicker Posterior Region	8 g	Single Anterior Region Double Occlusal Surface	2 mm 1st layer 4 mm 2nd layer	0 – 1 mm 
MG5 No Palatal Coverage Anteriorly	6.3 g	Single	4 mm	0 mm 
MG6 Thicker Anterior & Posterior Regions	8.7 g	Double Anterior Region & Occlusal Surface	2 mm 1st layer 4 mm 2nd layer	0 – 1 mm 

*Palatal flange refers to the outline of a mouthguard extending over the palatal mucosa adjacent to the upper dentition.

2.2.2 Mouthguard Thickness Measurements

Seven anatomical points, both anterior and posterior, were selected on each mouthguard to obtain dimensional thickness (Figure 2-1a-b). The position of these points (excluding Point iii) were similar to those published previously by Farrington et al. (2016) who investigated thickness in relation to fabrication technique. Each point was measured ten times using an electronic caliper gauge, resolution range ± 0.01 mm (External Digital Caliper 442-01DC Series, Moore and Wright, UK) for consistency and the mean (± SD) was recorded. The gauge was zeroed after each measurement for calibration. The thickness of the anterior region equated to the mean value of points (i) - (iii) and the thickness of the posterior region equated to the mean value of points (iv) - (vii) (Figure 2-1 a-b; Table 2-2). Overall mouthguard thickness was obtained from the mean of all points (i-vii) (Table 2-2).

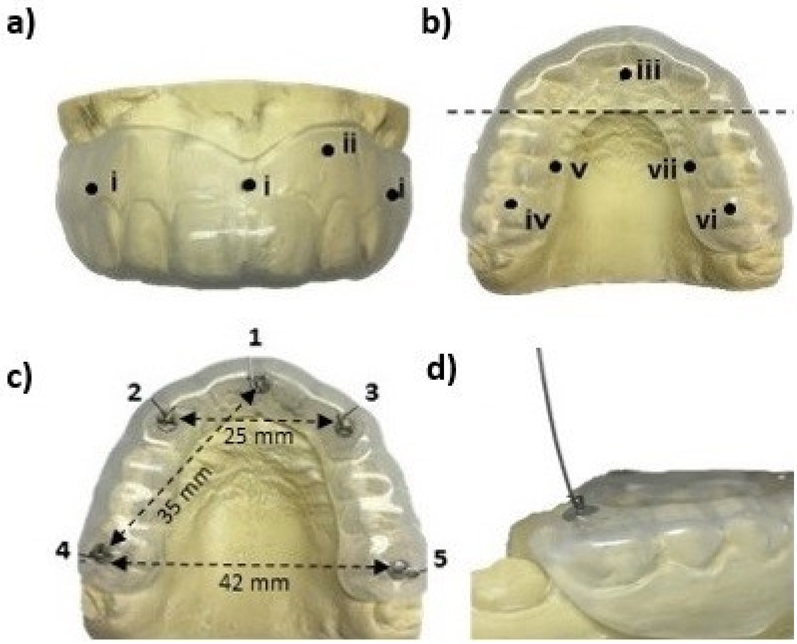

Figure 2-1: Thickness measurements at seven anatomical points in (a) the anterior region (i-iii) and (b) posterior region (iv-vii). Part (c) sites 1 – 5 show the location of the orthodontic brackets on a maxillary mouthguard: (1) palatally at the interdental space between the two central incisors (2-3) palatally at the central axis of the right and the left canine (4-5) occlusally at the centre of the first right and left molar. Part (d) illustrates attached orthodontic stainless steel wire to a bracket at the region of the left molar.

Table 2-2: Description of the points where mouthguard thickness was measured.

Point	Description
i	Anterior Labial Aspect, perpendicular to the occlusal plane, at the gingival margin, located at: the interdental space between the two central incisors; the central axis of the right canine and the left canine.
ii	Labial Sulcus, at a point 5 mm, perpendicular to the occlusal plane, in line with the gingival margin, located at the interdental space between the central and lateral incisors.
iii	Anterior Palatal Aspect, perpendicular to the occlusal plane, at the gingival margin, located at the interdental space between the two central incisors.
Total anterior region (1) – equates to the mean values of points i, ii & iii	
iv & vi	First Right (iv) and Left (vi) Molar, at the apex of the mesio – palatal cusp.
v & vii	Posterior Palatal Aspect, perpendicular to the occlusal plane, at the gingival margin, located at the interdental space between the first and second premolars.
Total posterior region (2) – equates to the mean values of points iv, v, vi & vii	

2.2.3 Mouthguard Retention Measurements

Retention was measured at different regions of the mouthguards using a Hounsfield H10KS Tensometer fitted with a 1 kN load cell (Hounsfield Test Equipment Ltd., Surrey, UK). The H10KS was controlled with QMat Professional Material Testing Software. Orthodontic brackets (Cat No: DB22-0478, DB Orthodontics, Silsden, UK) were secured with adhesive (Araldite® Rapid, Basel, Switzerland) onto each mouthguard at five specific sites (Figure 2-1c; Table 2-3). Then hard stainless steel wires, 0.35 mm Ø and 120 mm length, were attached to them (K. C. Smith Ortho Ltd. Hertfordshire, UK) (Figure 2-1d).

Table 2-3: Sites where orthodontic brackets were attached onto each mouthguard.

Site	Description
1	Anterior palatal aspect, just above the gingival margin, located at the interdental space between the two central incisors.
2	Anterior palatal aspect, just above the gingival margin, located at the central axis of the right canine.
3	Anterior palatal aspect, just above the gingival margin, located at the central axis of the left canine.
4	Centre of the occlusal aspect of the first right molar.
5	Centre of the occlusal aspect of the first left molar.

The dental model was secured to a stainless steel plate (150 x 220 mm) placed over the base of the Hounsfield testing machine. In order to connect the mouthguards into the grips of the testing apparatus, a novel rig (80 x 80 mm) was fabricated (Figure 2-2). Location holes allowed the wires to be parallel to each other and perpendicular to the occlusal plane when secured to the rig with terminal strips.

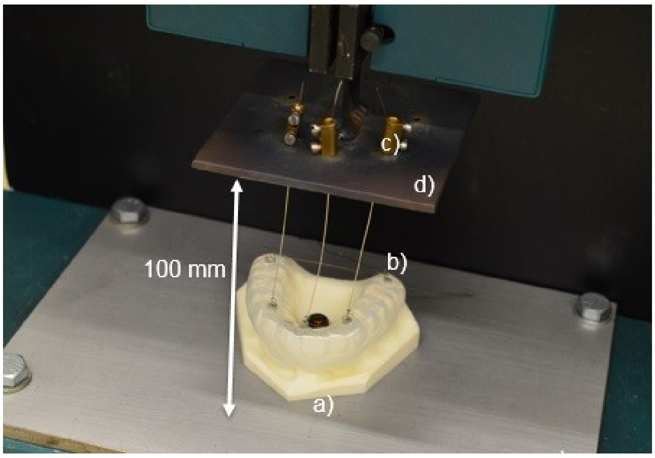

Figure 2-2: The testing rig consists of: (a) dental model and mouthguard; (b) orthodontic brackets and wire; (c) terminal strips; (d) novel rig; (e) stainless steel plate.

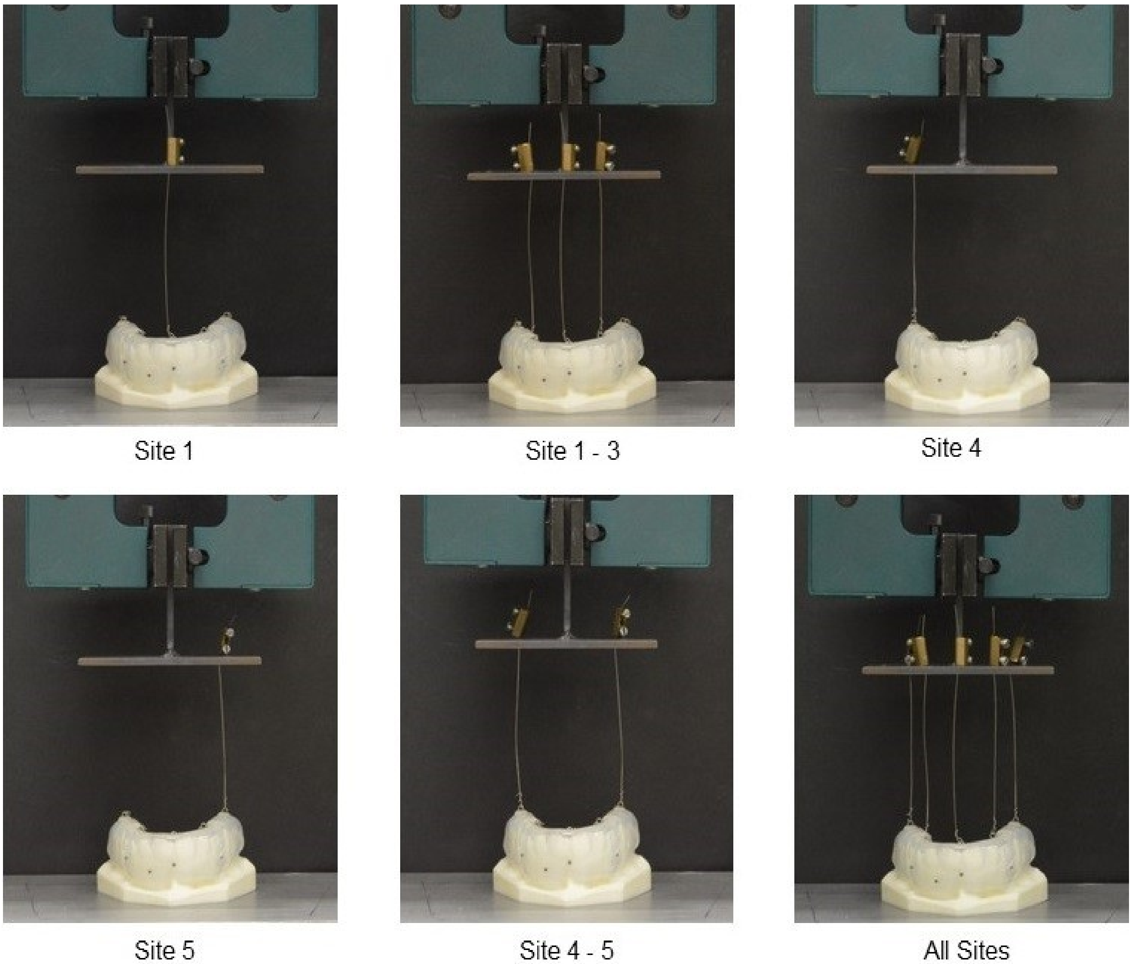

Figure 2-3: Illustration of the testing rig and all loading scenarios to test retention at different sites of the mouthguards.

The maximum force (N) required to fully displace a mouthguard from the model represented the retention force of devices at different sites as illustrated in Figure 2-3. All mouthguards were pulled away from the model by a displacement movement at a constant rate of 50 mm/min. Ten force measurements were recorded for each site and then an overall mean value was obtained (Figure 2-4). In order to reduce the variability within the testing procedure, after each measurement the load and extension were zeroed and the mouthguard was fitted back onto the model. An overall retention value was obtained by grouping the maximum forces recorded at all loading scenarios (Table 2-4).

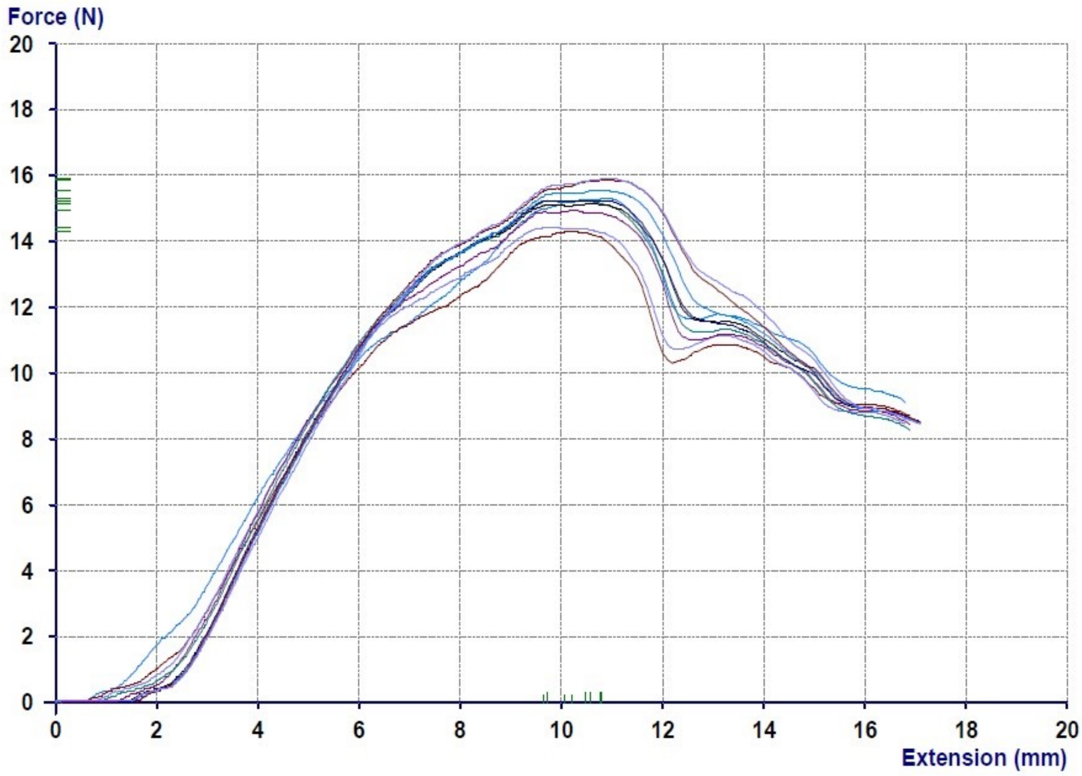

Figure 2-4: Illustration of the peak retention force in ten tests required to displace a mouthguard when load was applied at the region of the left molar.

Table 2-4: Retention force region in relation to retention force sites.

Retention Force Region	Measurement site
Anterior	Mean of Site 1 & Site (1 - 3)
Posterior	Mean of Site 4, Site 5 & Site (4 - 5)
Total	Mean of All Sites

Retention tests were then repeated under wet (saliva) condition. Each mouthguard and the dental model were immersed in 500 ml artificial saliva solution for 30 seconds prior to testing. Following each loading scenario, the mouthguard was immersed again in saliva solution for 30 seconds in order to keep it damp. The saliva was mixed according to a basic formulation consisting of: Water (1 L), Sodium Chloride (0.4 g), Potassium Chloride (0.4 g), Potassium Dihydrogen Orthophosphate (0.218 g) and Disodium Hydrogen Phosphate (1.192 g). The primary investigator conducted a test-retest reliability check on three randomly selected mouthguards under both dry and wet conditions. A second researcher also repeated the tests independently with the same three mouthguards under the same conditions.

2.2.4 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, Version 22.0. Armonk (IBM Corp., New York, US) and Microsoft Excel (2013). Histogram plots, Shapiro - Wilk normality test and box plots showed that the retention forces were not normally distributed and hence non-parametric statistical tests were applied. Wilcoxon Signed Ranks test was performed to compare the retention under dry and wet conditions. Differences in displacement force between mouthguards were identified with non-parametric Kruskal-Wallis test (multiple pairwise Mann-Whitney U post-hoc tests). The level of significance (α) was set at 0.05. Trend analysis using coefficient of determination (R^2) examined the correlation between thickness and retention of mouthguards. Due to the non-parametric nature of the data Spearman correlation was used. Additionally, Cronbach Alpha test was performed to examine the reliability of the results.

2.3 Results

Sixty retention force measurements were obtained for each mouthguard design. The retention forces recorded at the anterior region were significantly higher under wet condition compared to dry condition (p < 0.001) (Table 2-5). In contrast, the retention reported at the posterior region and the total retention were lower under wet condition compared to dry condition (p < 0.001).

Table 2-5: Median retention forces (N) for all mouthguards when tested under dry and wet condition.

Retention Force Region	**Retention under Dry Conditions**		**Retention under Wet Conditions**		**% Difference**	**Z-score**	***p***
	Median (N)	**Range (N)**	**Median (N)**	**Range (N)**			
Anterior	6.28	14.97	6.72	11.57	6.55 %	-4.363	0.001*
Posterior	5.75	15.71	3.99	13.67	44.11 %	-11.511	0.001*
Total	6.40	15.77	5.62	13.83	13.88 %	-4.618	0.001*

*Statistical difference between dry and wet conditions.

Figures 2-5 to 2-7 illustrate the differences between the retention at the anterior and posterior region as well as the total retention within mouthguard designs. However, no significant differences were found between the pairs of MG1, MG3 and MG4 under dry condition ($p > 0.121$). Additionally, the pairs of MG1 - MG4 ($p = 0.856$) and MG3 - MG6 did not differ in retention under wet condition ($p = 0.106$). Overall, the most retentive mouthguard design was found to be MG6 (11.36 ± 2.96 N (Dry) and 9.91 ± 3.48 N (Wet)) and the least retentive was MG5 (3.50 ± 1.93 N (Dry) and 3.49 ± 1.90 N (Wet)) (Figure 2-7; Table 2-6).

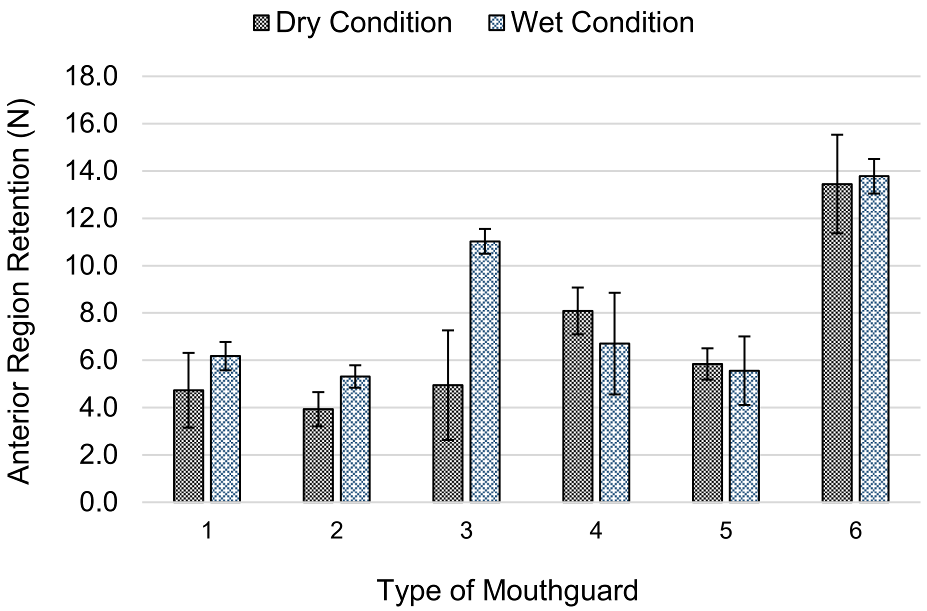

Figure 2-5: Mean retention forces (N) for each mouthguard design at the Anterior Region under both dry and wet conditions; with error bars representing standard deviation.

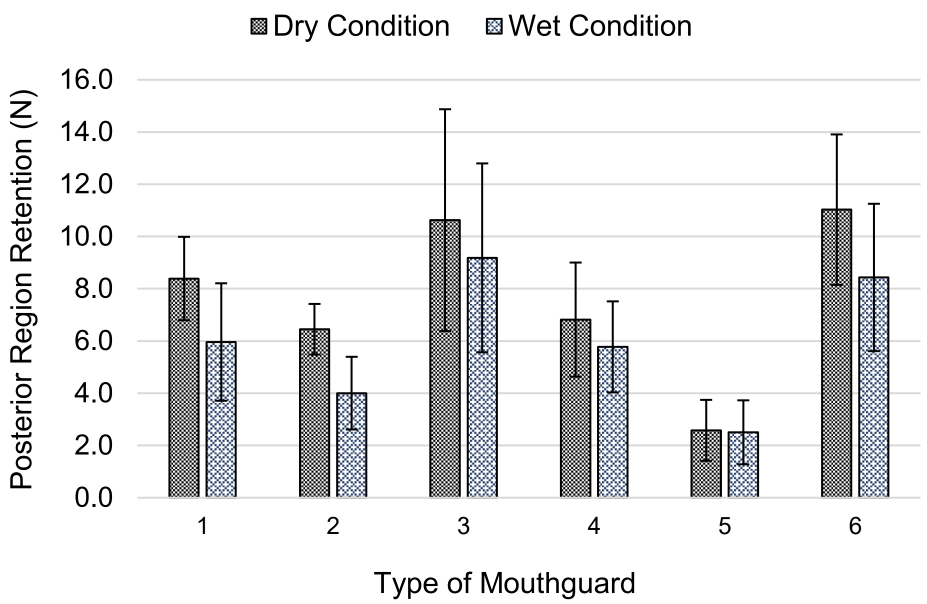

Figure 2-6: Mean retention forces (N) for each mouthguard design at the Posterior Region under both dry and wet conditions; with error bars representing standard deviation.

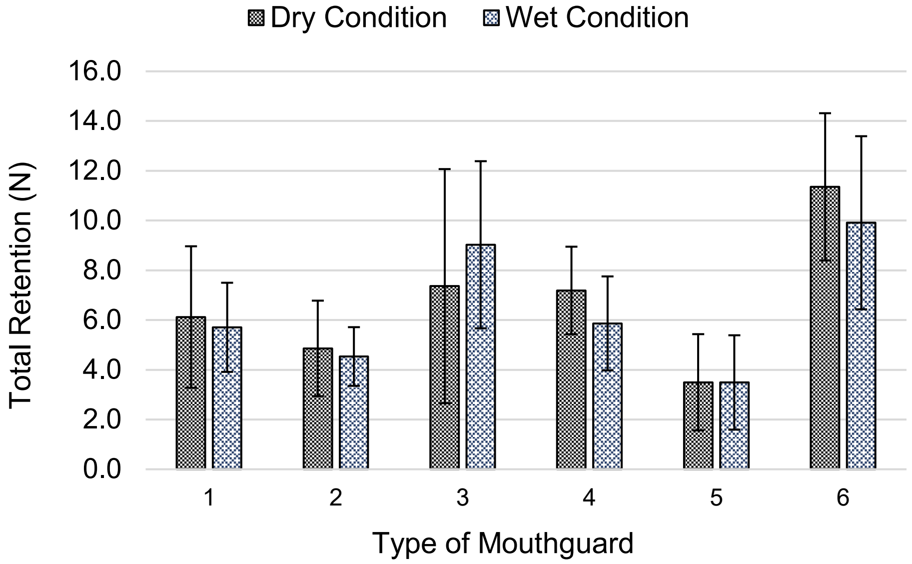

Figure 2-7: Mean total retention forces (N) for each mouthguard design under both dry and wet conditions; with error bars representing standard deviation.

MG2 and MG5 had the lowest mean total thickness of 2.02 mm and 1.96 mm and total retention of 3.50 N – 4.86 N (Dry) and 3.49 N – 4.53 N (Wet) (Table 2-6). The remainder of the mouthguard designs had a mean thickness of 2.40 mm or greater and showed higher retention of 6.12 N – 11.36 N (Dry) and 5.71 N – 9.91 N (Wet).

Table 2-6: Mean ± SD of the total retention (N) and final thickness (mm) for each mouthguard design under both dry and wet condition.

MG	Dry Condition Retention (N)	Wet Condition Retention (N)	Total MG Thickness (mm)
1	6.12 ± 2.84	5.71 ± 1.79	2.66 ± 0.49
2	4.86 ± 1.92	4.53 ± 1.18	2.02 ± 0.46
3	7.36 ± 4.71	9.03 ± 3.36	2.40 ± 0.37
4	7.19 ± 1.76	5.87 ± 1.89	2.42 ± 0.61
5	3.50 ± 1.93	3.49 ± 1.90	1.96 ± 0.47
6	11.36 ± 2.96	9.91 ± 3.48	2.59 ± 0.51

A positive relationship between mouthguard thickness and retention was found under both dry ($R^2 = 0.64$) and wet conditions ($R^2 = 0.55$) (Figure 2-8). Thus, 64.0% of the variability in mouthguard retention could be explained by thickness when dry and 55.0% when wet.

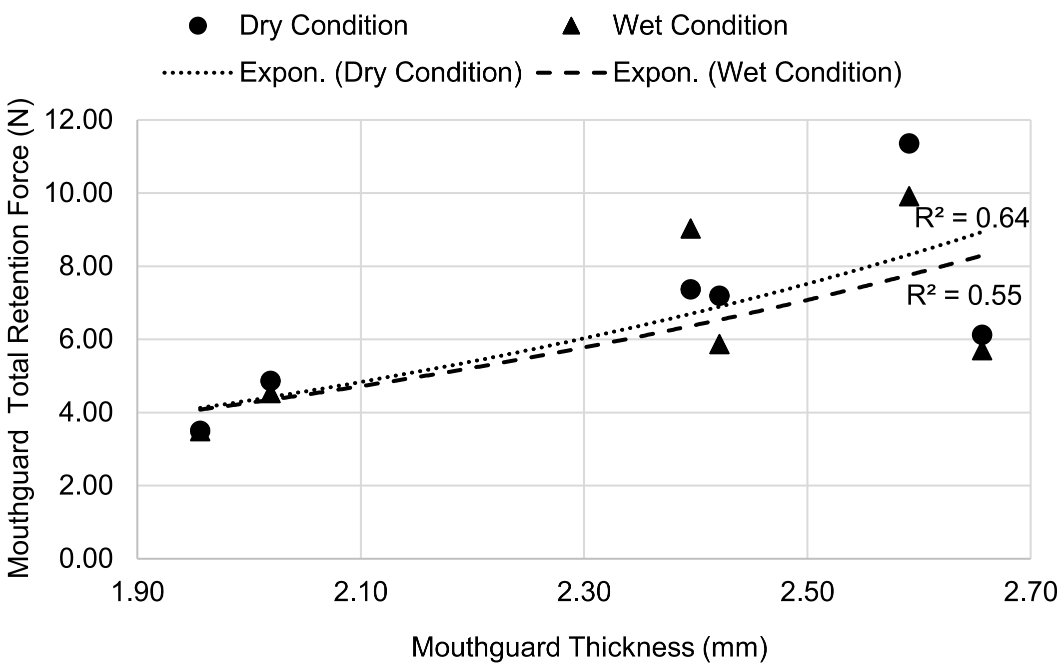

Figure 2-8: Relationship between thickness (mm) and retention (N) of the various mouthguard designs.

A total of 180 force measurements were recorded from MG1, MG2 and MG6 in both conditions to assess reliability. The primary researcher demonstrated high repeatability ($\alpha \geq 0.909$), although this was reduced when a second researcher conducted the displacement tests on the same three mouthguard designs ($\alpha \geq 0.848$).

2.4 Discussion

The current investigation proposed a new method to examine the retention of mouthguards. The findings demonstrated that the selected devices differed significantly in retention and key factors for these differences were mouthguards' thickness and design as well as the condition (dry or wet) under which tests were performed. It was unexpected that retention of mouthguards at the posterior region and total retention were higher under dry compared to wet (saliva) condition as viscosity of saliva is believed to improve retention of dental devices (Darvell and Clark, 2000). Therefore, the initial hypothesis that retention recorded under wet condition would be higher was rejected. A possible reason for these results was that the artificial saliva solution did not include a glycoprotein called mucin, which consists of 3 – 18 sugar units and it is secreted in the oral cavity (Slomiany et al., 1996). Adding mucin to the saliva formula could have increased its viscosity to reflect the oral environment more appropriately. Nevertheless, the retention recorded at the anterior region was statistically higher under wet in comparison to dry condition. This could be due to the anatomy of dental structures such as the length of the anterior teeth and the presence of labial undercuts, which could naturally increase mouthguards' retention.

Statistical differences between mouthguard designs in terms of their ability to withstand displacement forces were found. The highest retention at all points and under all conditions was shown by MG6, which had two layers of EVA blanks at the anterior region and the occlusal surfaces. In contrast, MG5 was the least retentive mouthguard, made of a single 4 mm EVA blank with no palatal coverage behind the anterior teeth. A positive correlation between mouthguard thickness and retention was found under dry ($R^2 = 0.64$) and wet ($R^2 = 0.55$) conditions. Having a double layer mouthguard (EVA blanks of 2 mm and 4 mm) increased the final thickness of the devices. For instance, MG3, MG4 and MG6 had a mean thickness above 2.40 ± 0.37 mm, which was more than the devices fabricated with a single EVA layer (MG2 and MG5; < 2.02 mm). Additionally, these three designs demonstrated retention of 5.71 ± 1.79 N to 9.91 ± 3.48 N that was higher than thinner mouthguards (MG2 and

MG5, 4.53 ± 1.18 N and 3.49 ± 1.90 N respectively). Although MG1 was made of one EVA blank, the device was characterised with a 4 mm palatal flange, which contributed to an overall increase in its thickness (2.66 ± 0.49 mm) and demonstrated level of retention similar to MG3, MG4 and MG6. Additionally, it was identified that MG1 required higher pulling force compared to MG2 (no palatal flange) under both dry and wet conditions (Figure 2-7; Table 2-6). These findings contradict previous literature, which identified that having a mouthguard with palatal outline did not influence retention level (Maeda et al., 2006; Gebauer et al., 2011; Nozaki et al., 2013). However, previous studies reported these statements based on questionnaires and participants' perception of retention level, which could be highly subjective. The present study demonstrated that fabricating mouthguards with two EVA blanks is recommended when the chosen mouthguard design has a reduced palatal flange. By using this technique, comfort and speech may improve as previously suggested by Maeda et al. (2006) and Gebauer et al. (2011) and simultaneously high level of retention will be maintained.

Similar to the current study, Del Rossi et al. (2008) proposed a test, which also recorded the maximum force of mouthguard displacement by positioning a metal wire behind the central incisors and attaching it to a strain gauge. However, the level of retention was investigated at only one site of the mouthguard, whereas the present study proposed five sites of the mouthguard to measure devices' retention at different regions (e.g. anterior and posterior). Del Rossi et al. (2008) evaluated the effects of colour on mouthguard fit. They fabricated the same mouthguard design but in five different colours, using 3 mm EVA blank and the same pressure-forming equipment as in the present study. The devices were tested at two angles, 90˚ and 135°, to the transverse plane to mimic the angle of mouthguard removal used by athletes and demonstrated using dark coloured blanks could provide better fit and adaptation due to their ability to absorb greater amount of infrared energy during the thermoforming process. Additionally, it is important to consider that the addition of coloring agents could increase the solidification process of the EVA blank and have an effect on the mechanical properties of the material after thermoforming. Del Rossi et al. (2008) suggested that mouthguards with better fit might limit the chewing forces naturally applied by an individual to keep a loose mouthguard in position, thereby prolonging the life of the device. Additionally, coloured devices are easy to see, which makes it easier to recognize if a participant is missing their mouthguard during a game. However, a number of clubs and societies prefer a clear mouthguard so that if serious bleeding or injury occurs within the oral environment, it can be viewed and noted. Maeda et al. (2009) conducted retention tests of three custom-made mouthguards with different outline. Similar to Del Rossi et al. (2008), they also measured retention at only one site of the devices (upper left molar). However, instead of using wires to connect the mouthguard to the testing machine, the authors attached a screw and washer jig. Maeda et al. (2009) recorded retention forces when the mouthguards started to separate from the tooth cervical margin, which makes it challenging to compare their findings to the present study that measured the maximum force required to fully displace a mouthguard. Maeda et al. (2009) did not assess the influence of mouthguard thickness on retention but the effect of pressure-forming and vacuum-forming technique. Additionally, the authors tested three custom-made mouthguards, all fabricated with a 3.8 mm EVA blank. They reported that a pressure-formed customised mouthguard with no palatal outline performed better than a mouthguard with 4 mm palatal extension (3.8 mm EVA blank) (133 ± 31 gf < 139 ± 24 gf, $p > 0.05$), which contradicts the present findings. However, when the mouthguards were vacuum-formed, the device with 4 mm palatal flange showed better fit than the other two mouthguards (one with palatal flange and one with increased buccal extension). It is important to take into account the features leading to limited dislodgement of mouthguards during use. If a mouthguard is poorly fitted and not retentive, an athlete will try to keep it in position, which could cause distraction, speech and breathing impedances; consequently, having a negative effect on performance.

Previous studies alongside this study have stated that mouthguard design and fabrication technique have an impact on retention (Del Rossi et al., 2008; Maeda et al., 2009). A number of design characteristics such as having no palatal flange, minimum final thickness of 2.5 mm, the presence of two EVA layers, pressure thermoforming during fabrication and the use of darker colours EVA blanks have been highlighted to improve fit and retention as well as improve users' comfort by limiting speech and breathing impairments. However, there is no standardised method procedure to examine retention of mouthguards, which could be important in order to test whether different custom-made devices provide sufficient retention. Thus far, the main differences in experimental procedures have been related to mechanism of testing, conditions (dry and wet) at which tests have been performed and method of reporting the results. For instance, the present study proposed the securing orthodontic brackets and wires to a mouthguard and connecting the wires to a rig into the grips of a testing machine, whereas previous studies used screw and washer (Maeda et al., 2009) or a chain with a fishhook attached to the tested device (Del Rossi et al., 2008). It is difficult to conclude which technique provides the most accurate measurement of retention due to differences in experimental procedures, investigated mouthguard designs and the influential factors examined (e.g. thickness, outline of the device, fabrication technique or colour).

Following the techniques used in the current study, it is recommended that future work should consider the use of Nano – Rock liquid die stone to create a master model. The polyurethane casting has hydrophilic properties, which prevent absorption of the artificial saliva solution and are likely to be similar to that of tooth surfaces and oral mucosa. Using a conventional gypsum cast would have not allowed that level of hydrophilicity. Further research should investigate more influencing factors on mouthguard retention such as variations in dental anatomy, alignment of the teeth and the presence of undercuts as dental arch dimensions differ with age, gender and ethnicity (Nojima et al., 2001; Kook et al., 2004; Gafini et al., 2011). The current study did not consider the effect of anatomical differences of the dental arch as only one master cast with no misaligned teeth was examined. However, during testing it was noted that the force required to pull mouthguards away was also dependent on how difficult it will be for the device to overcome an undercut.

Maeda et al. (2009) also agreed that mouthguards fully engaging the cervical undercut area of the dentition demonstrated better accuracy of fit compared to devices with limited extension in the buccal region. Hence, it could be suggested that individuals with deeper undercuts would naturally contribute to the increase of retention level; however, this needs to be further investigated. Improvements in the current test methodology are required to propose a better representation of the oral environment and mimic the angle at which mouthguard users apply forces to remove their device. Further work should use a larger sample size including different manufacturing techniques and existing materials to identify which mouthguard parameter has a predominant impact on retention and where the cut-off point is for sufficient retention force.

2.5 Conclusions

The present study demonstrated the use of a novel rig to measure retention of six custom-made mouthguard designs. This investigation aimed to obtain accurate measurements of retention and it is the first one to examine the retention at different regions of the device under two conditions – dry and wet. The findings demonstrated that mouthguard retention could be altered by changes in design. Techniques such as using two EVA blanks, finishing the outline at the gingival margins or appreciably decreasing the thickness of the palatal flange should be considered during fabrication of custom-made mouthguards in order to maintain retention and improve comfort. Higher retention was recorded at the anterior region in the presence of an artificial saliva solution, which showed that the oral environment conditions should be considered during testing of mouthguards. These findings have important implications for sports participants, who require mouth protection. By minimising the possibility of device dislodgement, the users may gain confidence wearing a mouthguard and hence this will lead to an increase in their usage.

The design of custom mouthguards could be a key factor associated not only with comfort but also with breathing impedances. Currently, there are mixed views regarding the effects of mouthguards on performance. Therefore, the following chapter of this thesis will examine the influence of selected custom-made mouthguard designs from this study, which demonstrated a high level of retention, on physiological parameters such as respiratory flow and blood lactate accumulation. In addition, it will also assess participants’ preferences in terms of design and their opinion in relation to comfort.

Chapter 3 : The Effects of Various Customised Mouthguard Designs on Physiological Parameters and Comfort in Male Rugby Players

3.1 Introduction

Rugby has been classified as a high-risk sport due to multiple tackles, rucks and collisions taking place during competition and training days (FDI, 1990; Quarrie et al., 2005; Ilia et al., 2014). A number of epidemiological studies have assessed the prevalence of dental trauma within adult and junior rugby players and highlighted the need of personal protective equipment (Muller-Bolla et al., 2003; Jagger et al., 2010; Nicol et al., 2011; Schildknecht et al., 2012; Ilia et al., 2014). Ilia et al. (2014) reported that 64.9% of rugby union players (*N* = 240; 24.1 ± 5.7 yrs) had sustained a dental injury, which was similar to the finding of Schildknecht et al. (2012), who reported 54.4% within a larger population (*N* = 517; 23.1 yrs). Both studies highlighted that lacerations to the lips, cheeks and tongue were the most common dental injuries (44.5% (Ilia et al., 2014) and 54.2% (Schildknecht et al., 2012)). In relation to all orofacial traumas, 41.9% were related to the dentition (e.g. avulsion, luxation, crown or root fracture), with more than a half of them affecting the upper anterior teeth (Ilia et al., 2014). Findings emphasised that the use of mouth protection should be further reinforced and encouraged amongst rugby players.

Despite the recommendations and regulations of mouth protection, it is often difficult for players to tolerate mouthguards. An investigation of mouthguard awareness and compliance within rugby demonstrated that only 53.9% (*N* = 65; males; 22.2 ± 5.66 yrs) of the players used mouthguards during training and competition (Boffano et al., 2012). In addition, 32.5% of the participants reported that they had never used a mouthguard. The most common issues were associated with impedances in communication (79.6%), followed by difficulty to close the lips (22.2%) and breathing obstruction (16.7%). Likewise, it was also reported that the most frequent complaints were related to breathing (24.9%) and speaking (15.1%) (Ilia et al., 2014). Participants' acceptance of mouthguards could be increased if they were provided with a well-fitted device (Collares et al., 2014; Ilia et al., 2014). Thus, the design of mouthguards should be considered in order to limit issues with comfort, breathing and communication. It is important that dental practitioners not only promote the advantages of customised mouthguards but also educate their patients about variations in design. Furthermore, they should provide information about the risk of injuries and the long-term benefits of investing in a custom-made device in comparison to the post-injury cost of dental treatment (Levin and Zadik, 2012).

Previous work in relation to different mouthguard types on various respiratory parameters and power output have been reported (Chapter 1: Table 1-2). However, they have all followed different methodologies in terms of exercise protocols, sport-specificity and assessment of physiological biomarkers and power output (Caneppele et al., 2017; Ferreira et al., 2018). Caneppele et al. (2017) conducted a meta-analysis to examine the influence of mouthguard use on performance. They reported that maximum oxygen uptake (VO_{2max} ml/kg/min) and maximum minute ventilation (VE_{max} L/min) did not differ statistically when participants exercised without a device or used only specific mouthguard types (upper or lower jaw 'boil-and-bite'; upper, lower or upper-lower jaw custom-made). However, the majority of previous studies that investigated field-based team-sports participants examined the influence of 'boil-and-bite' mouthguards compared to no mouthguard and/ or custom-made mouthguards (Bourdin et al., 2006; Duarte-Pereira et al., 2008; Queiroz et al., 2013; Drum et al., 2016). Some of them agreed that using custom-made devices led to less interferences during performance compared to using stock or 'boil-an-bite' types (Bourdin et al., 2006; Duarte-Pereira et al., 2008; Drum et al., 2016). Queiroz et al. (2013) were the only study to report improvements in both VO_{2max} and aerobic capacity. A 17.5% significant increase in VO_{2max} was found when female soccer players (*N* = 25; 18 – 22 yrs) performed a

20-m shuttle-run maximal exertion test with a customised device compared to no mouthguard. Wearing the device led to higher VO_{2max} than wearing stock and ‘boil-an-bite’ mouthguards whilst exercising. Nevertheless, the study did not provide any details about the mouthguard fabrication techniques and design. Collares et al. (2014) also examined the use of a customised mouthguard in soccer players (males; 16.2 ± 0.55 yrs) using a 20-m shuttle-run test. However, they found no changes in aerobic metabolism with a mouthguard compared to running without a mouthguard.

Only a few previous studies have addressed the use of exercise protocols, which aim to improve ecological validity by replicating the physiological demands of the participants’ sport. For instance, Piero et al. (2015) used a friction-loaded cycle ergometer to examine the effects of a customised mouthguard on submaximal and maximal parameters related to performance in road male cyclists (34 ± 6 yrs). The authors found no differences in cardio-respiratory and metabolic parameters, although cycling economy (Δ VO_2 / Δ work rate) was found to improve when a custom-made mouthguard was used (9.5 ± 1.1 vs 10.3 ± 1.1 ml/min/W, $p = 0.006$). Similarly, Schulze et al. (2018) highlighted the importance of using a sport-specific exercise by designing a protocol to assess the influence of a ‘vented’ mouthguard (VentMG) on cardiopulmonary parameters in 14 handball professional males (17.43 ± 0.76 yrs). They attempted to mimic a match by setting a handball-specific course consisting of nine exercise stations (2 x medium intensity rounds (230 seconds) with a 30-second break and then a minute later 2 x high intensity rounds (200 seconds) with a 30-second break). Wearing VentMG did not cause any significant airflow obstructions.

There is a limited literature, which has addressed the necessity of a rugby-specific exercise protocol to investigate the influence of mouthguards on cardiopulmonary parameters. This is recommended in order to achieve more ecologically valid findings to the participants and contribute to the current knowledge within mouthguard research. Rugby union is played in two halves of 40 minutes with a 10-minute break and involves two teams of 15 players (8 forwards and 7 backs) (Jenkins and Reaburn, 2000). Well-developed aerobic and anaerobic capabilities are required to sustain the physical demands of the game (e.g. intermittent running, sprinting and tackling, combined with walking and jogging) (Cunniffe et al., 2009; Ziv and Lidor, 2016; Brazier et al., 2018). Cunniffe et al. (2009) used a global positioning system (GPS) to determine that elite rugby union players spend 66.5 – 77.8% of their time standing or walking (0 – 6 km/h) and about 2.5% high-speed running (18 – 20 km/h) and sprinting (> 20 km/h). Hence, high-intensity intervals and strength endurance activities should be considered as main components in rugby-specific protocols. Most commonly, improvements in speed and distance covered are monitored through submaximal or maximal shuttle runs and intermittent sprint intervals (Galvin et al., 2013; Dubois et al., 2018). These types of exercise should also be considered when studies examine the effects of mouthguards in order to achieve valid measurements in relation to the sport.

The aim of the present chapter is to use a newly designed exercise protocol to examine the influence of custom-made mouthguards on physiological parameters and comfort in rugby union males. Participants' respiratory flow and blood lactate will be recorded whilst they perform with and without a mouthguard. The study will assess parameters including absolute and relative oxygen uptake, minute ventilation, respiratory exchange ratio, heart rate and blood lactate in order to investigate whether changes in mouthguard design may affect physiological performance. In addition, participants' perception of comfort level will be examined via a questionnaire. It is hypothesised that variations in mouthguard design will not influence physiological parameters and comfort-related issues whilst performing with and without any of the selected customised mouthguards.

3.2 Methods

Prior to any experimental work, ethical approval was obtained and granted from the School of Healthcare Science, Faculty of Science and Engineering, Manchester Metropolitan University (Ethics Number: SE151683). Additionally, the procedures included in the study design followed the ethical principles outlined in the Declaration of Helsinki (World Medical, 2013). Participants were provided with detailed information about the study objectives, full protocol and the possible risks and benefits of taking part prior to obtaining informed consent. All participants had the right to withdraw at any time during the study.

Initially, 19 male rugby players were recruited for the study, however five of them could not complete all sessions due to injuries during their competition season, which left 14 participants to complete the full study protocol. All participants were part of the Manchester Metropolitan University Rugby Union team and trained for 5-10 hours per week; with 11 ± 3.90 yrs of experience in rugby and 10 ± 3.83 yrs of competing. The anthropometric data of the sampled population are shown in Table 3-1.

Table 3-1: Description of the sampled population (*N* = 14).

Variable	**Mean**	**±**	**SD**
Age (yrs)	20		1
Mass (kg)	92		13
Height (cm)	182		8
Body Mass Index	28		2
Hb (g/dL)	16.0		1.1
Lung Function (%)	90		8
VO_{2max} (ml/kg/min)	50		4

During the first initial visit, medical and dental assessments were completed to ensure none of the participants had any present injuries, related cardiovascular problems, temporomandibular disorder or any trauma of the oral and facial structures that would affect their involvement within the study (Appendix A). Additionally, all participants were physically fit, taking no form of medication, which could affect airflow, muscle fatigue and HR. A capillary finger prick blood sample was taken to determine the levels of haemoglobin (Hb) (HemoCue® 201+ System, Crawley, England). If any signs of anaemia (Hb < 13 g/dl) were present, the participants were excluded from the study.

3.2.1 Fabrication of Mouthguards

A dental clinician took alginate dental impressions (Tropicalgin®, Zhermack SpA, Italy) of both the maxillae and the mandible for each participant. The impressions were immediately disinfected (DISINFIN med®, Bracon Ltd, Heathfield, UK) and dental casts for fabrication of mouthguards were produced by pouring vacuum mixed Kaffir-D dental stone (3:1 powder/ liquid ratio) (John Winter & Co Ltd., Halifax, UK). All casts were then duplicated in model resin (Rhino Rock, DB Orthodontics, Silsden, UK) to create dental models with higher strength and multiple use.

Three mouthguard devices were fabricated for each participant in the dental laboratories at Manchester Metropolitan University by the same technician (the author of this thesis). All mouthguards were thermoformed on a Biostar machine (BIOSTAR®, SCHEU-DENTAL GmbH, Iserlohn, Germany) (Figure 3-1) at 4.8 bars applied pressure and made of clear ethylene vinyl acetate (EVA) blanks of 120 mm Ø (diameter) (Bracon Ltd, Heathfield, UK).

Figure 3-1: Thermoforming process of mouthguards; (a) heating chamber; (b) secured EVA blank; (c) participant's dental cast.

The heating temperature was set at 220°C with the heating time dependant on the thickness of the blank (50 s, 90 s and 120 s for 2 mm, 4 mm and 5 mm respectively) inputted via coding following manufacturer's instructions. Figure 3-2 shows the selected mouthguard designs (MG1, MG4, MG6), which demonstrated high retention levels during the tests evaluated in Chapter 2.

Figure 3-2: Three customised mouthguards (MG1, MG4 and MG6) used by all participants.

MG1 was fabricated from a 5 mm single EVA blank and it had a 4 mm gingival flange, similar to the mouthguard proposed by Gebauer et al. (2011). In comparison, MG4 and MG6 were fabricated with two EVA layers (2 mm and 4 mm) and were trimmed around the palatal gingival margins. The designs of MG4 and MG6 were adapted from previous studies by Morales et al. (2015)

and Takeda et al. (2014). MG4 consisted of a double layer at the occlusal surfaces of the posterior region only, whereas MG6, which was designed for a rugby player with mal-aligned teeth, had a double EVA layer covering all anterior teeth and the occlusal surfaces of the posterior teeth. MG1 and MG4 were finished at the distal surfaces of the first maxillary molars, whereas MG6 extended over the second maxillary molars. All devices had a full buccal flange. The mouthguards were disinfected, placed into storage boxes and labelled with the participant’s reference number. The labels were colour coded depending on the mouthguard design.

3.2.2 Baseline Measurements and Aerobic Fitness Assessment

To control for variability in the data, the participants were asked to record their dietary intake and physical activity levels 24 hr prior to every laboratory testing session. Additionally, they were told to restrain from caffeine and alcohol, and fast at least 2 hr before testing.

In order to assess any differences in aerobic fitness pre and post exercise sessions with mouthguards, two maximal exertion tests were performed (at the start and at the end of the study). Primary baseline measurements during these sessions included: stature, body mass, blood pressure, heart rate (HR), Hb, blood lactate (BLa) and lung capacity. Blood pressure and resting HR were recorded with a standard automatic blood pressure monitor (OMRON M2, Kyoto, Japan). Then, capillary finger prick blood samples were taken twice to measure Hb and BLa levels. A portable analyser (Lactate Pro, Arkray, Japan) was used to analyse the BLa samples. Participants’ lung capacity was assessed through spirometry tests where participants wore a nose clip and exhaled as much air as they could (Gold Standard MicroPlus Spirometer, Micro Medical Ltd., Kent, UK).

The VO_{2max} tests involved an incremental exercise on a treadmill (Woodway GmbH, Weil am Rhein, Germany) until maximal exhaustion. Participants performed a 10 min warm-up run, which helped selecting a suitable running speed for each individual (11 ± 0.70 km/h). The test started at 0% treadmill incline, which was increased by 1% every minute, whilst the speed remained unchanged. During the last 10 s of each one minute stage, participants were asked to rate their level of perceived exertion (RPE) on a standard Borg scale (6 – No Exertion to 20 – Maximal Exertion). The same researcher provided verbal encouragement to help participants achieve maximal exertion during the VO_{2max} tests. The test was terminated when the participant jumped to the sides of the treadmill. Finally, a 10 min cool down was completed and a 3 min post-exercise BLa sample was taken.

The gas exchange parameters assessed during each test session (alongside performance with mouthguards) included VO_2 (absolute L/min and relative to body mass ml/kg/min), VE (L/min) and respiratory exchange ratio (RER). Prior to the start of a test, participants were fitted with a facemask, which was connected to a breath-by-breath analyser (METALYZER® 3B, CORTEX, Leipzig, Germany). Following manufacturer's instructions, volume sensor pre-calibration of the gas analyser was performed prior to each session using a 3-litre calibration syringe (Hans Rudolph™, Inc, Series 5530, Shawnee, US). Additionally, HR (bpm) was monitored with a HR sensor (Polar H7, Polar Electro Ltd, Warwick, UK). A baseline reading of the above measurements was taken by asking the participants to remain at rest for one minute prior to exercise. This not only excluded the effect of early VO_2 kinetics but also allowed to examine any possible variations of the biomarkers prior to each trial.

3.2.3 Sport Specific Rugby Protocol

The stages of the present study are described in Figure 3-3. Visits 3 – 6 included four laboratory-based test sessions during which participants performed a rugby-specific protocol with randomly allocated mouthguard or without a mouthguard. No information about the differences in mouthguards characteristics and design was provided in order to avoid any bias.

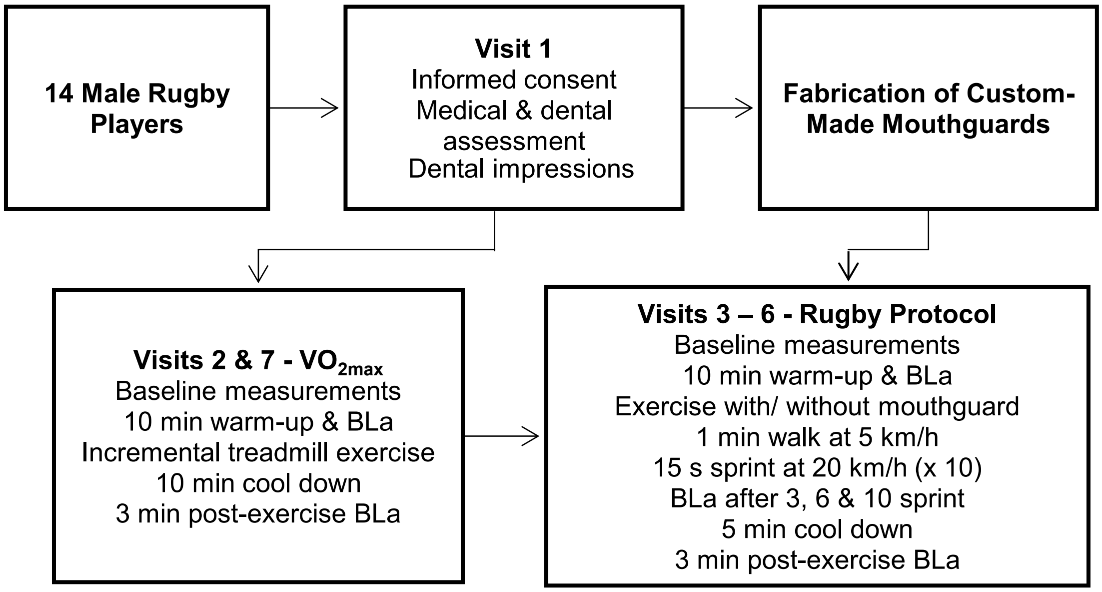

Figure 3-3: Flowchart illustrating the present rugby-specific study design.

At the beginning of each rugby-specific session, body mass, HR and BLa were recorded. Participants performed a standardised 10 min warm-up run. The main protocol consisted of four alternating intervals that were repeated ten times – 1 min walk at 5 km/h, acceleration from 5 km/h up to 20 km/h, a 15 s sprint at that speed and deceleration from 20 km/h down to 5 km/h (Table 3-2). The choice of speeds (5 km/h and 20 km/h) was based on proposed speed zones that were recorded previously during elite rugby union games (Cunniffe et al., 2009).

Respiratory parameters as described in Section 3.2.2 were measured, using the same breath-by-breath analyser procedure. Capillary finger prick BLa samples were taken at rest, post warm-up, alongside the end of third, sixth and tenth sprints and 3 min post-exercise. Blood sampling at these specific intervals was performed following the study of Gharbi et al. (2014) who examined the effect of 1 – 10 sprint repetitions on BLa concentration.

Table 3-2: Exercise protocol performed by all participants with each of the three mouthguards and without a mouthguard.

Interval	Speed	Time
Warm-up		
Walking	5 km/h	5 min
Jogging	8 to 12 km/h	5 min
Exercise Intervals	**(x 10)**	
i) Walking	5 km/h	1 min
ii) Acceleration	up to 20 km/h	15 s
iii) Sprint	20 km/h	15 s
iv) Deceleration	down to 5 km/h	15 s
Cool Down		
Walking	5 km/h	2 min
Jogging	9 km/h	2 min
Walking	4 km/h	1 min

In addition, immediately following each sprint, participants were asked to provide their perceived rate of exertion using a Borg scale as they did during the VO_{2max} tests. After the final sprint, a 5 min cool down including walking and light jogging was performed.

3.2.4 Assessment of Comfort

Following each test session, participants were asked to wear the mouthguard provided during training and competitions (if any) until their next laboratory visit. They were then given a questionnaire (Appendix B) that was designed to assess their perception of comfort, thickness, retention, breathability, communication, effect on concentration, protection and the likelihood of wearing the provided mouthguard again. These aspects were addressed by previous studies, which aimed to examine comfort within different mouthguard types (Brionnet et al., 2001; Duarte-Pereira et al., 2008; von Arx et al., 2008;

Duddy et al., 2012; Lee et al., 2013; Liew et al., 2014). The present study used a scale of 1 – 10 to rate each of the characteristics, with 10 having the greatest merit. The final evaluation of each mouthguard characteristic was based on the mean score of all participant responses.

3.2.5 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, Version 24.0. Armonk (IBM Corp., New York, US) and Microsoft Excel (2013). Prior to any analysis, distribution of the data was checked with histogram plots, Shapiro-Wilk normality test and Levene's test for homogeneity of variance. Statistical significance was set at $p \leq 0.05$.

In order to obtain the maximum values of VO_2 (L/min and ml/kg/min), VE (L/min), RER and HR, rolling averages of the last 30 seconds of the VO_{2max} test breath-by-breath measurements were calculated. Then, a paired samples t-test was used to compare the parameters of the first and second VO_{2max} sessions.

Equation 3.1 was used to calculate the intensity (V%) of the rugby-specific protocol as a percentage of the achieved VO_{2max} (ml/kg/min) ($V2$) and HR_{max} ($V2$) during the maximal exertion test; $V1$ equals either the absolute maximum VO_2 (ml/kg/min) or the absolute maximum HR achieved during each trial of performing the protocol.

$$Equation\ 3.1: \qquad \%\,V = \frac{V2 - V1}{V2}\ x\ 100$$

If parametric assumptions were met, repeated measures ANOVA (within subjects) with post-hoc (Bonferroni) or paired samples t-test were performed to identify the effect of mouthguards on the following parameters: VO_2 (L/min), VO_2 (ml/kg/min), VE (L/min), RER, HR (bpm), BLa (mmol/L) and RPE. Wilcoxon test was used when data were non-parametric. All cardio-pulmonary measurements were analysed using rolling averages of each sprint, walking interval, acceleration and deceleration intervals. Additionally, the absolute maximum values for HR of each sprint, walking interval, acceleration and deceleration intervals were analysed.

Percentage change of the same variable between different conditions (No MG, MG1, MG4 and MG6) was calculated following Equation 3.2, where $X1$ results from one condition and $X2$ from another.

$$Equation\ 3.2: \qquad \%\ Change = \frac{X2 - X1}{X2}\ x\ 100$$

Due to the nature of BLa measurements and the difficulty to achieve equal baseline values prior to exercise, additional analyses were performed to find the increase/ decrease of BLa values between intervals. For example, the difference between post warm-up BLa and BLa after the third sprint was calculated by subtracting the former from the latter.

The questionnaire data was analysed by performing Wilcoxon test with significance level set at $p \leq 0.05$.

Sample size calculations were conducted with G*Power. The following assumptions were made: (i) use of repeated measures ANOVA, within-subjects (ii) comparison of four groups (same population at three mouthguard conditions and without a mouthguard) (iii) examining the changes in seven parameters at those four conditions (VO_2 L/min and ml/kg/min, VE, RER, HR, BLa, RPE) $\alpha = 0.05$ and (iv) effect size of 0.80. It was found that a minimum of 12 participants were required.

3.3 Results

3.3.1 VO_{2max} Tests

Participants' level of aerobic fitness in terms of VO_{2max} measurements did not differ significantly between the start and the end of the study (Table 3-3). However, there was a 5.7% increase in VO_{2max} (ml/kg/min) and 8.3% higher level of BLa.

Table 3-3: Mean ± SD of cardiopulmonary parameters recorded at maximal exertion (*N* = 14).

VO_{2max} test	VO_2 (L/min)	VO_2 (ml/kg/min)	VE (L/min)	RER	HR (bpm)	BLa (mmol/L)
1	4.58 ± 0.64	50 ± 5	175.3 ± 19.9	1.12 ± 0.04	194 ± 9	11.0 ± 2.9
2	4.68 ± 0.75	53 ± 6	173.8 ± 19.5	1.13 ± 0.01	195 ± 8	12.0 ± 2.0
p	0.164	0.193	0.373	0.193	0.361	0.219

3.3.2 Effects of Mouthguards on Physiological Parameters

Table 3-4 shows that intensity of the rugby protocol varied between 84 – 93% in terms of VO_2 (ml/kg/min) and 92 – 96% in terms of HR. Overall, the highest effort in relation to both parameters was found whilst performing with MG1 design; although not statistically significant.

Table 3-4: Intensity of rugby exercise protocol relative to participants' maximum oxygen uptake (VO_{2max} ml/kg/min) and maximum heart rate (HR_{max} bpm) (*N* = 14).

	No MG			MG1			MG4			MG6		
	Mean		SD	Mean		SD	Mean		SD	Mean		SD
%VO_{2max}	84%	±	4.88	93%	±	10.67	88%	±	9.04	84%	±	5.57
%HR_{max}	93%	±	3.17	96%	±	5.21	95%	±	5.74	92%	±	3.36

During the first walking interval, VO_2 (L/min and ml/kg/min) without a mouthguard was statistically higher compared to wearing MG4 and MG6 (10.7%, $p = 0.048$) (Appendix C1). Although not significant, the VO_2 (L/min and ml/kg/min) recorded with MG1 was the highest in comparison to the other two mouthguard designs (up to 7.8%, $p \geq 0.104$).

Whilst sprinting, no statistical changes in VO_2 (L/min and ml/kg/min) were reported (Table 3-5; Appendix C3). However, it should be considered that performing with MG4 demonstrated up to 5.7% non-significant increase compared to MG1 and MG6. In relation to VE, having no mouthguard during the first sprint only showed 16.0% higher ventilation than whilst wearing MG1 ($p = 0.031$), but no other differences were reported.

During the last deceleration interval, significantly greater VO_2 (L/min and ml/kg/min) was recorded with MG1 in comparison to No MG condition ($p = 0.030$) (Appendix C4). Additionally, although the percentage increase was not statistically higher, performing with MG1 led to greater VO_2 (L/min and ml/kg/min) than performing with MG4 and MG6 (up to 9.2%).

In relation to maximum and mean HR values, wearing no device demonstrated significantly higher measurements compared to wearing any of the three mouthguards during the first three walking intervals (up to 7.5%, $p \leq 0.006$). Significant HR increase without a device was also reported during the third acceleration in comparison to MG6 (6.4%, $p = 0.044$) (Appendix C2), during the second sprint in comparison to MG6 again (3.4%, $p = 0.045$) and during the first and second deceleration intervals in comparison to MG4 and MG6 (up to 16.5%, $p \leq 0.005$). No statistical differences within HR were observed during exercise with the selected mouthguards. Additionally, the percentage difference between conditions was low (up to 5.4% during walking; 2.5% during accelerating; 1.7% during sprinting; 1.4% during decelerating).

Table 3-5: Mean ± SD of VO_2, VE and Max ± SD of HR during each sprint interval (1 – 10) whilst wearing No MG, MG1, MG4 or MG6 (N = 14).

	No MG			**MG1**			**MG4**			**MG6**			
	Mean		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
							VO_2 (L/min)						
1	2.73	±	0.31	2.62	±	0.53	2.75	±	0.45	2.58	±	0.44	0.327
2	3.20	±	0.45	3.32	±	0.64	3.28	±	0.48	3.13	±	0.47	0.657
3	3.21	±	0.47	3.28	±	0.73	3.32	±	0.49	3.15	±	0.49	0.980
4	3.19	±	0.46	3.35	±	0.77	3.37	±	0.46	3.16	±	0.49	0.437
5	3.18	±	0.45	3.33	±	0.81	3.32	±	0.41	3.18	±	0.47	0.656
6	3.23	±	0.44	3.31	±	0.72	3.33	±	0.42	3.20	±	0.48	1.000
7	3.23	±	0.47	3.42	±	0.63	3.33	±	0.51	3.24	±	0.48	0.551
8	3.19	±	0.48	3.37	±	0.64	3.28	±	0.50	3.23	±	0.42	1.000
9	3.21	±	0.41	3.30	±	0.61	3.32	±	0.50	3.16	±	0.48	1.000
10	3.17	±	0.43	3.27	±	0.58	3.26	±	0.46	3.11	±	0.40	1.000
							VE (L/min)						
1	**99.3**	**±**	**18.8***	**83.3**	**±**	**15.1***	87.3	±	24.1	86.7	±	13.8	**0.031**
2	114.8	±	23.6	112.3	±	23.7	106.4	±	32.6	112.1	±	17.7	1.000
3	120.6	±	21.1	115.5	±	24.1	119.6	±	22.9	116.1	±	21.1	0.796
4	120.8	±	26.5	120.5	±	26.2	120.4	±	34.9	120.1	±	24.1	1.000
5	124.9	±	23.6	118.9	±	23.2	119.9	±	34.0	125.0	±	24.4	0.296
6	127.2	±	26.9	123.5	±	26.7	122.7	±	36.3	125.3	±	23.2	0.659
7	125.9	±	25.6	126.8	±	24.0	120.1	±	34.7	127.0	±	23.0	1.000
8	128.9	±	23.0	129.4	±	23.0	123.6	±	35.5	128.2	±	22.9	1.000
9	126.9	±	22.0	128.5	±	23.4	122.8	±	34.8	124.5	±	23.6	1.000
10	126.5	±	22.4	123.4	±	25.2	118.2	±	35.3	127.3	±	23.6	1.000
							HRmax (bpm)						
1	162	±	12	159	±	12	158	±	12	156	±	13	0.111
2	**168**	**±**	**10†**	165	±	12	163	±	12	**162**	**±**	**13†**	**0.045**
3	168	±	11	165	±	12	165	±	13	165	±	14	0.217
4	168	±	12	167	±	12	168	±	12	166	±	12	0.248
5	169	±	11	169	±	13	169	±	10	168	±	12	0.263
6	171	±	11	169	±	11	169	±	10	169	±	11	0.749
7	172	±	11	170	±	10	169	±	9	170	±	10	0.272
8	173	±	12	172	±	11	170	±	9	171	±	10	0.833
9	173	±	11	170	±	10	172	±	11	170	±	8	1.000
10	172	±	11	171	±	10	171	±	10	171	±	8	1.000

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by:* = No MG and MG1; † = No MG and MG6.

A significant increase of BLa accumulation was recorded post warm-up when no mouthguard was worn in comparison to using any mouthguard design (31.0 – 44.0%, p = 0.001) (Figure 3-4). These statistical differences also occurred following the third sprint. Despite the lack of statistical significance throughout the rest of the protocol, the lowest accumulation of BLa was recorded with MG1; up to 14.0% lower value compared to when MG4 was used and up to 8% lower compared to MG6.

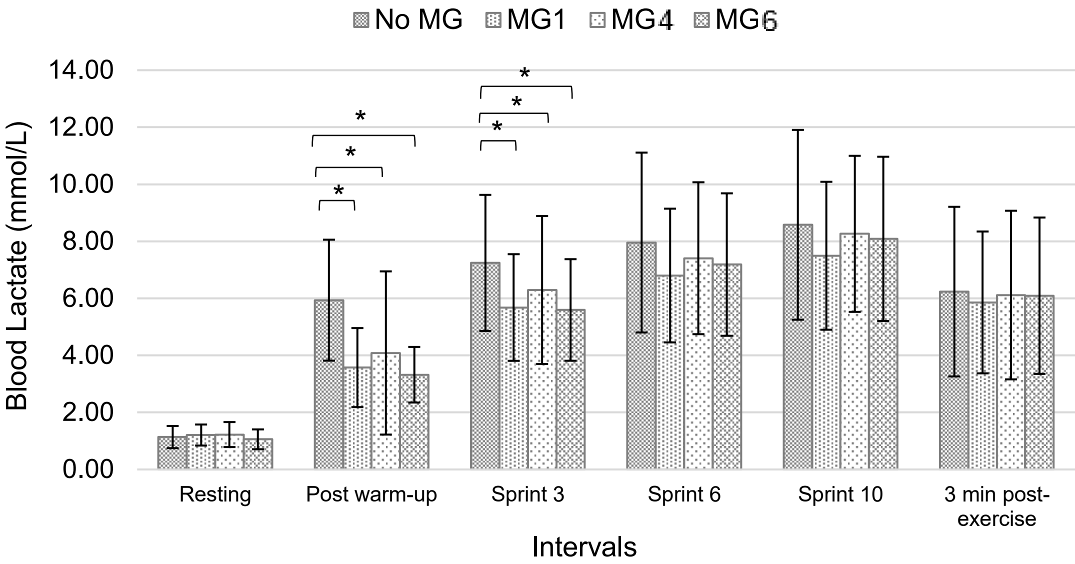

Figure 3-4: Blood lactate levels measured at rest, throughout the rugby protocol and 3 min post-exercise whilst wearing no mouthguard (No MG) and any of the three selected designs (MG1, MG4 or MG6) (N = 14). Error bars represent the standard deviation. Statistical differences ($p < 0.05$) are highlighted by: * = MG1 and MG4; MG1 and MG6; MG4 and MG6.

Participants' perception of exertion demonstrated that exercising without a mouthguard was significantly harder than whilst using MG1 or MG4 ($p \leq 0.05$). Although significant differences were recorded only during the first half of the protocol, Figure 3-5 illustrates that the same trend was followed throughout the whole protocol. In contrast, wearing the three mouthguards demonstrated similar perceived exertion; starting with rating of 9.0 ± 0.00 ('Very Light'), which increased to 15.33 ± 0.58 ('Hard/ Heavy') after the last sprint.

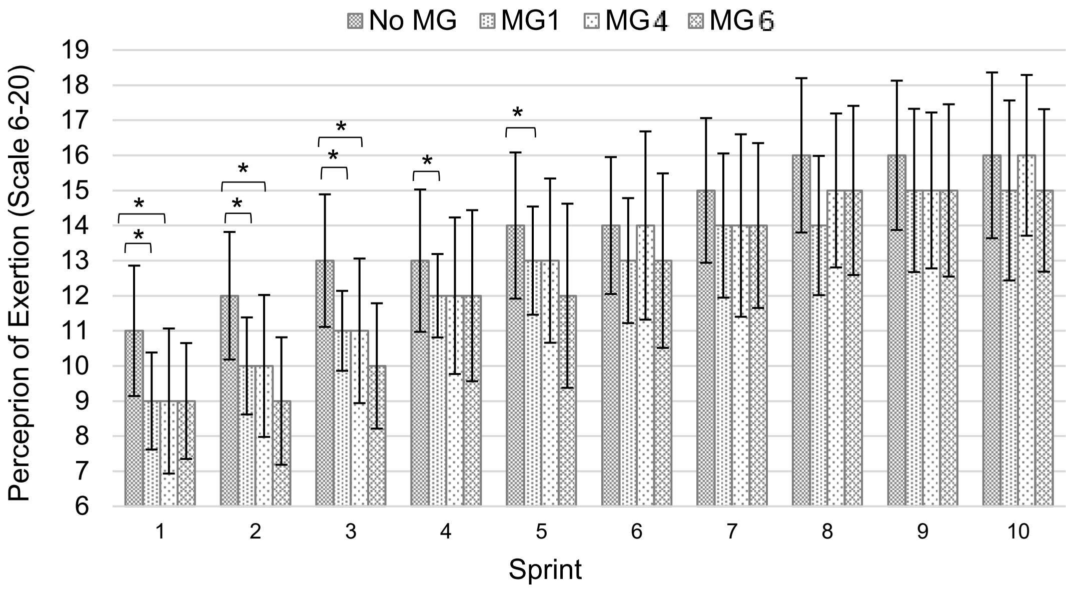

Figure 3-5: Participants' perception of exertion following each sprint (1 – 10) (*N* = 14). Error bars represent the standard deviation. Statistical differences ($p < 0.05$) are highlighted by: * = No MG and MG1; No MG and MG4.

3.3.3 Level of Comfort

Table 3-6 shows participants' usage of the three mouthguard designs prior to completing a questionnaire to assess mouthguards' level of comfort. However, due to incomplete questionnaire, there was some missing data in relation to the period each device was used for. Most participants wore the mouthguards for 2 hr or less. MG1 and MG6 were used similarly for both training and competition by 35.0% of the participants, whereas MG4 was used the least (21.4% participants during training and 7.1% during competition).

Table 3-6: Number of participants using each mouthguard (MG1, MG4, MG6) for ‘2 hr or less’, ‘3 – 5 hr’ or ‘6 hr or more’ during training and/or competition.

Type	2 hr or less	3 – 5 hr	6 hr or more	Use during training	Use during competition
MG1	9	3	1	5	4
MG4	10	1	1	3	1
MG6	8	1	2	5	4

MG4 was significantly more comfortable compared to the other two devices ($p \leq 0.017$) (Figure 3-6). In addition, it was rated more retentive than MG1, causing less interferences with concentration ($p \leq 0.041$). However, MG6 had the highest retention score and participants felt it provided the highest protection level, which differed significantly to MG1 ($p = 0.019$). In terms of the total rating score (mean of all characteristics), MG1 was rated statistically lower than MG4 and MG6 (7.55 ± 0.85; 8.21 ± 1.00 and 7.58 ± 1.70 respectively; $p \leq 0.039$). Likewise, participants were more likely to wear MG4 or MG6 again rather than MG1 ($p \leq 0.049$). Nevertheless, regardless of the design, 73.0% of the participants were likely to use the devices again.

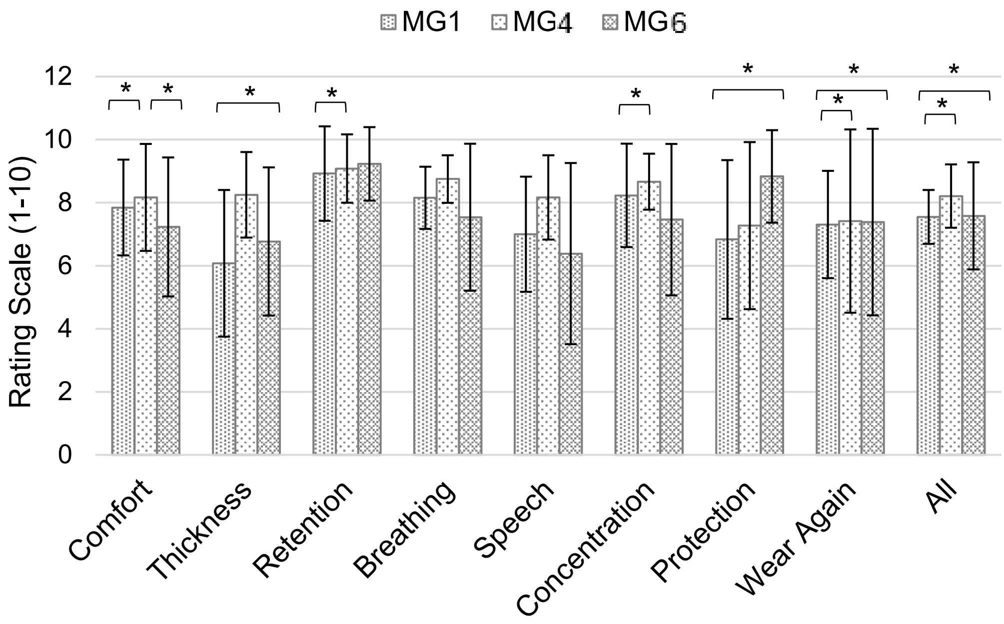

Figure 3-6: Participants' rating of mouthguard designs (MG1, MG4 and MG6) on a scale of 1-10 with error bars representing the SD (N = 14). Statistical differences ($p < 0.05$) are highlighted by: * = MG1 and MG4; MG1 and MG6; MG4 and MG6.

3.4 Discussion

To the authors' knowledge, this is the first study to examine the effects of three different custom-made mouthguards on physiological parameters in male rugby union players performing a submaximal sprint protocol. Wearing no mouthguard demonstrated statistically greater VO_2 (L/min and ml/kg/min) compared to MG4 and MG6 during the first walking interval but lower VO_2 (L/min and ml/kg/min) compared to MG1 during the last deceleration interval. Additionally, VE was greater without a mouthguard than with MG1 during the first sprint. The reported findings did not demonstrate any trend and no other significant differences were observed in terms of consumption of oxygen and ventilation. Therefore, it could be concluded that wearing any of the three mouthguards did not obstruct respiratory flow during performance of the rugby exercise protocol. As reported in Section 3.3.2, significant increase in the mean and maximum HR was recorded over the first three repetitions of the four exercise intervals whilst performing without a device. However, wearing any of the three mouthguards did not have any influence on HR. Although statistically higher respiratory parameters were reported only when no mouthguard was used, it was noted that HR, levels of BLa and perceived exertion were also significantly higher than whilst participants performed with mouthguards. Therefore, it could be suggested that the increase in airflow was due to a greater participants' exercise effort rather than due to wearing no device.

In order to further examine the effects of mouthguards, calculations of percentage differences within parameters between the mouthguard conditions were made. Although not significant, MG1 led to 7.8 – 10.2% higher VO_2 (L/min and ml/kg/min) in comparison to wearing MG4 or MG6 during walking, accelerating and decelerating. However, having MG4 showed 5.7% higher oxygen uptake than having MG1 or MG6 whilst sprinting. Additionally, there was a trend throughout the protocol of a lower BLa accumulation whilst wearing MG1 compared to all other conditions, but significant only to No MG post warm-up and post third sprint. This showed that a design with a 5 mm single layer EVA and a 4 mm palatal flange contributed to increase airflow dynamics and lower BLa concentration, although not significantly, in comparison to having no mouthguard or designs such as MG4 and MG6.

Analysing cardiopulmonary parameters during each of the four exercise intervals was important as it provides more detailed observations. For example, examining acceleration has previously been proposed to be more important than sprinting due to the short sprint duration (85% of all sprints under 30 m) (Johnston et al., 2014). Therefore, breaking down the exercise protocol allowed reflection on valuable activity elements.

Two previous studies have examined the effects of 'boil-and-bite' and custom-made mouthguards in rugby participants. As with the present research, they both included males only, who performed an exercise activity during randomised sessions with and without mouthguards. Bourdin et al. (2006) tested 19 team players, 16 of whom played rugby (27 ± 4.8 yrs; 91.4 ± 18.6 kg). They were asked to perform three maximal 6 s cycling sprints with a 4 minute rest to assess submaximal aerobic work and then cycle until maximal exhaustion. The authors found no significant influence of wearing a 'boil-and-bite' or customised mouthguards on forced vital capacity, ventilation and maximum power between sessions. It should be considered that in comparison to the present study, they did not use breath-by-breath analysis; instead, they collected expired gas during the last 30 s of each stage. This led to more limited findings in terms of cardiopulmonary parameters than the current study. Additionally, although Bourdin et al. (2006) showed that BLa exceeded 9 mmol/L post VO_{2max} test, they did not examine the differences in BLa concentrations with and without devices. Only the shape of their proposed custom-made device was similar to MG4 from the present study. Both devices had an increased thickness over the occlusal surfaces of the posterior teeth. However, Bourdin et al. (2006) used polymethyl methacrylate on a metal framework to construct their mouthguard, instead of much more commonly used EVA. In contrast, the custom device proposed by Duarte-Pereira et al. (2008) was a laminated pressure-formed mouthguard, made of EVA. The authors evaluated the performance of 10 rugby players (21-23 yrs; 84 kg) through counter-movement jumps and rebound jumps of 15 s over a force platform. There were no significant differences within the players' power during both jumps but a tendency to attain lower performance with fitted mouthguards was reported. Although Duarte-Pereira et al. (2008) used vertical jumps as an assessment protocol, which are also important elements in rugby, however their findings were not evaluated in detail. They reported no significant effects on spirometry parameters such as forced expiratory volume and forced vital capacity when a device was worn. In contrast to the present study, respiratory flow was not examined during the exercise activity. Both Bourdin et al. (2006) and Duarte-Pereira et al. (2008) addressed rugby specific exercise, commonly used during training and competition games, such as cycling sprints and vertical jumps. However, their protocols were much shorter than the protocol proposed in the present study. It could be argued that if they examined the same parameters over a longer exercise protocol, their findings could have highlighted further observations. The role of multiple-sprint protocols to assess players' performance has been previously documented and hence it was decided to use this type of activity to assess changes in the aerobic metabolism (Jenkins and Reaburn, 2000). Gharbi et al. (2014) demonstrated that when participants performed 10 x 30 m shuttle sprints, there was a 4.5% speed decrement compared to 1 x 30 m sprint. Therefore, to limit any speed variations and avoid pacing differences between the sprints, the study protocol consisted of pre-set speeds for all intervals, which was advantageous during interpretation of the current findings.

The level of participants' aerobic fitness in relation to VO_{2max} was slightly lower than previously reported VO_{2max} (55.3 ± 1.2 ml/kg/min) values within professional rugby union players (*N* = 14; 26 ± 1.9 yrs; 97.6 ± 13.2 kg) (Dubois et al., 2018). However, it was close to the VO_{2max} (53.8 ± 3.4 ml/kg/min) of elite-level rugby 7s players (*N* = 18; 21.9 ± 2.0 yrs; 89.7 ± 7.6 kg) (Higham et al., 2013). This demonstrated good aerobic fitness of participants in comparison to players competing at first division level. Although at the end of the study, VO_{2max} and BLa post-exercise were higher than those recorded at the beginning of the study, these were not statistically different. Therefore, it could be concluded that there was no training effect on participants' aerobic metabolism that resulted from the consecutive sprint sessions.

GPS tracking data reported that during a game of elite rugby union teams players perform at ~ 80 – 85% VO_{2max} and ~ 88% HR_{max} (Cunniffe et al., 2009). This was also confirmed by a more recent study that examined elite rugby officials and observed most time spent between 81 – 90% HR_{max} with no significant difference between the two halves of the game (Blair et al., 2018). The intensity of the present protocol in terms of VO_{2max} (84 – 93%) and HR_{max} (92 – 96%) was close to what has been reported but still up to 8% higher. However, mean BLa concentration after the last sprint (8.10 ± 0.46 mmol/L) was within a range observed previously during a game (5.1 to 9.8 mmol/L) (McLean, 1992; Deutsch et al., 1998).

Prior to the start of the study, all participants had past experience of wearing different mouthguard types (e.g. 'boil-and-bite' and custom-made). This possibly contributed to a more accurate individual perception of comfort level. Overall, the most favoured design was MG4 that had no palatal flange and was thicker over the occlusal surfaces of the posterior teeth. Rugby is a sport where communication with teammates is important and the present findings showed that trimming the palatal flange up to the gingival margin was beneficial in relation to speech as shown within Figure 3-6. This was in agreement with previous findings (Maeda et al., 2006; Gebauer et al., 2011; Nozaki et al., 2013; Gomez-Gimeno et al., 2019). However, in terms of retention, MG6 had the highest score, which agreed with the findings presented in Chapter 2. Additionally, participants reported that MG6 provided the highest protection. This was the only device fabricated with two EVA layers that increased the anterior (labial) region thickness. Hence, participants perceived that this mouthguard would absorb greater impact force. Nevertheless, participants felt that MG6 impeded their comfort, breathing, speech and concentration the most, possibly due to being bulkier than MG1 and MG4. An important finding was that MG1, which is usually the most common mouthguard design, had the lowest rating and participants were least likely to wear it again. One reason could be the extended palatal flange that led to significantly lower comfort compared to MG4. In addition, participants thought that the thickness and protection level were not as good as in the other two mouthguards. These findings should be considered by dental technicians and suppliers of mouthguards in order to provide suitable devices for individuals participating in sports. User compliance with mouthguards may increase if participants are made aware of the variations in custom-made designs and their benefits.

Participants' perception of comfort could be a defining factor for their compliance and frequency of using the device. Similar to the current study, Collares et al. (2014) used a scale of 10 (with 10 having the greatest merit) to examine participants' opinion on a custom-made device fabricated with 3 mm EVA blank. The mouthguard design was identical to MG1 from this study (4 mm palatal flange; extending up to the distal surfaces of the first molars). However, their participants did not have previous experience of wearing any mouthguard type. Hence, the authors completed the assessment pre and post two weeks usage of the device. Collares et al. (2014) reported much lower score compared to the present study in terms of communication (pre 2.12 ± 1.55; post 3.83 ± 2.50) and breathing (pre 5.63 ± 2.52; post 6.80 ± 2.56).

Previous literature has reported that the physiological demands within rugby union vary depending on the players' position (Nicholas, 1997; Jenkins and Reaburn, 2000; Ziv and Lidor, 2016; Brazier et al., 2018). For instance, forwards have a greater dependency on anaerobic metabolism due to being involved in game elements that require greater body mass and absolute power and strength. In contrast, backs' demand higher aerobic work to be able to perform repetitions of high-intensity sprints and quickly accelerate and decelerate (Brazier et al., 2018). However, the present study did not address players position, as one of the aims was to use a standardised protocol within all participants in order to reflect on the effect of mouthguard usage. The sports protocol within this study included four speed intervals (walking, acceleration, sprinting and deceleration) that were found to be essential during a rugby union game (Jenkins and Reaburn, 2000; Cunniffe et al., 2009; Ziv and Lidor, 2016; Dubois et al., 2018). It may be argued that five sprint bouts (5 – 15 s) with 25 – 30 s passive recovery are sufficient to examine the physiological demands in field-based sports (Nicholas, 1997; Cunniffe et al., 2009; Chaouachi et al., 2010; Gharbi et al., 2014). However, due to longer recovery intervals consisting of 1 min walking, the current protocol consisted of ten sprints.

The current research did not include any female participants. It has been established that males have different metabolic and respiratory rates to females that lead to variation in responses to exercise and higher aerobic power (Sheel et al., 2004; Harms, 2006). Hence, it was decided to avoid introducing another gender. In addition, despite investigating the use of three bespoke custom-made mouthguards, 'boil-and-bite' devices were not examined. It could be argued that this is a disadvantage due to the high popularity of this type of mouthguard amongst participants. In order to increase the validity of the current findings, future studies should include a larger sample size and divide the volunteers according to their position-specific demands. In addition, further work should make use of portable gas analysers in order to examine the effects of mouthguards on other rugby-specific qualities such as repeated shuttle run sprints and jump abilities; previously validated as an effective practice in rugby union (Buchheit, 2010; Darrall-Jones et al., 2015).

The amount of airway opening caused by different mouthguard deigns and how that contributes to airflow should be further investigated. For instance, Schultz Martins et al. (2018) used a cone beam computer tomography (CBCT) scan to examine the effects of jaw-repositioning customised device on the upper airway volume and width. They demonstrated a significant interaction between jaw repositioning, volumetric change and increase in both aerobic and anaerobic performance. Although, it is challenging to report optimal mouthguard dimensions due to individual differences in anatomy, future research should aim to propose an approximate range to be met by custom-made devices.

3.5 Conclusions

The present study is believed to be the first to report the effects of three customised mouthguard designs on breath-by-breath gas exchange and blood lactate concentration in rugby participants performing a newly designed rugby-specific exercise. Wearing any of the three selected custom-made mouthguards did not have an impact on oxygen consumption, ventilation, heart rate and blood lactate. However, having no device demonstrated significant increase in these parameters and participants' perceived exertion during the first three repetitions of the chosen exercise intervals. Therefore, using custom-made mouthguards led to lower exercise effort, which is beneficial for participants. It is worth mentioning, that although the parameters recorded whilst using any of the mouthguard designs did not demonstrate any significant differences, there was a trend of increased airflow and decreased blood lactate when MG1 was used compared to MG4 and MG6. In terms of participants' perceived comfort, MG4 was the most favoured design except for protection and retention, where participants preferred MG6.

The following chapter of this thesis will examine the use of the same customised mouthguards but within boxing participants following a sport-specific exercise protocol. Although mouthguards in boxing are mandatory, there is a great lack of research assessing their influence on physiological parameters.

Chapter 4 : The Effects of Various Customised Mouthguard Designs on Physiological Parameters and Comfort in Male Boxers

4.1 Introduction

Since the 2012 Olympic Games in London (UK), the participation in combat sports has grown continuously. A survey by Sport England (2012) found that people taking part in boxing increased by 46.0% during 2011 to 2012 and similarly England Boxing (2012) reported that there were 30,000 more participants compared with the year before. In addition, the Active People Survey showed that the number of people aged 16 - 25 yrs and 26+ yrs were higher than previous years, 79,100 and 87,300 respectively (England Boxing, 2012; Sport England, 2012). In the same year, participants in the USA (aged 6 yrs or older) also increased by 1.36 million (Statista, 2017).

Boxing is a contact sport, where participants are exposed to high repetitive punch forces. These forces have been reported to range from 2,381 ± 328 N for novice boxers to 4,800 ± 601 N for elite boxers (Smith et al., 2000; Ifkovits et al., 2015). A study over a 16 year period demonstrated that 89.9% of the injuries during a professional boxing fight were to the head, neck and face (Zazryn et al., 2003). The data reported was equivalent to 224.8 injuries per thousand-fight participations (427 boxing fight participations; males; 27.3 yrs) with the highest rate of injuries to the orbit region (45.8%). In the USA, 165,602 boxing injuries led to visiting an emergency centre over a 19 year period and 22.5% of them were related to the head and neck (Potter et al., 2011). People aged 18 - 24 yrs (N = 61 280, 37.0% of the total sample) were most likely to have an injury compared to other age groups. Additionally, it was reported that 87.0% of these traumas involved the face, orbit region and mouth; with fracture being the most common diagnosis (27.5%), followed by soft tissue injuries (26.0%). Furthermore, previous work found that contact sport athletes, such as boxers and wrestlers, have 33.0 - 72.0% chance of sustaining dental injuries (Kumamoto and Maeda, 2004). Emerich and Nadolska-Gazda (2013) assessed the frequency of dental trauma and reported 35.9% of the participated amateur boxers (*N* = 338; males) to have sustained tooth injuries; with crown fractures being the most common (40.7%), followed by tooth loss (21.9%). The authors divided the participants into four age groups – schoolboys (*N* = 49, 13.6 ± 0.8 yrs), juniors (*N* = 106, 15.6 ± 0.5 yrs), youths (*N* = 66, 17.5 ± 0.5 yrs) and seniors (*N* = 117, 21.2 ± 2.8 yrs). The senior boxers, with 5.7 ± 3.4 yrs of practice, were found to have the highest number of dental injuries (*N* = 52) compared to the rest of the groups (*N* = 13, *N* = 30, *N* = 26 starting from the youngest). Likewise, similar results were found by Al-Arfaj et al. (2016), who surveyed 124 participants (23.7 ± 5.4 yrs), and reported crown fracture and avulsion as the most predominant dental traumas in direct and non-direct sports participants; 34.8% and 12.0% respectively. Compliance with protective equipment, safety regulations and education about the risk of injuries are factors of high importance to reduce the rate of trauma in boxing (Potter et al., 2011).

In 2013, the Amateur International Boxing Association announced that head guards were no longer required for elite male amateur boxers aged 19 - 40 yrs, but the use of mouthguards was still compulsory during all bouts (Davis et al., 2017). However, a study found that during training 43.0% (*N* = 144) of boxers used mouth protection occasionally and 13.7% (*N* = 46) used no protection at all (Emerich and Nadolska-Gazda, 2013). In addition, Ifkovits et al. (2015) showed similar results during boxing, 34.1% (*N* = 74) mostly wore their mouthguards, 17.5% (*N* = 38) rarely to never and only 9.7% (*N* = 21) used their mouthguards at practice sessions. These results have demonstrated that sometimes the role of mouth protection is underestimated, and participants are not aware of the long-term consequences of dental injuries or are non-compliant. Taking preventative measurements to minimise the likelihood of orofacial trauma is vital at all times in boxing and the effectiveness of mouthguards as sport protective devices has been shown previously (Knapik et al., 2007; Galic et al., 2018).

Questionnaire–based studies have reported that if the level of mouthguard comfort and breathing impedances were improved, the popularity and use of the devices in contact sports would increase (Brionnet et al., 2001; Kececi et al., 2005; Raaii et al., 2011; Boffano et al., 2012; Lee et al., 2013; Liew et al., 2014). Lee et al. (2013) assessed the attitudes of taekwondo athletes competing at national and international level (*N* = 152; 109 males, 43 females). Their findings showed that 73% of the participants reported that mouthguards caused airway obstruction and 34.8% found them uncomfortable. A more recent study by Galic et al. (2018) surveyed 229 participants (59 water polo, 58 karate, 57 taekwondo, 55 handball; 157 males, 72 females; 12.9 ± 3.2 yrs) and reported that 37% thought wearing a mouthguard was unnecessary, 21.5% said it was uncomfortable and 5.2% had issues with breathing and communication. In order to achieve good impact absorption, mouthguards have often been fabricated thicker and this could feel bulkier, uncomfortable and have breathing issues due to thickness of 3-4 mm (Westerman et al., 2002; Yamada, 2006; Gawlak et al., 2015). However, fabrication techniques in terms of design could be applied to minimise the thickness of the palatal aspect to decrease interferences with speech and breathing and still maintain the protective properties of the device (Maeda et al., 2006; Nozaki et al., 2013). For example, Maeda et al. (2006) found a significant increase in comfort and improvements in breathing, communicating and swallowing when the palatal margin of the mouthguard was trimmed around the margins of the teeth (*N* = 17; 4 males, 13 females; 22.3 yrs) ($p < 0.01$). Nozaki et al. (2013) also supported the proposal of finishing the mouthguard at the palatal gingival line. The authors showed that there was an improvement in speech when such design was used compared to when participants wore a device with 4 mm palatal extension (*N* = 18; 7 males, 11 females; 19.2 yrs). However, these outcomes were based on the individuals' perception. Therefore, a more in-depth assessment of the effects of different mouthguard designs on the above issues is required.

Chapter 1 (Section 1.4) and Chapter 3 (Sections 3.1 and 3.4) of this thesis have examined the influence of different types of mouthguards on performance and physiological parameters within various sports. However, it was found that there is a distinct lack of literature addressing the effects of mouthguard design on respiratory parameters during boxing where the use of mouth protection is mandatory. Previous studies have mainly examined taekwondo and martial arts athletes wearing custom-made devices (Kececi et al., 2005; Cetin et al., 2009; Rexhepi and Brestovci, 2013; Yarar et al., 2013; El-Ashker and El-Ashker, 2015). Kececi et al. (2005) tested 11 males and 11 females from the Turkish taekwondo national team (16.0 ± 1.1 yrs; 6.7 ± 2.5 yrs of training) on a maximal exertion 20-m shuttle run with a portable breath-by-breath analyzer. They found no significant differences within oxygen uptake (VO_2 51.79 ± 2.12 vs 52.73 ± 1.81 ml/kg/min), minute ventilation (VE 106.32 ± 5.75 vs 108 ± 4.14 L/min), tidal volume (1.91 ± 0.08 vs 1.88 ± 0.07 L/min), respiratory exchange ratio (RER 1.11 ± 0.01 vs 1.12 ± 0.01) and heart rate (HR 198 ± 1.41 and 198 ± 1.83) whilst performing without and with a custom mouthguard. In addition, Cetin et al. (2009) also investigated the effects of a custom-made mouthguard in 11 male and 12 female taekwondo athletes (17.0 ± 1.34 yrs; 7.07 ± 2.84 yrs of training). However, in comparison to Kececi et al. (2005), they examined changes in anaerobic power and reported that using a customised mouthguard did not impair significantly sprinting time, squat jump, counter-movement jump, handgrip strength, isometric leg and back strength ($p > 0.05$). Nevertheless, Cetin et al. (2009) reported that when participants performed a 30-s Wingate anaerobic test on a cycle-ergometer, hamstring peak torque and average and peak power with a mouthguard showed a significant increase (146.3 ± 16.82 Nm vs 154.29 ± 21.47 Nm; 7.01 ± 0.88 vs 7.24 ± 0.99 W/kg; 9.09 ± 1.49 vs 9.54 ± 1.52 W/kg, respectively). Similarly, Yarar et al. (2013) used the same anaerobic test and observed no changes between wearing a mouthguard and no mouthguard in the average power output, right and left handgrip strength and VO_{2max} in eight senior combat sport males (22.0 ± 2.2 yrs) (530.16 ± 71.37 W vs 533.53 ± 76.59 W; 48.56 ± 5.10 kg vs 48.72 ± 5.88 kg; 49.90 ± 5.32 kg vs 48.63±6.96 kg; 53.37 ± 5.57 ml/min/kg vs 53.87 ± 6.42 ml/min/kg, respectively). However, the aforementioned studies have assessed parameters during a shuttle run, cycle ergometer test or upper and lower body strength exercises, which are not typical elements from taekwondo or any other combat sports.

Currently, only two studies have previously assessed the use of mouthguards in boxers. Rexhepi and Brestovci (2013) examined 20 male athletes participating in boxing and karate (21.4 ± 4.9 yrs; 71.8 ± 8.9 kg) who competed at an elite level. The participants were asked to perform a maximal exertion exercise on four occasions – without a mouthguard and with three different mouthguards. The highest oxygen consumption and minute ventilation were recorded when boxers used a custom PlaySafe device (VO_{2max} = 55.53 ± 5.57 ml/min/kg; VE = 143.2 ± 19.19 L/min) compared to maxillary boil-and-bite (VO_{2max} = 52.65 ± 6.48 ml/min/kg; VE = 130.1 ± 19.72 L/min), bi-maxillary mouthguard (VO_{2max} = 49.21 ± 7.03 ml/min/kg; VE = 111.41 ± 13.86 L/min) and without a mouthguard (VO_{2max} = 51.47 ± 6.49 ml/min/kg; VE = 132.42 ± 23.63 L/min). However, the study did not provide any details of thickness and design of the mouthguards. El-Ashker and El-Ashker (2015) also examined only male boxers (*N* = 18; 19.4 ± 2.0 yrs; 74.5 ± 5.1 kg; 3.8 ± 1.8 yrs of training) who performed a running protocol on a treadmill without a mouthguard and with two different types of the device - stock and customised. Similar to Rexhepi and Brestovci (2013) and Kececi et al. (2005), the authors used breath-by-breath analysis and reported that wearing a stock mouthguard could hinder VO_2 (ml/kg/min) during high intensity exercise, whereas custom-made devices showed no interferences.

Accurate assessment of any changes in physiological parameters in boxers could be limited when exercise machines such as treadmill and cycle ergometer are used due to lack of sports-related movements. Boxing is a combat sport, which involves punching one’s opponent and defensive movements involving the whole body, which suggests that possibly the most accurate way of monitoring respiratory flow is through a simulation of a boxing match (Davis et al., 2013; Davis et al., 2014). The number of rounds in amateur boxing differs, depending on the boxers’ category. For example, 3 x 2-min rounds for novice boxers, 4 x 2-min rounds for intermediate boxers and 3 x 3-min for open-class boxers (Davis et al., 2013; Slimani et al., 2017). Due to the nature of the sport, high levels of both aerobic and anaerobic fitness is required in order to maintain muscular strength, speed and defence during a boxing bout with short recovery intervals (El-Ashker and Nasr, 2012; Chaabene et al., 2014; Slimani et al., 2017).

Studies have previously investigated the effects of boxing exercise and training regimes on physiological responses, highlighting the importance of monitoring improvements in respiratory flow and muscle fatigue (Kravitz et al., 2003; Akalan et al., 2004; Ghosh, 2010; El-Ashker and Nasr, 2012; de Lira et al., 2013; Rexhepi and Brestovci, 2013; Lovell et al., 2013; Bruzas et al., 2014; Kim et al., 2014; Chaabene et al., 2014; Kamandulis et al., 2018). This is usually assessed through maximal exertion tests, which allow coaches to track changes in athletes' performance. For instance, Lovell et al. (2013) used a 30-s Wingate test to examine improvements in VO_{2max}, HR_{max} and blood lactate (BLa) levels of a world ranked mixed martial arts participant (25 yrs; 90.2 kg) prior to and post six weeks of preparation for a fight. Likewise, other past studies have also used the same parameters to assess metabolic changes in male boxers (El-Ashker and Nasr, 2012; Davis et al., 2014). Davis et al. (2014) proposed an exercise protocol that included simulation of a boxing match in a ring against handheld pads, which was based on video footage of past competitive matches. They demonstrated that the metabolic energy demands in semi-contact amateur boxing (3 x 2-min) are about 85.0% aerobic. The authors emphasised the importance of using exercise protocols that replicate the physiological demands of participants during a boxing match. In order to achieve accuracy in recording the assessed respiratory measurements, they also requested participants to wear their protective gear, including their own mouthguard. However, Davis et al. (2014) did not consider the possible effects of mouthguards whilst testing, which could have potentially affected their reported findings. As mentioned previously, different sports mouthguard types could have a different or no effect on cardio-pulmonary parameters. The findings of past literature have suggested that using customised devices does not have a negative impact on aerobic and anaerobic metabolism whilst performing. However, there is a lack of research investigating the features of custom-made devices, which could possibly minimise interferences with performance in terms of respiratory gas exchange. This is important especially in elite level athletes where small improvements in physiological biomarkers could be meaningful. The question whether custom-made mouthguards hinder physiological parameters by obstructing the airflow or improve physiological parameters through an increase of airflow should be addressed further.

Therefore, the aim of the current chapter was to investigate changes in respiratory flow, blood lactate accumulation and perception of comfort in male boxers using different custom–made mouthguard designs, which were tested in Chapter 2 of this thesis. This will be achieved by designing a new exercise protocol that accurately reflects individuals' responses in terms of absolute and relative oxygen uptake, minute ventilation, carbon dioxide production, respiratory exchange ratio, heart rate and blood lactate when no mouthguard or any of the three designs are used. Finally, participants will be asked to complete a questionnaire rating their experience in terms of possible impairments due to wearing a mouthguard. It is hypothesised that there will be no differences in the examined physiological parameters when exercise is performed with and without any of the mouthguards. In addition, the design of devices will not be an influential factor on the participant's perception of breathing and comfort-related issues.

4.2 Methods

Prior to any experimental work, ethical approval was obtained and granted from the School of Healthcare Science, Faculty of Science and Engineering, Manchester Metropolitan University (Ethics Number: SE151683). Additionally, the procedures included in the study design followed the ethical principles outlined in the Declaration of Helsinki (World Medical, 2013). Participants were provided with detailed information about the study objectives, full protocol and the possible risks and benefits of taking part prior to obtaining informed consent. All participants had the right to withdraw at any time during the study.

Initially, 20 elite male boxers, from the same boxing academy, were recruited for the study. Five of them withdrew from the study, due to work/ university commitments, one participant deceased and 14 completed all sessions. All participants trained for at least 10 hours per week and had previously worn a mouthguard. The average participation in sport was 7 ± 9.38 yrs, with a mean time of competing 5 ± 7.85 yrs. The anthropometric data of the sampled population are shown in Table 4-1.

Table 4-1: Description of the sampled population (*N* = 14).

Variable	**Mean**	**SD**
Age (yrs)	26	8
Mass (kg)	80	11
Height (cm)	178	4
Body Mass Index	25	4
Hb (g/dL)	16.0	0.9
Lung Function (%)	79	7
VO_{2max} (ml/kg/min)	54	7

Although the present study includes a different cohort of participants, the same study design and experimental procedures were included as documented in the previous study (Chapter 3, Sections 3.2.1, 3.2.2 and 3.2.4). Hence, the same medical and dental assessments (Appendix A) were completed to ensure none of the participants had any present injuries, related cardiovascular problems, temporomandibular disorder or any trauma of the oral and facial structures. Additionally, participants were required to be physically fit and taking no form of medication, which could affect airflow, muscle fatigue and HR. A capillary finger prick blood sample was also taken to determine the levels of haemoglobin (Hb) (HemoCue® 201+ System, Crawley, England) and if any signs of anaemia (Hb < 13 g/dl) were present, the participants were excluded from the study.

4.2.1 Fabrication of Mouthguards

The procedures from impression taking to mouthguards fabrication followed the same principles as described in Chapter 3, Section 3.2.1. In brief, a dental clinician took alginate dental impressions (Tropicalgin®, Zhermack SpA, Italy) of both the maxillae and the mandible for each participant. Dental casts for fabrication of mouthguards were produced firstly by pouring vacuum mixed Kaffir-D dental stone (3:1 powder/ liquid ratio) (John Winter & Co Ltd., Halifax, UK) and then duplicated in model resin (Rhino Rock, DB Orthodontics, Silsden, UK). The same three mouthguard designs were fabricated for each participant in the dental laboratories at Manchester Metropolitan University. All mouthguards were pressure-formed (BIOSTAR®, SCHEU-DENTAL GmbH, Iserlohn, Germany) and made of clear ethylene vinyl acetate (EVA) blanks, 120 mm Ø (Bracon Ltd, Heathfield, UK). Figure 4-1 shows the selected mouthguard designs.

Figure 4-1: Three customised mouthguards (MG1, MG4 and MG6) used by all participants.

To recap, the designs were selected based on previous literature and demonstrated high retention level during the tests evaluated in Chapter 2. MG1 was fabricated with a 5 mm single EVA blank and it had a 4 mm gingival palatal flange. In comparison, MG4 and MG6 were fabricated with two EVA layers (2 mm and 4 mm) and were trimmed around the palatal gingival margins. MG4 consisted of a double layer at the occlusal surfaces of the posterior region only, whereas MG6 had a double EVA layer covering all anterior teeth and the occlusal surfaces of the posterior teeth. MG1 and MG4 were finished at the distal surfaces of the first maxillary molars, whereas MG6 extended over the second maxillary molars. All devices had a full buccal flange. The mouthguards were disinfected, placed into storage boxes and labelled with the participant's reference number. The labels were colour coded depending on the mouthguard design.

4.2.2 Baseline Measurements and Aerobic Fitness Assessment

Laboratory temperature and humidity conditions were consistent during all visits, 20.0 ± 0.6 °C and 50 ± 4 %, respectively. In addition, to control any possible variability in the data, the participants were asked to record their dietary intake and physical activity levels 24 hr prior to every laboratory testing session. They were also told to restrain from caffeine and alcohol, and to fast at least 2 hr prior to testing.

Again, in order to assess any possible differences in the participants' aerobic fitness between the start and end of the study, two maximal exertion tests were performed. The primary baseline measurements during these sessions included: stature, body mass, blood pressure, HR, Hb, BLa and lung capacity. All of these measurements followed the procedures described in Chapter 3, Section 3.2.2, except for BLa and lung capacity. Calibrated Biosen C-line Analyser (BIOSEN C-Line™, EKF Diagnostics, Cardiff, UK) was used to analyse the BLa samples instead of a portable BLa analyser. Spirometry tests were completed with the use of a Vitalograph (Vitalograph® Ltd., Buckingham, UK). Participants were wore a nose clip and exhaled as much air as possible, maintaining the breath out for approximately 6 seconds. The percentage Vital Capacity was calculated by the ratio between Forced Expiratory Air Volume at 1 second and the Forced Vital Capacity (maximum amount of air exhaled).

The VO_{2max} tests involved the same incremental exercise on a treadmill (Woodway GmbH, Weil am Rhein, Germany) until maximal exhaustion. Following a 10 min warm-up run, the test started at 0% treadmill incline, which was increased by 1% every minute, whilst the speed remained unchanged (10 ± 0.65 km/h). During the last 10 s of each one-minute stage, participants were asked to rate their level of perceived exertion (RPE) on a standard Borg scale (6 – No Exertion to 20 – Maximal Exertion). The same researcher provided verbal encouragement to help participants achieve maximal exertion during the VO_{2max} tests. The test was terminated when the participant jumped to the sides of the treadmill. Finally, a 10 min cool down was completed and a 3 min post-exercise BLa sample was taken.

The gas exchange parameters assessed during each test session (alongside performance with mouthguards) included absolute (L/min) and relative oxygen uptake (VO_2 ml/kg/min), carbon dioxide production (VCO_2 L/min) minute ventilation (VE L/min) and respiratory exchange ratio (RER). Similar to the previous study, participants were fitted with a facemask, which was connected to a pre-calibrated breath-by-breath analyser (METALYZER® 3B, CORTEX, Leipzig, Germany). Additionally, HR (bpm) was monitored with a HR sensor (Polar H7, Polar Electro Ltd, Warwick, UK). A baseline reading of the above measurements was taken by asking the participants to remain at rest for one minute prior to exercise in order to exclude the effect of early VO_2 kinetics.

4.2.3 Repeatability of Sport Specific Boxing Protocol

A repeatability study of the newly designed protocol was conducted to assess the consistency of the examined physiological parameters. Five participants of the sampled population (21 ± 2 yrs; 81 ± 14 kg) were randomly selected to repeat the test on three occasions without wearing a mouthguard.

Each visit lasted an hour and consisted of the following: baseline measurements (body mass, HR and BLa), a warm-up and a boxing specific protocol. During the warm-up, participants were instructed to throw random combinations of punches over 2 x 3-min, with 1 min break. The sport specific protocol included 4 x 3-min boxing rounds, with 1 min rest after each round.

During each 3 min round, participants were given a verbal signal every 6 seconds and they performed a combination of four straight maximum punches (120 punches per round) against a punch bag (50 cm Ø x 140 cm, 60 kg) (PRO–BOX Colossus Punch Bag, JPLennard Ltd., UK). This was in order to control the intensity of each individuals' punches and limit any possible pacing. Hence, this was an attempt to achieve a standardised exercise intensity between testing sessions. Additionally, after each combination of punches, participants moved from the right-hand side to the left-hand side of the punch bag in order to increase the exercise intensity. A pair of 10 oz (0.28 kg) boxing gloves (Lonsdale, Shirebrook, UK) was provided to the participants; however, all participants used their own hand wrappings and training sportswear.

The same respiratory parameters as described in Section 4.2.2 were measured, using the same breath-by-breath analyser procedure. Similarly, capillary finger prick BLa samples were taken, however BLa level was measured at rest, post warm-up, following the end of each boxing round and 5-min post-exercise. In addition, immediately after each boxing round participants were asked to rate their perceived rate of exertion on a Borg scale.

4.2.4 Sport Specific Boxing Protocol

All participants (*N* = 14) had to perform four sessions consisting of the protocol described previously in Section 4.2.3. Figure 4-2 illustrates an example of a participant performing the boxing-specific protocol. During each session, participants were randomly assigned to perform with one of the three mouthguards or without a mouthguard. In order to minimise the placebo effect, they were not provided with any information about the differences in the characteristics and design of mouthguards. The stages that all participants went through in order to complete the study are summarised in Figure 4-3.

Figure 4-2: Breath–by–breath data collection through a facemask during the boxing-specific exercise protocol.

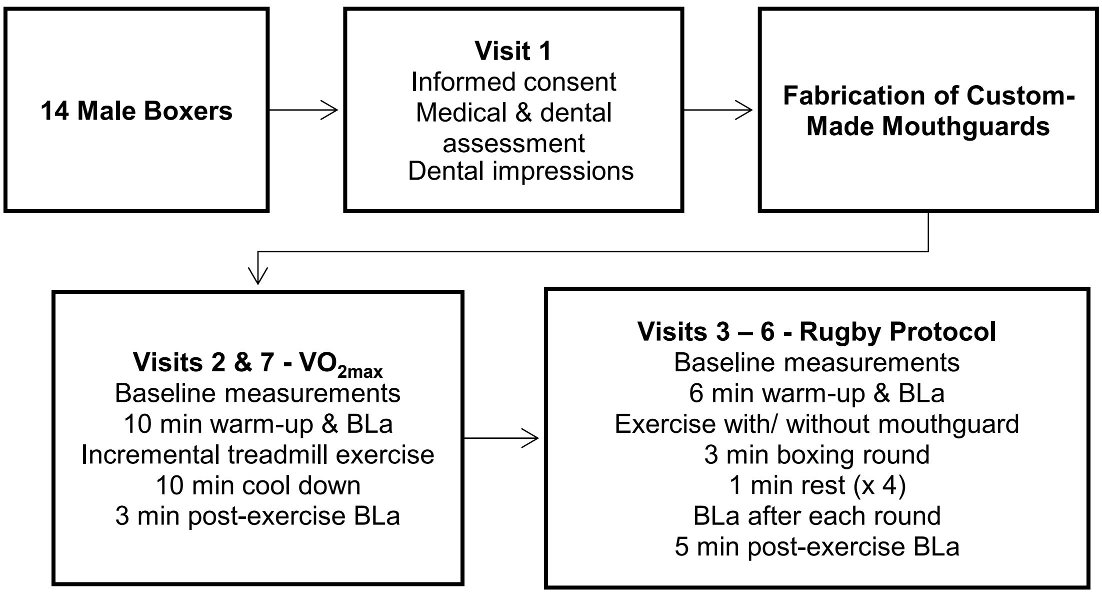

Figure 4-3: Flowchart illustrating the boxing protocol study design.

4.2.5 Assessment of Comfort

Following a mouthguard session, participants were asked to wear the device during training and competitions (if any) until their next laboratory visit. Similar to the previous study (Chapter 3, Section 3.2.4), they were then given a questionnaire (Appendix B) to assess their perception of comfort, thickness, retention, breathability, communication, effect on concentration, and the likelihood of wearing the mouthguard again. A scale of 1 – 10 to rate each of the characteristics was given, with 10 having the greatest merit. The final evaluation of each mouthguard characteristic was based on the mean score of all responses. As mentioned previously, the examined mouthguard characteristics in terms of participants' individual perception were selected based on previously published literature (Brionnet et al., 2001; Duarte-Pereira et al., 2008; Duddy et al., 2012; Lee et al., 2013; Liew et al., 2014).

4.2.6 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, Version 24.0. Armonk (IBM Corp., New York, US) and Microsoft Excel (2013). Prior to any analysis, distribution of the data was checked with histogram plots, Shapiro-Wilk normality test and Levene's test for homogeneity of variance. Statistical significance was set at $p < 0.05$.

In order to obtain the maximum values of VO_2 (L/min and ml/kg/min), VCO_2 (L/min), VE (L/min) and HR (bpm), rolling averages of the last 30 seconds of the VO_{2max} test breath-by-breath measurements were calculated. Then, a paired samples t-test was used to compare the parameters of the first and second VO_{2max} sessions.

If parametric assumptions were met, repeated measures ANOVA (within subjects) with post-hoc (Bonferroni) or paired samples t-test were performed to identify the effect of mouthguards on the following parameters: VO_2 (L/min and ml/kg/min), VCO_2 (L/min), VE (L/min), RER, HR (bpm), BLa (mmol/L) and RPE. Wilcoxon test was used when data were non-parametric. All cardio-pulmonary measurements were analysed:

(i) rolling averages of the last 30 seconds of each round;
(ii) rolling averages of the last 30 seconds of each resting interval;
(iii) absolute maximum values of each round;
(iv) absolute maximum values of each resting interval;
(v) % differences of (i) and (iii) relative to the measurements obtained from the VO_{2max} test (vi), where participants showed higher aerobic fitness. Equation 4.1 and 4.2 were used:

$$Equation\ 4.1: \qquad \%\ Difference = \frac{vi - i}{vi}\ x\ 100$$

$$Equation\ 4.2: \qquad \%\ Difference = \frac{vi - iii}{vi}\ x\ 100$$

Percentage change of the same variable between different mouthguard conditions (No MG, MG1, MG4 and MG6) was calculated following Equation 4.3, where *V*1 results from one condition and *V*2 from another.

$$Equation\ 4.3: \qquad \%\ Change = \frac{V2 - V1}{V2}\ x\ 100$$

In order to further examine the repeatability of the exercise protocol, Equation 4.4 was used to find the coefficient of variation (CoV) of the main variables (VO_2 (L/min), VE, HR, BLa and RPE), where $\bar{x}$ is the mean value and SD is the standard deviation:

$$Equation\ 4.4: \qquad CoV = \frac{SD}{\bar{x}}\ x\ 100$$

Levene's test for homogeneity of adjusted CoV scores (between subjects) was also performed.

Due to the nature of BLa measurements and the difficulty to achieve equal baseline values prior to exercise, additional analyses were performed to find the increase or decrease of lactate values between intervals. For example, the difference between post warm-up BLa and BLa after the first round was calculated by subtracting the former from the latter.

The questionnaire data was analysed by performing Wilcoxon test with significance level set at $p \leq 0.05$.

Sample size calculations were conducted with G*Power. The following assumptions were made: (i) use of repeated measures ANOVA, within-subjects (ii) comparison of four groups (same population at three mouthguard conditions and without a mouthguard) (iii) examining the changes in eight parameters at those four conditions (VO_2 L/min and ml/kg/min, VE, VCO_2, RER, HR, BLa, RPE) $\alpha = 0.05$ and (iv) effect size of 0.80. It was found that a minimum of 12 participants were required.

4.3 Results

4.3.1 VO_{2max} Test

The first maximal effort exercise showed significantly lower VO_{2max} (7.8%) and lower HR_{max} (1.0%) than the second test at the end of the study (Table 4-2). Although BLa levels did not show significant changes, a 9.6% increase was recorded.

Table 4-2: Mean ± SD of cardiopulmonary parameters recorded at maximal exertion (*N* = 14).

VO_{2max} test	**VO_2 (L/min)**	**VO_2 (ml/kg/min)**	**VE (L/min)**	**VCO_2 (L/min)**	**HR (bpm)**	**BLa (mmol/L)**
1	**3.87 ± 0.40***	**50 ± 7***	152.5 ± 21.9	4.52 ± 0.45	195 ± 12	12.2 ± 2.7
2	**4.05 ± 0.50***	**54 ± 7***	154.8 ± 24.6	4.58 ± 0.52	197 ± 11	13.5 ± 2.6
p	**0.022**	**0.032**	0.868	0.132	0.527	0.257

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * VO_{2max} test 1 and VO_{2max} test 2.

4.3.2 Repeatability of Boxing Protocol

There were no statistical differences between mean VO_2 (L/min), VE, BLa and maximum HR when the exercise protocol was repeated three times without a mouthguard ($p \geq 0.055$) ($N = 5$) (Table 4-3). Additionally, the CoV for VO_2 (L/min), VE, HR and RPE did not differ significantly ($p \geq 0.055$). For all trials, the participants' perception of exertion was the same, starting at 'Light' (RPE = 11) in Round 1 to 'Somewhat hard' (RPE = 14) in the last round ($p \geq 0.317$).

Table 4-3: Mean ± SD of VO_2, VE and blood lactate and Max ± SD of HR for each round (R1-R4) without a mouthguard. The coefficient of variation (CoV) for the assessed variables of each trial are presented in percentages.

Parameter	Trial 1	Trial 2	Trial 3	*p*
VO_2 (L/min)				
R1	2.56 ± 0.56	2.11 ± 0.71	2.29 ± 0.61	0.503
R2	2.50 ± 0.61	2.34 ± 0.77	2.35 ± 0.50	0.981
R3	2.50 ± 0.59	2.41 ± 0.83	2.44 ± 0.42	1.000
R4	2.55 ± 0.65	2.39 ± 0.63	2.40 ± 0.83	0.579
CoV	22.1%	29.8%	23.7%	0.056
VE (L/min)				
R1	72.2 ± 17.3	60.9 ± 18.1	62.1 ± 22.8	0.269
R2	78.6 ± 21.1	69.7 ± 23.3	69.1 ± 22.7	0.189
R3	80.7 ± 26.3	72.2 ± 27.5	70.8 ± 17.2	0.224
R4	84.2 ± 29.3	73.8 ± 29.4	77.3 ± 21.6	0.115
CoV	28.4%	34.2%	29.1%	0.338
BLa (mmol/L)				
R1	3.17 ± 0.65	2.39 ± 0.67	3.20 ± 1.14	0.750
R2	3.31 ± 0.56	2.56 ± 0.67	3.58 ± 1.66	0.182
R3	3.56 ± 0.84	2.72 ± 0.81	3.78 ± 1.96	0.638
R4	3.82 ± 0.41	2.71 ± 0.76	3.76 ± 1.88	0.068
CoV	**17.9%†**	**17.9%¥**	**26.4%†¥**	**0.001**
HR (bpm)				
R1	167 ± 18	164 ± 21	155 ± 22	0.210
R2	174 ± 18	168 ± 19	159 ± 21	0.055
R3	175 ± 20	173 ± 16	166 ± 21	0.086
R4	179 ± 20	176 ± 12	170 ± 20	0.174
CoV	10.2%	9.9%	12.5%	0.088

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: † = Trial 1 and Trial 3; ¥ = Trial 2 and Trial 3.

BLa during the second trial was 25.0% lower than the values recorded at Trial 1 and 27.0% than Trial 3. However, the differences within participants were not significant ($p \geq 0.068$). Similarly, the differences of BLa values between rounds did not differ statistically ($p \geq 0.465$). Nevertheless, BLa in Trial 3 was associated with greater variability than in Trials 1 and 2 ($p \leq 0.001$) (Table 4-3). Appendix D1 and D2 include all data of mean ± SD and max ± SD of the assessed cardiopulmonary parameters for each boxing round and resting interval.

4.3.3 Effects of Mouthguards on Physiological Parameters

Across all conditions, there was no difference in VO_2 (L/min) during all intervals ($p \geq 0.623$) (Table 4-4 and 4-5). Despite the lack of statistical significance, VO_2 (L/min) was found to be slightly higher when mouthguards were used compared to having no mouthguard (increase of 3.5% with MG1, 2.7% with MG4 and 3.1% with MG6). During the first round, the VE was 17.0% higher when participants wore MG6 compared to No MG and 20.2% higher than MG4 ($p < 0.002$) (Table 4-4; Table 4-5). RER was found to be 5.8% lower when MG1 was used compared to No MG ($p < 0.003$) (Appendix D3).

Table 4-4: Mean ± SD of VO_2 and VE, and Max ± SD of HR for each round (R1-R4) whilst wearing no mouthguard (No MG) or any of the three selected designs (MG1, MG4 or MG6).

Parameter	No MG	MG1	MG4	MG6	*p*
VO_2 (L/min)					
R1	2.60 ± 0.40	2.65 ± 0.42	2.61 ± 0.47	2.69 ± 0.42	1.000
R2	2.60 ± 0.50	2.71 ± 0.40	2.75 ± 0.37	2.72 ± 0.51	0.623
R3	2.57 ± 0.47	2.72 ± 0.37	2.64 ± 0.45	2.64 ± 0.51	0.901
R4	2.62 ± 0.45	2.67 ± 0.41	2.69 ± 0.36	2.68 ± 0.56	1.000
VE (L/min)					
R1	**73.7 ± 16.4†**	73.4 ± 15.7	**71.7 ± 13.2¥**	**86.2 ± 17.5†¥**	**0.002**
R2	79.3 ± 20.8	78.9 ± 15.9	78.9 ± 14.1	81.3 ± 23.5	1.000
R3	79.5 ± 20.5	81.3 ± 18.2	79.2 ± 15.3	79.7 ± 22.3	1.000
R4	83.3 ± 21.5	83.3 ± 18.1	82.1 ± 14.7	82.9 ± 24.1	1.000
HR (bpm)					
R1	159 ± 16	160 ± 18	156 ± 22	165 ± 22	0.096
R2	164 ± 23	166 ± 17	164 ± 22	165 ± 16	1.000
R3	166 ± 23	173 ± 18	167 ± 22	166 ± 17	0.074
R4	166 ± 22	**165 ± 16¢**	171 ± 19	**169 ± 16¢**	**0.041**

Significant main effects are highlighted in bold. Group differences ($p < 0.05$) are highlighted by: † = NoMG and MG6; ¢ = MG1 and MG6; ¥ = MG4 and MG6.

Table 4-5: Mean ± SD of VO_2 and VE, and Max ± SD of HR for each resting interval whilst wearing no mouthguard (No MG) or any of the three selected designs (MG1, MG4 or MG6).

Parameter	No MG	MG1	MG4	MG6	*p*
VO_2 (L/min)					
Pre-test	0.63 ± 0.12	0.64 ± 0.13	0.60 ± 0.14	0.59 ± 0.17	0.157
Rest 1	1.77 ± 0.31	1.83 ± 0.34	1.79 ± 0.28	1.80 ± 0.28	0.100
Rest 2	1.76 ± 0.33	1.88 ± 0.28	1.79 ± 0.26	1.84 ± 0.35	1.000
Rest 3	**1.69 ± 0.27***	**1.85 ± 0.30***	1.89 ± 0.48	1.89 ± 0.48	**0.046**
Rest 4	1.70 ± 0.34	1.78 ± 0.32	1.82 ± 0.35	1.85 ± 0.58	0.145
Post-test	0.96 ± 0.22	0.97 ± 0.20	1.00 ± 0.17	1.11 ± 0.37	0.124
VE (L/min)					
Pre-test	**23.2 ± 3.9#†**	22.7 ± 5.0	**19.7 ± 3.9#**	**20.5 ± 6.2†**	**0.018**
Rest 1	51.3 ± 10.8	46.1 ± 14.2	49.4 ± 9.1	51.8 ± 11.2	0.363
Rest 2	54.1 ± 13.7	55.7 ± 9.9	52.7 ± 10.5	54.5 ± 12.9	1.000
Rest 3	52.8 ± 11.7	55.9 ± 10.5	52.2 ± 11.0	57.0 ± 16.2	1.000
Rest 4	51.5 ± 12.9	54.9 ± 11.7	53.3 ± 11.8	54.6 ± 19.6	0.815
Post-test	34.7 ± 5.9	36.1 ± 7.6	33.8 ± 5.9	36.5 ± 10.3	1.000
HR (bpm)					
Pre-test	105 ± 20	103 ± 16	108 ± 33	102 ± 17	0.701
Rest 1	155 ± 23	157 ± 21	153 ± 21	158 ± 16	1.000
Rest 2	163 ± 24	166 ± 17	161 ± 16	163 ± 23	1.000
Rest 3	164 ± 23	168 ± 16	165 ± 22	166 ± 16	1.000
Rest 4	166 ± 23	168 ± 19	168 ± 16	171 ± 16	1.000
Post-test	135 ± 21	143 ± 23	140 ± 24	139 ± 25	0.276

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * = No MG and MG1; # = No MG and MG4; † = No MG and MG6.

When the absolute maximum values of each interval were compared, in the fourth round, the recorded HR with MG6 was 2.3% significantly higher compared to MG1 (p = 0.041). Additionally, at the minute prior to exercise having no mouthguard showed higher values than MG4 in terms of VO_2 (L/min and ml/kg/min), VE and VCO_2 ($p \leq 0.007$) (Appendix D4). However, that did not have an influence on any changes within parameters for the rest of the boxing protocol ($p \geq 0.073$).

By using Equation 4.1 and 4.2 to calculate the percentage difference of mean and maximum values relative to VO_{2max} results, it was found that in the first round, VE was 17.7% higher with MG6 compared to MG4 (p = 0.002). None of the other assessed variables showed any statistical differences ($p \geq 0.101$).

Although there were no significant differences in the accumulation of BLa, there was an increase when wearing MG1 compared to No MG, MG4 and MG6 by 15.0%, 14.2% and 8.3% respectively (p = 1.000) (Figure 4-4).

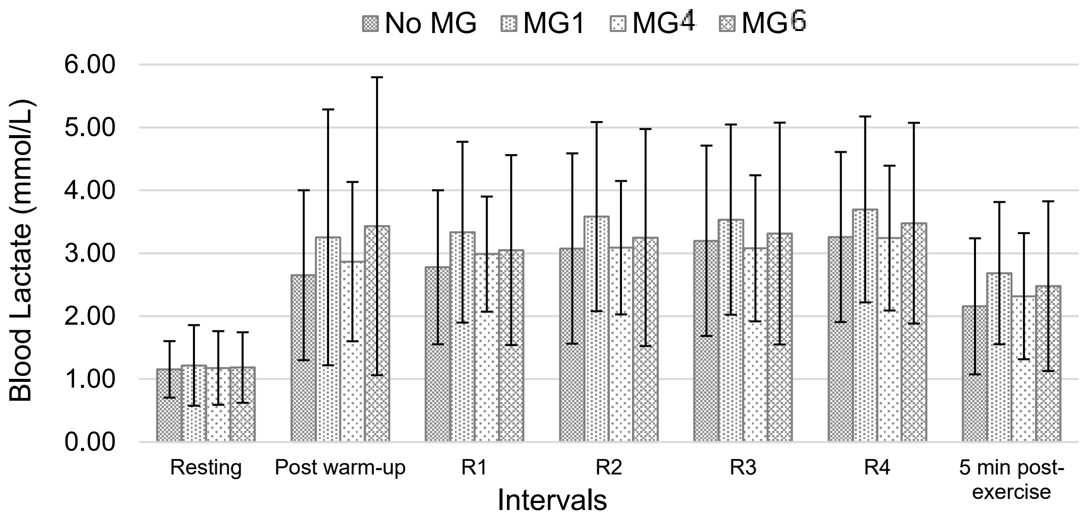

Figure 4-4: Differences in blood lactate levels at rest, throughout the boxing protocol and 5 min post-exercise whilst wearing no mouthguard (No MG) and three selected designs (MG1, MG4 or MG6) (N = 14). Error bars represent the standard deviation.

At the end of the boxing exercise protocol, individuals reported a level of exertion between ‘Light’ and ‘Somewhat hard’ when performing without a mouthguard (12.69 ± 4.83), whereas when a mouthguard was used, their perceived rate of exertion was ‘Hard’ (13.54 ± 2.44); yet no significant difference was found (p = 0.498) (Figure 4-5).

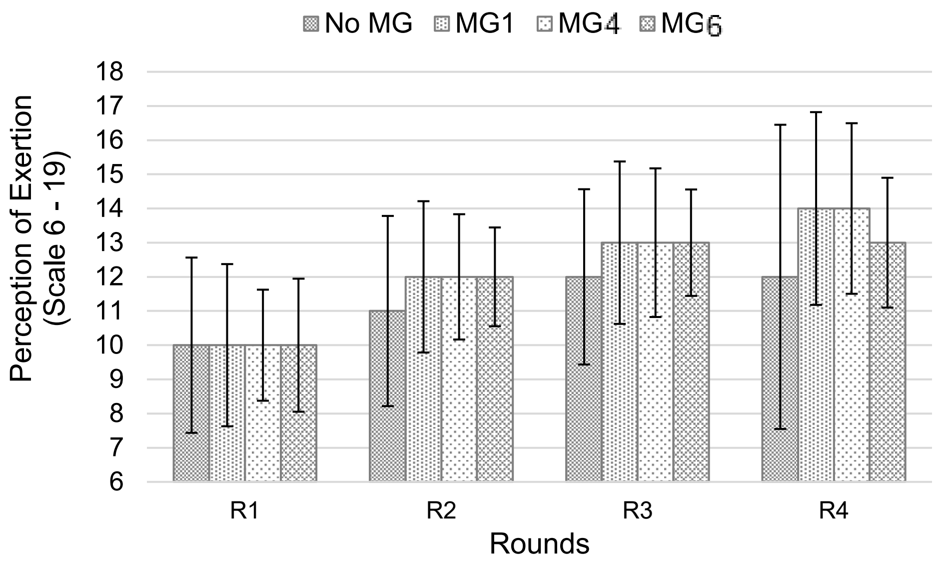

Figure 4-5: Participants’ perception of exertion after each round (R1, R2, R3, R4) (N = 14). Error bars represent the standard deviation.

4.3.4 Level of Comfort

Table 4-6 shows the usage of the three mouthguards before participants were asked to assess devices’ level of comfort. The results were based on 13 participants due to one not completing the questionnaire. More than half of the cohort used the mouthguards in their regular training. However, the majority of participants (61.5%) wore MG1 and MG4 for ‘2 hr or less’ and 53.8% wore MG6 for the same period.

Table 4-6: Number of participants using each mouthguard (MG1, MG4, MG6) for ‘2 hr or less’, ‘3 – 5 hr’ or ‘6 hr or more’ during training and/ or competition (*N* = 13).

Type	2 hr or less	3 – 5 hr	6 hr or more	Use during training	Use during competition
MG1	8	1	4	8	1
MG4	8	2	3	9	3
MG6	7	3	3	7	0

Overall, MG1 was rated with a total score of 8.92 ± 0.76 compared to MG4 and MG6, with 7.66 ± 0.67 and 7.79 ± 0.84 respectively ($p \leq 0.039$) (Figure 4-6). MG1 was also reported to be the most comfortable and participants were most likely to wear it again ($p \leq 0.049$). Additionally, the participants perceived MG1 as more retentive with the least influence on concentration. In terms of thickness, MG6 received the lowest rating compared to MG1 and MG4 (Figure 4-6).

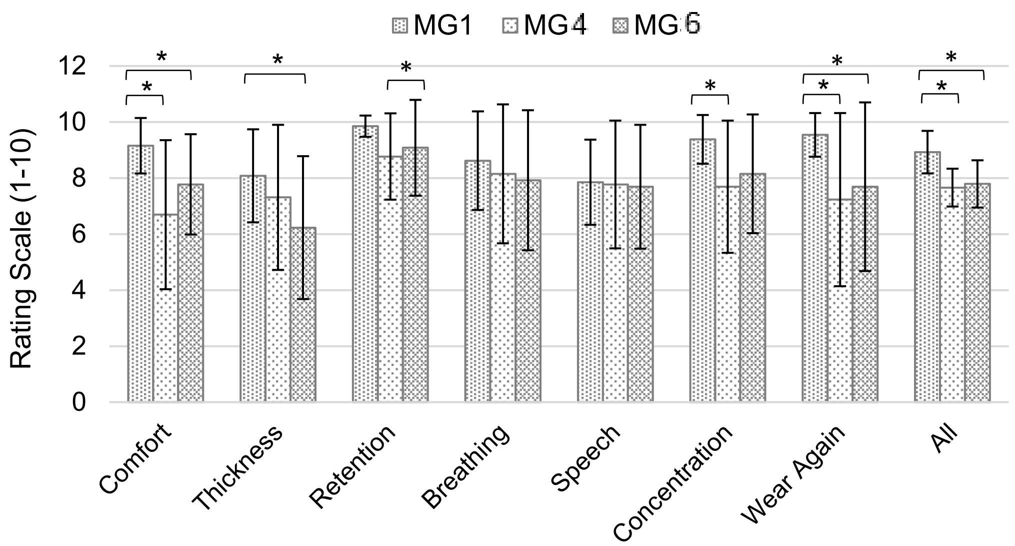

Figure 4-6: Participants’ rating of mouthguard types (MG1, MG4 and MG6) on a scale of 1-10 with error bars representing the standard deviation (*N* = 13). Statistical differences ($p < 0.05$) are highlighted by: * MG1 and MG4; MG1 and MG6.

4.4 Discussion

The current study is believed to be the first to investigate the influence of three different customised mouthguards on physiological parameters and comfort in elite male boxers within a specific boxing protocol. Participants demonstrated comparable aerobic fitness in terms of VO_{2max} assessment, which was important in order to minimise the training effect between testing sessions. The recorded VO_{2max} was within the typical range for male boxers (49 to 65 ml/kg/min) reported in a literature review by Chaabene et al. (2014). However, there was a 7.0% increase of VO_{2max} at the end of the study that could be explained either by improvements in performance or participants being more motivated to demonstrate better results. As proposed by Schulze et al. (2018), the sub-maximal effort of the boxing exercise was calculated based on the VO_{2max} and HR_{max} achieved during the maximal exertion tests. The findings showed an average intensity across all individuals and conditions of 79.0 – 83.0% VO_2 (ml/kg/min) and 85.0 – 88.0% HR at the end of the last boxing round. This outcome was comparable to a study by Finlay et al. (2018), who reported 79% VO_2 (ml/kg/min) and 89% HR post 3 x 3 min boxing-specific exercise protocol. They tested senior elite amateur boxers (*N* = 9; 21 ± 4 yrs; 69.7 ± 8.2 kg; VO_{2max} = 55.0 ± 6.1 (ml/kg/min); HR_{max} = 189 ± 5 b/min) who performed an exercise designed according to national analysis from the 2012 London Olympic Games.

There were no significant differences in VO_2 (L/min and ml/kg/min) during all boxing rounds. Overall, wearing any of the mouthguard designs demonstrated a greater respiratory flow compared to No MG, with MG1 showing the highest increase of 2.4 – 6.4%. Minimal variation of 1.1% – 3.7% was recorded within VO_2 (L/min and ml/kg/min) when the three mouthguard conditions were compared. Wearing MG6 demonstrated 4.6% greater VE than having no device, which was statistically different only after the first round. Additionally, higher readings (1.0 – 5.0%) of all respiratory parameters and HR were recorded with MG6 compared to the other two mouthguards, which could possibly be due to the mouthguard causing higher strain whilst performing.

During all resting (recovery) intervals, VO_2 (L/min and ml/kg/min) was 0.2 – 18.2% higher and HR was 1.4 – 5.8% lower when performing without a device compared to wearing the three mouthguards. The findings suggested that during recovery intervals, participants could benefit from removing their mouthguards to facilitate greater gas exchange. This is important to reduce the metabolic oxygen demand during the one minute between boxing rounds. However, it should be noted that MG4 demonstrated the lowest percentage changes in VO_2 (L/min and ml/kg/min) compared to No MG, which could be due to the lack of gingival flange and possible jaw opening (2 – 3 mm). Prior to the exercise activity, mean VE was reported significantly higher without a mouthguard than with MG4 (13%) and MG6 (9.4%). This was also found in the maximum VO_2 (L/min and ml/kg/min), VE and VCO_2 recorded during the minute prior to exercise but only compared to MG4. These changes could have been influenced by the variation of workload during the self-selected 10 min warm-up rather than the type of mouthguard worn.

Although there were no significant differences within accumulation of BLa, substantially higher levels were recorded during all boxing rounds performed with MG1 compared to No MG, MG4 and MG6 (8.3 – 15.0%) (Table 4-4). In addition, wearing no device demonstrated the lowest BLa levels throughout the protocol, followed by MG4. The meaningful value of the present findings follow a previous study that examined physiological changes in 17 elite male boxers (19.47 ± 1.26 yrs; 73.8 ± 5.1 kg) pre and post training programme (El-Ashker and Nasr, 2012). The authors identified a drop of 16.0% in BLa accumulation post training (pre 8.7 and post 7.3 mmol/L) and considered that as a significant outcome that showed aerobic improvements. Hence, despite the lack of significance in the current study, the reported increase in BLa indicated higher anaerobic work whilst using a mouthguard compared to no mouthguard. Furthermore, El-Ashker and Nasr (2012) demonstrated a change of 9.6% VO_{2max} post-training (58.2 ± 6.9 vs 64.6 ± 7.2 ml/kg/min) and 7.9% resting HR (73.1 ± 2.7 vs 67.3 ± 1.9 b/min). These differences in VO_2 and HR were greater than the changes reported in the present study during the boxing rounds. Therefore, it could be proposed that the recorded differences influenced by mouthguard design were not substantial enough to conclude that there was a negative effect in terms of respiratory flow. Similarly, Lovell et al. (2013) reported a 14.0 – 15.0% improvement in VO_{2max} following a training regime of a professional fighter, which was again higher than the current findings.

Following the evaluation of previous studies in Chapter 1, Section 1.4, it is clear that there are controversial views about the influence of mouthguards on performance and physiological parameters. Boxing is one of the sports where mouth protection is mandatory and yet there is a lack of research assessing the effects of mouthguard types during exercise. El-Ashker and El-Ashker (2015) were the first to examine elite male boxers using a stock and custom-made mouthguard whilst running on a treadmill. The authors asked their participants to run with and without a mouthguard and complete two 10 min stages (medium and high intensities). Their breath-by-breath analysis demonstrated that VO_2 (ml/kg/min) was significantly reduced 12.0 – 13.5% with the use of stock mouthguard (43.54 ± 5.68 ml/kg/min) at higher intensities compared to a custom mouthguard (50.37 ± 5.34 ml/kg/min) or no mouthguard (49.48 ± 3.65 ml/kg/min). El-Ashker and El-Ashker (2015) highlighted that the decrease in VO_2 was due to a stock mouthguard causing obstruction. In contrast, the present study evaluated that a decrease in VO_2 could also identify lower exercise effort during performance, which is beneficial for participants. This conclusion was based on the analysis of HR and BLa measurements, which demonstrated an increase with simultaneous increase in VO_2. These findings show higher strain during performance. Although the two studies differed in terms of mouthguard types and exercise protocols, there was a similarity within the design of the custom-made mouthguards. El-Ashker and El-Ashker (2015) vacuum-formed a 4 mm EVA blank and fabricated a customised mouthguard, which was similar to MG1 from the present study. At high intensity running, they reported 1.9% decrease in VO_2 (ml/kg/min) and 3.8% increase in VE when using this mouthguard type compared to no mouthguard. In comparison, following the last boxing round of the current study protocol, an increase in both VO_2 (ml/kg/min) and VE was reported when

MG1 was used compared to no mouthguard (2.9% and 0.5% respectively). Nevertheless, both studies showed that there was no substantial differences when male boxers used such design.

Although a treadmill test may create high physiological values, this type of exercise activity does not specifically address the characteristics of a boxing match. It could be argued that the newly developed protocol in the present study could provide more valid results to determine the effect of customised mouthguards on changes in airflow, HR and BLa. The exercise protocol was designed based on a number of repeatability tests, which demonstrated superior repeatability of respiratory flow measurements and BLa levels ($p \geq 0.055$). The examined variables were selected according to previous literature, which investigated the physiological demands of boxing, indicating metabolic changes. In an attempt to mimic an amateur boxing match in laboratory conditions without a boxing ring and opponent, it was decided to increase the number of rounds in the protocol (4 x 3-min), instead of using the usual duration of a bout (3 x 2-min, 3 x 3-min or 4 x 2-min). In addition, to compensate for the lack of dynamic and defensive movements, the number of punches per minute was 60, which was higher than punches recorded during a real Olympic amateur boxing (~ 20) and Commonwealth boxing (~ 37.5) (Smith, 2006; Davis et al., 2017). However, the exercise intensity in the current study was controlled by instructing the participants to perform the same punching combination with the same type of punches in a controlled time interval (6 s).

The chosen protocol 4 x 3-min was more relevant to a performance of amateur boxers rather than professional boxers. In professional boxing, competitors may reach up to twelve boxing rounds, which consequently affects the physical demands and endurance until the end of the bout. Therefore, future protocols could increase the number of boxing rounds in order to reflect on the true effects of wearing a mouthguard and its influence on respiratory flow and BLa accumulation during longer boxing matches. Nevertheless, the findings of the present study may have important implications in relation to increasing the use of custom-made mouthguards as no significant negative impact on airflow was recorded. This could further encourage players to use mouthguards during both training and competition. Additionally, coaches should be aware of the benefits of custom-made devices and educate not only the athletes but also parents of young boxing participants.

Thirteen of the participants completed a comfort questionnaire (rating scale 1-10) assessing the three mouthguards after using and/ or training with each device. MG1 was pressure-formed using a single 5 mm EVA blank and it extended 4 mm in the palate. It was the most favoured design by all participants and showed significantly higher score compared to MG4 and MG6 in terms of comfort (9.00 ± 1.00 vs 6.73 ± 2.83 vs 7.67 ± 1.83) and tendency to wear again (9.64 ± 0.67 vs 7.36 ± 3.17 vs 7.50 ± 3.06) (p < 0.05). Additionally, MG1 retention level and concentration were rated statistically better than MG4, which had no palatal extension (9.91 ± 0.30 vs 8.73 ± 1.62 and 9.27 ± 0.90 vs 7.90 ± 2.56 respectively) (p < 0.05). Participants' perception in terms of retention confirmed the findings reported in Chapter 2, Section 2.3 that MG1 demonstrated significantly higher retention level than MG4. However, these findings contradict to Maeda et al. (2006) and Nozaki et al. (2013) who suggested that trimming the palatal extension up to the gingival margin improved communication, breathing and swallowing. However, it should be considered that participants from the two past studies did not take part in any sports and they were not asked to perform any form of exercise prior to assessing the changes in mouthguard design via questionnaires. MG6 had the lowest score in regards to thickness, however participants still agreed that it provided better comfort (7.67 ± 1.83 vs 6.73 ± 2.83), retention (9.00 ± 1.76 vs 8.73 ± 1.62) and lower interference with concentration (8.00 ± 2.28 vs 7.90 ± 2.56) compared to MG4; although these differences were not significant. The fact that MG4 was reported to cause slightly less obstruction with breathing and speech than MG6 could be explained by its lower thickness. Both designs were finished at the palatal gingival line, however, MG6 had two layers of EVA blanks (2 mm and 4 mm) that covered the labial portion of the anterior teeth and the occlusal surfaces of the posterior. As a result, MG6 felt thicker than MG4, which had one layer of 4 mm EVA covering the anterior teeth and a double layer over the posterior teeth. Out of all participants in the present study, one had previously worn a custom-made mouthguard and the rest had used only ‘boil-and-bite’ types, which varies greatly in thickness and design due to self-moulding. They all confirmed that the former provided better fit, felt more comfortable and was much more retentive. Gawlak et al. (2014, 2015) also supported this opinion. Both studies provided 21 males (16 – 35 yrs), participating predominantly in Brazilian jiu-jitsu and muay thai, with five custom-made and three ‘boil-and-bite’ mouthguards. The participants were asked to change a mouthguard every six weeks and then assess certain characteristics of each type. Gawlak et al. (2014) showed that the mean total score of all characteristics was highest when participants used an injection moulded custom mouthguard (Corflex) (35.095; scale 1-3, with 3 being the highest merit), followed by pressure-formed device with two Erkoflex EVA layers (34.048). Out of the three ‘boil-and-bite’ mouthguards, Protech scored slightly higher usability than Shock Doctor (30.429 vs 30.287 respectively). However, all custom devices were rated to be better than the ‘boil-and-bite’ device. The same results were reported in terms of comfort and protection by Gawlak et al. (2015).

Similar to Chapter 3, the current research did not include any females and did not examine ‘boil-and-bite’ types of mouthguards. Further work should divide the participants into categories according to their competition level as it could be suggested that mouthguards could make a difference to one’s performance but only at certain levels of competition. Due to difficulties with transporting equipment and accommodating testing sessions within the timetable of the boxing academy, where participants trained, the study was completed in the university physiological laboratories. Future studies, should consider using portable breath-by-breath and BLa analysers that could be used in training environment or boxing rings (Ghosh, 2010; Thomson and Lamb, 2017). In addition, using such equipment could allow the participants to perform movements that are more dynamic and / or perform against an opponent (trainer / coach), which would increase the replication of a boxing match. Future work should also include anaerobic activities such as strength exercise or the use of pressure sensors measuring impact to gather more data about the anaerobic abilities of the participants (Thomson and Lamb, 2017). To measure changes in punch force, wireless wrist accelerometers could be included (Thomson and Lamb, 2017) or a boxing bag fitted with special device to register the force (kg) and number of punches as well as the energy output (J) (Bruzas et al., 2018; Kamandulis et al., 2018).

Finally, as training under hypoxic conditions is increasing its popularity in boxing (Ruddock, 2015), it could be advantageous if further work considers the geographical differences in relation to altitude where tests are performed. It is known that the decrease of air pressure could affect physical performance (Vogt and Hoppeler, 2010; Ruddock, 2015). Therefore, simulation of such conditions would increase the scientific knowledge of the use of mouthguards in relation to respiratory physiological parameters under such conditions.

4.5 Conclusions

The present study examined the influence of three custom-made mouthguards on cardiopulmonary parameters in boxers performing a newly designed exercise protocol. During all boxing rounds, there were no significant differences in oxygen uptake with and without a mouthguard. In addition, having a mouthguard did not affect significantly accumulation of blood lactate and perception of exertion. Overall, MG4 design with missing palatal flange and increased posterior thickness reported the lowest interferences compared to wearing no mouthguard or the other two designs. However, during the rest intervals, oxygen uptake was 0.2 – 18.2% higher and heart rate was 1.4 – 5.8% lower without a device compared to wearing a mouthguard. Based on these findings, it is recommended for participants to remove their mouthguards during the one-minute recovery between rounds in order to facilitate oxygen consumption. Although MG1 showed the highest interferences within physiological parameters, this device was most favoured according to participants' perceived level of comfort. The current study demonstrated that wearing a custom-made device does not have a negative influence on airflow and blood lactate accumulation. Additionally, a single layer mouthguard with 4 mm palatal flange was found more comfortable than devices with no palatal flange. In order to increase the use of custom-made mouthguards, participants in boxing should be aware of these positive findings.

During the past decade, training under moderate or high altitude conditions has increased in popularity due to proposed beneficial effects on aerobic fitness. To the authors' knowledge, currently, there are no published studies investigating the possible impact of this type of training in boxing and mouthguards. Therefore, the following chapter will examine the effects of participants during hypoxic conditions in relation to normoxic conditions.

Chapter 5 : The Effects of Customised Mouthguard Designs on Physiological Parameters during Exercise under Hypoxic Conditions in Male Boxers

5.1 Introduction

Mixed views in terms of wearing different mouthguard types and their effects on physiological parameters, power output and comfort have been reported (discussed in Chapter 1, Section 1.4; Chapter 3, Sections 3.1 and 3.4; Chapter 4, Sections 4.1 and 4.4). The variation of findings could be related not only to types and designs of mouthguards but also to differences within types of sport, performance level of participants, exercise protocols and equipment used. Figure 5-1 illustrates past studies assessing the influence of mouthguards on respiratory and performance at various geographical locations with different altitude. The majority of the studies have taken place at altitudes below 500 m where the concentration of oxygen in the air is close to what is accepted as normal (20.93%; normoxia). However, there were some investigations that tested participants at moderate altitudes up to 2363 m. This should be considered when evaluating previous findings due to the influence of such surrounding environment on athletes' oxygen uptake and physical activities, especially during initial exposure to attitudes above the sea level (hypoxic condition). At moderate (~ 2000 - 3000 m) and high altitudes (~ 3000 - 5500 m), the oxygen concentration (FO_2), barometric pressure and partial pressure of oxygen (PO_2) are lower compared to sea level, which simultaneously reduces the density of oxygen molecules in the air and could lead to negative effects in performance (McArdle et al., 2015) (Figure 5-2). Previous studies, examining the effects of altitude, have demonstrated a decrease in participants' arterial oxygen saturation, which has led to an increase in cardiac output or occurrence of hyperventilation reaction in an attempt to partially offset the reduction of PO_2 (Péronnet et al., 1991; Gore et al., 1997; Wehrlin and Hallén, 2006; Clark et al., 2007; Gore et al., 2008; Hamlin et al., 2008; Chapman et al., 2011; Nassis, 2013; McArdle et al., 2015; Girard et al., 2017).

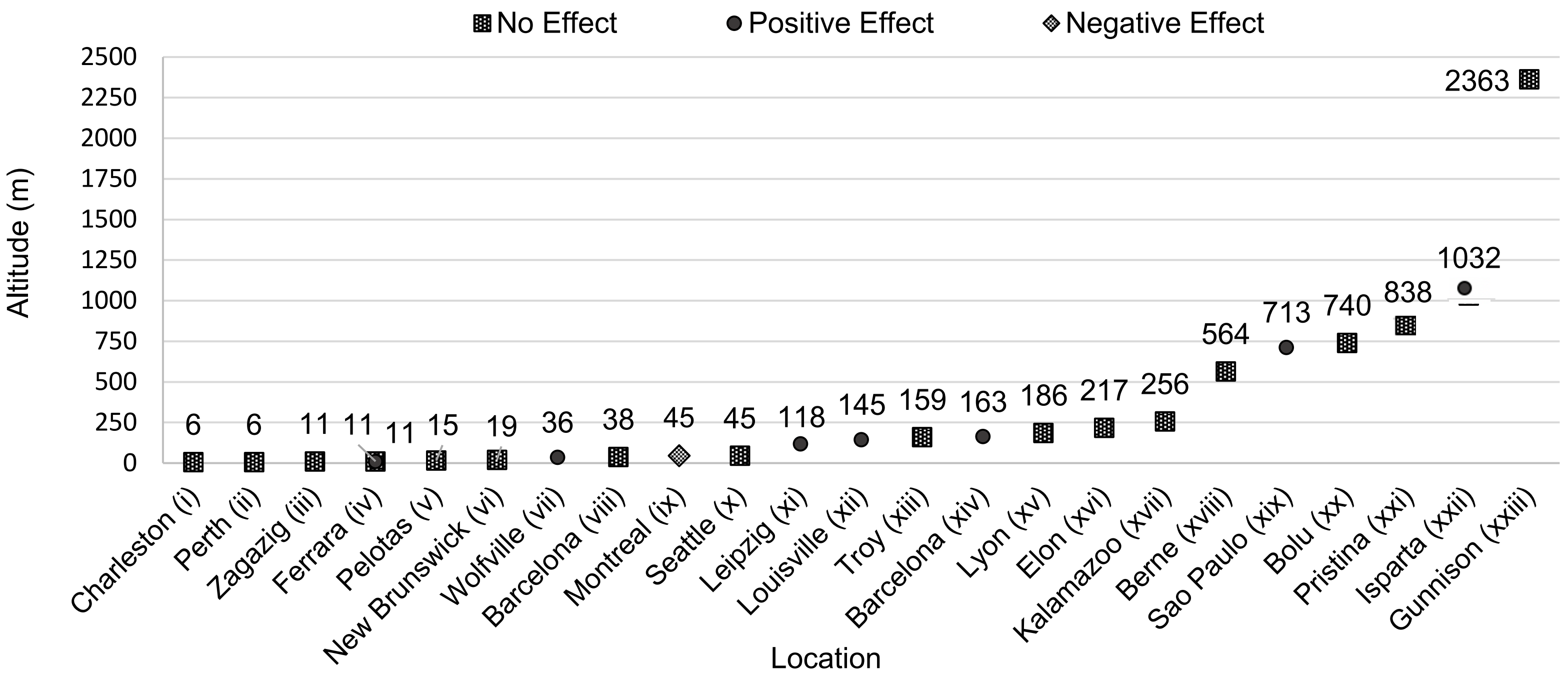

Figure 5-1: Altitude of locations where studies have examined the influence of mouthguards on physiological parameters and performance (regardless of mouthguard type); (i) Garner and McDivitt (2015); (ii) Gebauer et al. (2011); (iii) El-Ashker and El-Ashker (2015); (iv) Piero et al. (2015); (v) Collares et al. (2014); (vi) Golem et al. (2017); (vii) Schultz Martins et al. (2018); (viii) Duarte-Pereira et al. (2008); (ix) Delaney and Montgomery (2005); (x) Duddy et al. (2012); (xi) Schulze et al. (2018); (xii) Bhatt (2015); (xiii) Green et al. (2018); (xiv) Morales et al. (2015); (xv) Bourdin et al. (2006); (xvi) Bailey et al. (2015); (xvii) Hanson et al. (2018); (xviii) von Arx et al. (2008); (xix) Queiroz et al. (2013); (xx) Yarar et al. (2013); (xxi) Rexhepi and Brestovci (2013); (xxii) Kececi et al. (2005) and Cetin et al. (2009); (xxiii) Drum et al. (2016). The reported altitudes were searched by the author at MAPS.IE (2018).

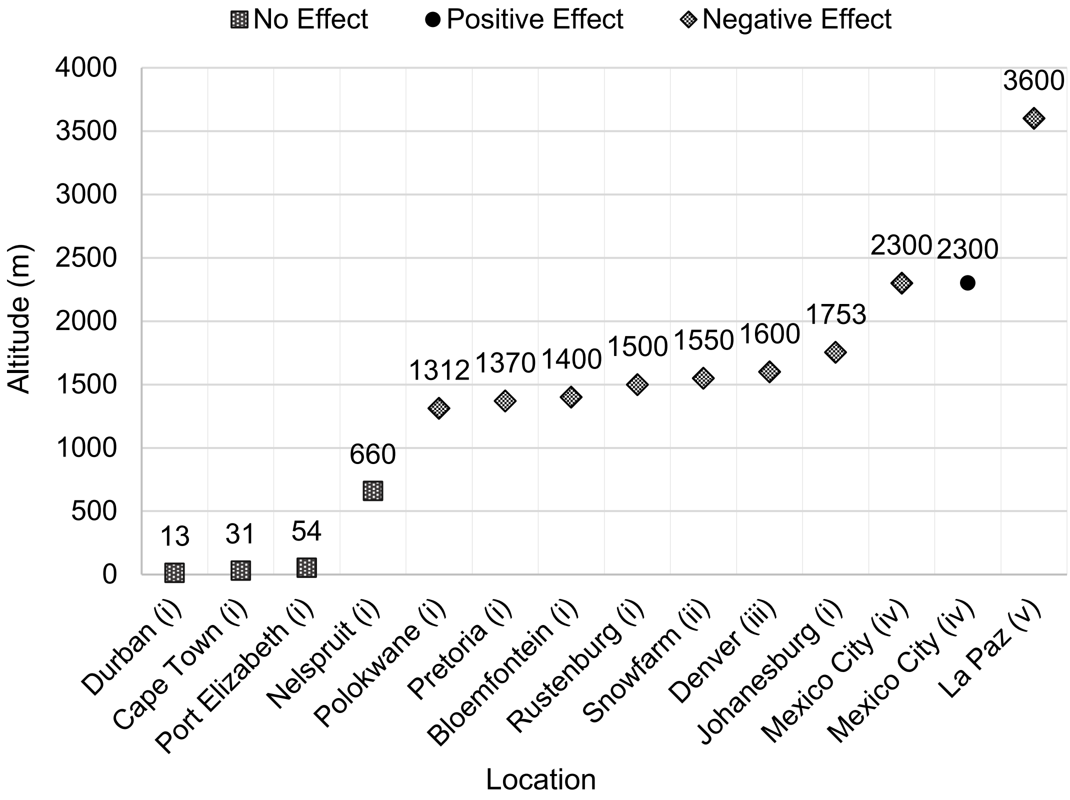

Figure 5-2: Effects of competing at geographical locations with altitude between 13 m - 3600 m on athletes' performance (data presented from: (i) Nassis (2013); (ii) Hamlin et al. (2008); (iii) Garvican et al. (2014); (iv) Péronnet et al. (1991); (v) Brutsaert et al. (2000)).

Since mouthguards have been previously associated with having a possible influence on respiratory flow, it would be beneficial to examine as to whether wearing a device during hypoxic conditions could affect any physiological parameters in comparison to normoxia within the same participant. These performance findings could benefit athletes and coaches, especially if the athlete may participate in a competition at a higher altitude than their training location.

One of the main reasons for previous studies to investigate the influence of moderate and high altitudes on performance has been related to the geographical locations of international championships (e.g. Olympic and Commonwealth Games, Rugby World Cup, World Boxing Championships, etc.) (Gore et al., 2008; Turner et al., 2014). Previous studies have examined sports such as football (Brutsaert et al., 2000; Bradley et al., 2009; Nassis, 2013; Garvican et al., 2014), rugby (Morton, 2006; Hamlin et al., 2008; Galvin et al., 2013; Billaut and Buchheit, 2013), short and long distance running (Péronnet et al., 1991; Gore et al., 1997; Dufour et al., 2006; Chapman et al., 2011) and cycling (Clark et al., 2007). Nassis (2013) analysed football performance during 64 matches of the 2010 World Cup in South Africa, where matches were performed at altitudes from 13 m (Durban) to 1753 m (Johannesburg). The study showed that athletes' endurance was affected significantly above 1200 m and the distance covered during a match was 3.1% lower. For instance, at 1401 – 1753 m the distance covered was 103.6 ± 5.2 km and 106.9 ± 4.3 km at sea level. The process of acclimatization causes the number of red blood cells to increase, which explains this outcome as it allows the players to utilize oxygen better than the players training at sea level (Ruddock, 2015). Additionally, studies have reported a decrease in maximal oxygen uptake (VO_{2max}) results with increase of altitude in both trained and untrained athletes due to a reduction in the arterial oxygen saturation (Gore et al., 1997; Wehrlin and Hallén, 2006; Levine et al., 2008; McArdle et al., 2015). Levine et al. (2008) found that the VO_{2max} of endurance athletes decreased with 0.5% - 1.0% every 100 m above the sea level, whereas McArdle et al. (2015) recorded a decrease of 7.0 – 9.0% every 1000 m above 600 m. Therefore, if athletes are competing at moderate altitudes or above, several days of acclimatization are recommended, which would allow adaptation to the reduced PO_2 and minimise the interferences with performance (Schuler et al., 2007; Gore et al., 2008; Hamlin et al., 2008; Levine et al., 2008; Nassis, 2013).

However, that is not always possible and recent research has used equipment such as 'altitude training' masks, oxygen filtration machines, altitude tents and hypoxic chambers to simulate altitude conditions (Hamlin et al., 2008; Levine et al., 2008; Galvin et al., 2013; Ruddock, 2015). These practices have not only attempted to investigate the effects of hypoxia on exercise activities (acclimation) but also have helped athletes with their training prior to competition (Hamlin et al., 2008; Galvin et al., 2013; McArdle et al., 2015).

Galvin et al. (2013) suggested that such training is recommended in the pre-season period to achieve temporary performance gains for a period of four weeks. Coaches and athletes have attempted to use temporary training at altitude conditions as a strategy to enhance cardiopulmonary parameters and performance at ambient air (Millet et al., 2010; Billaut and Aughey, 2013; McLean et al., 2014; Ruddock, 2015). Turner (2015) and Ruddock (2015) proposed that using simulated altitude equipment in the training of boxers could be beneficial for their performance but more research is required to support the advantages of this method. The authors believe that the aim of exercising at hypoxic conditions as part of a conditioning programme should improve athletes' ability to consume, transport and utilize oxygen. Singh et al. (2014) showed that haematological responses could improve after training at location as high as 1200 m (~ 18% oxygen in the air). They reported a significant 11.9% increase in participants' haemoglobin (Hb) levels and 7.1% in haematocrit (Hct) levels (control group N = 10, 28.7 ± 4.5 yrs; altitude training group N = 19, 29.6 ± 4.5 yrs; male soldiers), which indicated improvements in physiological adaptation leading to beneficial effects due to increase of oxygen transportation. In contrast, the findings of Friedmann et al. (1999) demonstrated that endurance training at moderate altitude (1800 m, St. Moritz, Switzerland) did not increase Hb levels, even when additional iron supplementation was taken by participants. To the authors' knowledge, this was the only study that examined national level boxers who performed an incremental treadmill test until maximal exhaustion at moderate altitude location (experimental group N = 9, 24.2 ± 2.9 yrs, 72.6 ± 17.1 kg and control group N = 7, 23.8 ± 2.6 yrs, 77.1 ± 11.9 kg). There is a lack of evidence-based literature addressing the effects of hypoxia training on boxers and more specifically the effects on respiratory parameters during normoxic and hypoxic conditions whilst participants use their mouthguards.

Therefore, the primary aim of the present chapter is to investigate changes in respiratory and blood lactate (BLa) influenced by the use of custom-made mouthguards and hypoxic environment. This would enhance the scientific knowledge from previous published literature, which reported that custom-made devices have no impact on airflow or could potentially lead to improvements in physiological parameters, regardless of the environmental conditions (e.g. lower FO_2) (Kececi et al., 2005; Bourdin et al., 2006; Duarte-Pereira et al., 2008; Cetin et al., 2009; Gebauer et al., 2011; Duddy et al., 2012; Queiroz et al., 2013; Collares et al., 2014; El-Ashker and El-Ashker, 2015; Garner and McDivitt, 2015; Morales et al., 2015; Piero et al., 2015). More specifically, the present chapter will evaluate the differences in physiological performance within a cohort of boxers during both normoxic and hypoxic conditions without and with two selected custom-made mouthguards. The study will examine whether specific features of the mouthguard designs could impair performance whilst performing under simulated moderate altitude conditions. Such findings could suggest features in customised mouthguards, which may benefit training or competing at different environment to sea level. It is hypothesised that using different customised mouthguards whilst performing at simulated hypoxic conditions does not affect airflow parameters such as oxygen uptake (VO_2 L/min and ml/kg/min), minute ventilation (VE), carbon dioxide production (VCO_2), respiratory exchange ratio (RER) as well as heart rate (HR) and BLa in male boxers. Additionally, performing under hypoxic environment will cause impairments within these parameters.

5.2 Methods

Prior to any experimental work, ethical approval was obtained and granted from the School of Healthcare Science, Faculty of Science and Engineering, Manchester Metropolitan University (Ethics Number: SE1617150). Additionally, the procedures included in the study design followed the ethical principles outlined in the Declaration of Helsinki (World Medical, 2013).

Participants, who took part in the previous study (Chapter 4), were invited to participate in the present follow-up study. A total of seven participants completed all testing sessions and their anthropometric data are shown in Table 5-1.

Table 5-1: Description of the sampled population (*N* = 7).

Variable	**Mean**	**±**	**SD**
Age (yrs)	25		7
Mass (kg)	82		11
Height (cm)	179		5
Body Mass Index	26		3
Hb (g/dL)	15		1
Lung Function (%)	76		5
VO_{2max} (ml/kg/min)	47		5

None of the participants had any present injuries, related cardiovascular problems, temporomandibular disorder or any trauma of the oral and facial structures. Participants were asked to attend three laboratory testing sessions at approximately the same time of day and perform the same boxing exercise protocol as under normoxic conditions (Chapter 4). In order to avoid any training effect, there was a minimum period of one week between visits.

5.2.1 Characteristics of Mouthguards

Two custom-made mouthguard designs were selected based on participants' perception of comfort that was reported in Chapter 4, Section 4.3.4 (Figure 5-3). MG1 and MG6 were rated with the highest total score and participants were most likely to wear them again. During each session, the participants randomly chose to wear one of the devices or have no device. As the participants had been involved within the previous study, they were asked to use two of the custom-made mouthguards that they received whilst testing under normal conditions.

Figure 5-3: The two customised mouthguards (MG1 and MG6) used by all participants.

To recap, MG1 was fabricated from a 5 mm single EVA blank and similar to the mouthguard used within a study by Gebauer et al. (2011), which had a 4 mm extension into the palate. MG6 was fabricated from two EVA layers (2 mm and 4 mm) that covered all anterior teeth and the occlusal surfaces of the posterior teeth as used within a study by Takeda et al. (2014). MG6 was trimmed around the palatal gingival margins and it was extended over the second maxillary molars, whereas MG1 was finished at the distal surfaces of the first maxillary molars. All devices had a full buccal flange.

5.2.2 Simulation of Hypoxic Conditions

All participants performed an exercise protocol under simulated hypoxic conditions in an environmental chamber (Design Environmental Ltd., Gwent, UK) at Manchester Metropolitan University, Cheshire Campus. The chamber was controlled via Contour Software (Design Environmental Ltd., Gwent, UK) and programmed at FO_2 = 16.77%, which corresponded to 6000 ft/ 1829 m altitude above sea level (609.05 mmHg barometric pressure; PO_2 = 127.47 mmHg). The temperature and the humidity were set to the same values as when participants were tested under normoxic conditions, 20˚C and 50% respectively. A maximum deviation of ± 1 of the chosen settings was accepted to allow for any variations throughout the duration of the data collection. Prior to testing, all participants were provided with a participant information sheet. They were also informed verbally regarding safety and the measurements taken during the study whilst they were under hypoxic conditions. Participants wore a pulse oximeter to monitor oxygen saturation levels, which was strapped around their chest and connected to their ear lobe (Model 8000Q2 Ear Clip SNSR, NONIN Medical Inc., North Plymouth, USA). If oxygen saturation levels dropped below 94% and the participant experienced any sensation of dizziness, numbness or superficial tingling, they were removed immediately from the environmental chamber. Additionally, if whilst testing, the temperature and humidity in the chamber fell outside the range of ambient conditions (10˚C - 27˚C & 30% - 70%, respectively), and if the FO_2 of 16.77% dropped down to 15%, the test were terminated and participants removed. During testing, participants' HR was monitored with a HR sensor (Polar H7, Polar Electro Ltd, Warwick, UK) to determine the degree of any physiological stress.

5.2.3 Sport Specific Boxing Protocol

Participants were asked to record their dietary intake and physical activity levels 24 hr prior to each session. Additionally, they were advised to fast and drink at least one litre of water 2 hr prior to testing and refrain from caffeine and alcohol in order to prevent any stimulation to the participant or influence the results obtained. Each of the three laboratory visits lasted approximately one hour and consisted of the following: baseline measurements, a 10 min warm-up and a 16 min sport specific study protocol.

A finger prick blood sample was taken prior to each testing session to examine the levels of Hb of all participants. A portable Hb analyser (HemoCue® 201+ System, Crawley, England) was used and if the Hb levels were less than 13.0 g/dl (grams per decilitre), which is the diagnostic of anaemia, the participants were excluded from the study (WHO, 2011). Additionally, the levels of Hct were checked following the procedure described by Billett (1990). In brief, a microhaematocrit tube was filled with approximately 0.05 ml of blood and spun in a microhaematocrit centrifuge for 5 min (HaematoSpin 1400, Hawksley and Sons Ltd., Sussex, UK). Then, a microhaematocrit reader (Hawksley and Sons Ltd., Sussex, UK) was used to measure the percentage of red blood cells. If the levels were lower than the normal range for men (40.0 – 54.0%), the participants were not allowed to continue with the study. In addition, body mass, blood pressure and BLa measurements were taken.

The sport specific protocol that was reported previously in Chapter 4 (Section 4.2.3) was replicated in the present chapter under hypoxic conditions. During data collection, verbal encouragement was provided to help participants maintain their maximum effort whilst throwing punches. The same gas exchange parameters (VO_2 L/min and ml/kg/min, VE L/min, VCO_2 L/min and RER) were recorded with the same calibrated breath-by-breath analyser (METALYZER® 3B, CORTEX, Leipzig, Germany) as in the previous study (Chapter 4, Section 4.3.3 and 4.3.4). This allowed comparison of the findings under both normal and hypoxic conditions within the same participants. In addition, it would also highlight any respiratory effects of wearing a mouthguard or not under both conditions, which has not been evaluated previously. Figure 5-4 illustrates the testing environment with a participant performing the exercise protocol. Similarly to the previous study, finger prick BLa sample was taken following each boxing round (Biosen C-line Analyser, Cardiff, UK) and participants were asked to rate their perceived exertion (RPE) on a standard Borg scale (6 – No Exertion to 20 – Maximal Exertion). In order

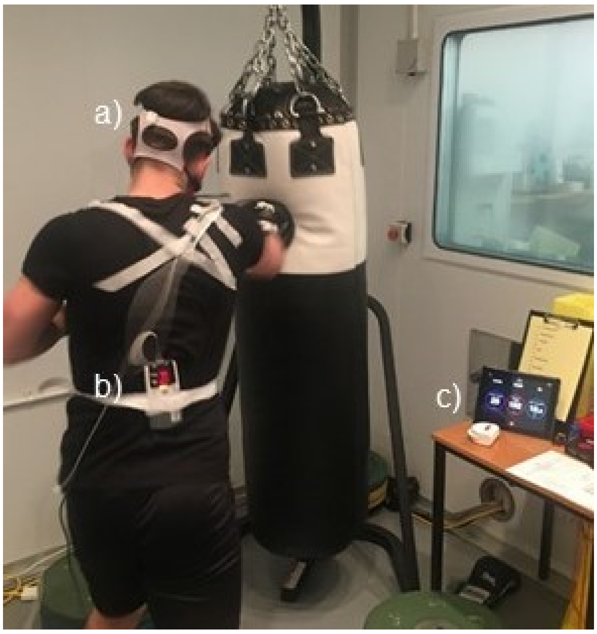

Figure 5-4: Participant performing a boxing-specific exercise protocol under hypoxic conditions; (a) mask connected to a breath-by-breath analyser; (b) pulse oximeter with wire connected to the ear lobe; (c) live feedback from punch tracker.

to prevent any bias, participants were not provided with any information about the possibility of increased exercise effort under altitude conditions.

5.2.4 Assessment of Punch Velocity

In order to monitor the consistency of punching velocity between sessions, punch tracker sensors (Hykso®, Costa Mesa, California, US) were placed on the participants' wrists and secured with their own hand wraps (Figure 5-5). In addition, the type of punches was monitored to check whether participants performed straight punches as instructed at the beginning of each session. The sensors were connected to an iPad tablet (Apple Inc., Cupertino, California, US) via Bluetooth, which allowed the participants to follow their results and maintain their maximum effort during exercising. Participants were able to see live feedback of their average velocity, punch count and intensity score.

Figure 5-5: Left image shows two punch tracker sensors (Hykso®, Costa Mesa, California, US). Right image shows an example of the results for punch count, intensity score and average velocity following a 3-minute boxing round.

5.2.5 Statistical Analysis

Equation 5.1 was used to calculate the exertion/ intensity (%*V*) of the boxing-specific protocol as a percentage of the achieved VO_{2max} (ml/kg/min) (*V*2) and HR_{max} (*V*2) during the last maximal exertion test performed in the previous study (Chapter 4). *V*1 equals either the absolute maximum VO_2 (ml/kg/min) or the absolute maximum HR achieved during each trial (under normoxic and hypoxic conditions).

$$Equation\ 5.1: \qquad \%\,V = \frac{V2 - V1}{V2}\ x\ 100$$

Statistical analyses were performed using IBM SPSS Statistics, Version 24.0. Armonk (IBM Corp., New York, US) and Microsoft Excel (2013). Prior to any analysis, distribution of the data was checked with histogram plots, Shapiro-Wilk normality test and Levene's test for homogeneity of variance. Statistical significance was set at $p \leq 0.05$.

If parametric assumptions were met, repeated measures ANOVA (within subjects) with post-hoc (Bonferroni) or a paired samples t-test were performed to identify the effect of mouthguards on the following parameters at hypoxic conditions: VO_2 (L/min and ml/kg/min), VCO_2 (L/min), VE (L/min), RER, HR (bpm), BLa (mmol/L) and RPE. Wilcoxon test was used when data were non-parametric (including perceived exertion). All cardio-pulmonary measurements were analysed using:

(i) rolling averages of the last 30 seconds of each round;
(ii) rolling averages of the last 30 seconds of each resting interval;
(iii) absolute maximum values of each round;
(iv) absolute maximum values of each resting interval.

Due to the nature of BLa measurements and the difficulty to achieve equal baseline values prior to exercise, additional analysis were performed to find the increase or decrease of lactate values between intervals. For example, the difference between post warm-up BLa and BLa after the first round was calculated by subtracting the former from the latter. Additionally, percentage change between the three different conditions (No MG, MG1 and MG6) was calculated following Equation 5.2, where *X*1 results from one condition and *X*2 from another.

$$Equation\ 5.2: \qquad \%\ Change = \frac{X2 - X1}{X2}\ x\ 100$$

Two-way repeated measures ANOVA (within subjects), 3 x 2 design, was performed to find any significant interactions between both factors - change of mouthguard design and change in the environment (normoxia and hypoxia). Prior to performing the test, data were checked for approximate normal distribution (Shapiro-Wilk), any significant outliers and sphericity (Mauchly's test). The three mouthguard conditions refer to No MG(n), MG1(n) and MG6(n) at normoxia and No MG(h), MG1(h) and MG6(h) at hypoxia. Equation 5.3 was used to find the percentage difference between measurements:

$$Equation\ 5.3: \qquad \%\ Change = \frac{MG(h) - MG(n)}{MG(h)}\ x\ 100$$

The recorded punch tracker data were re-organised in terms of punches thrown with the left and right hand during each boxing round. Paired samples t-test for data with normal distribution and Wilcoxon test for data with non-normal distribution were performed to compare the punch velocities (km/h).

5.3 Results

The levels of Hb (14.8 ± 0.1 g/dl) and Hct (42.3 ± 1.9 %) recorded at the beginning of the three hypoxic sessions were within the normal ranges for men. Additionally, there was no significant increase/ decrease of the two parameters between the three mouthguard conditions ($p > 0.072$). Participants' body mass (80.8 ± 0.7 kg) did not differ significantly to the body mass recorded during previous testing under normal conditions (82.7 ± 0.1 kg) ($p = 0.066$).

5.3.1 Effects of Different Mouthguard Designs on Physiological Parameters under Hypoxic Conditions

Table 5-2 shows that wearing either MG1 or MG6 did not influence VO_2 (L/min) significantly during the boxing rounds. However, VE was 8.7% higher when participants performed the third round without a mouthguard compared to MG1 ($p = 0.036$). It was identified that during the first two recovery intervals (Rest 1 and Rest 2) VO_2 (L/min and ml/kg/min) and VE were statistically greater whilst no mouthguard was worn compared to MG1 and MG6 (Table 5-3; Appendix E1).

Table 5-2: Mean ± SD of VO_2 (L/min) and VE (L/min) and Max ± SD of HR (bpm) for each round (R1-R4) without a mouthguard (No MG) and with two different mouthguard designs (MG1, MG6) under hypoxic condition (N = 7).

		No MG			MG1			MG6			
Parameter		**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
VO_2	**R1**	2.87	±	0.28	2.75	±	0.75	2.91	±	0.79	0.575
	R2	3.12	±	0.55	2.95	±	0.49	2.93	±	0.73	0.339
	R3	3.11	±	0.35	2.93	±	0.44	2.99	±	0.53	0.063
	R4	2.99	±	0.35	2.95	±	0.40	3.05	±	0.53	0.209
VE	**R1**	94.3	±	28.8	91.3	±	28.1	94.3	±	34.2	1.000
	R2	108.1	±	26.1	101.8	±	26.4	102.3	±	44.7	0.234
	R3	**108.1**	**±**	**23.6***	**98.7**	**±**	**20.3***	104.0	±	12.69	**0.036**
	R4	106.5	±	16.7	116.7	±	22.1	109.4	±	13.92	0.236
HR	**R1**	**174**	**±**	**13#**	169	±	21	**168**	**±**	**14#**	**0.018**
	R2	180	±	12	176	±	17	175	±	13	0.068
	R3	181	±	12	178	±	16	178	±	13	0.394
	R4	184	±	10	180	±	13	183	±	17	1.000

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * = No MG and MG1; # = No MG and MG6.

Table 5-3: Mean ± SD of VO_2 (L/min) and VE (L/min) and Max ± SD of HR (bpm) for each resting interval without a mouthguard (No MG) and with two different mouthguard designs under hypoxic condition (MG1, MG6) (*N* = 7).

	No MG			**MG1**			**MG6**			
Parameter	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
VO_2										
Pre-test	0.79	±	0.15	0.79	±	0.17	0.77	±	0.20	1.00
Rest 1	**2.17**	**±**	**0.27***	**1.97**	**±**	**0.30***	1.95	±	0.35	**0.035**
Rest 2	**2.29**	**±**	**0.33*#**	**2.08**	**±**	**0.19***	**1.94**	**±**	**0.29#**	**0.018**
Rest 3	2.26	±	0.25	2.06	±	0.22	2.06	±	0.22	0.076
Rest 4	2.00	±	0.32	1.98	±	0.19	2.03	±	0.28	1.000
Post-test	1.16	±	0.23	1.29	±	0.32	1.19	±	0.18	0.782
VE										
Pre-test	27.5	±	6.3	26.3	±	5.2	26.7	±	6.4	1.000
Rest 1	**66.8**	**±**	**16.1*#**	**59.1**	**±**	**12.5***	**60.0**	**±**	**14.5#**	**0.011**
Rest 2	**75.9**	**±**	**18.6*#**	**57.7**	**±**	**10.5***	**59.9**	**±**	**10.3#**	**0.024**
Rest 3	76.3	±	16.6	65.0	±	10.1	67.4	±	12.7	0.053
Rest 4	68.0	±	12.2	65.9	±	8.1	65.5	±	13.9	1.000
Post-test	41.5	±	11.3	42.8	±	10.8	42.8	±	8.4	1.000
HR										
Pre-test	107	±	15	105	±	18	102	±	12	0.147
Rest 1	**169**	**±**	**15#**	164	±	22	**161**	**±**	**13#**	**0.102**
Rest 2	175	±	14	173	±	18	167	±	11	0.073
Rest 3	180	±	11	174	±	16	170	±	11	0.833
Rest 4	180	±	10	175	±	15	176	±	14	0.110
Post-test	145	±	18	141	±	27	148	±	18	0.189

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * = No MG and MG1; # = No MG and MG6.

During No MG condition, the consumption of VCO_2 during Rest 2 was 19.0% significantly higher compared to MG6 and HR was approximately 4.0% higher during the first round and resting interval, which suggested that participants were exercising harder without a mouthguard (Table 5-2; Appendix E1). There were no statistical differences in the RER between the three mouthguard conditions when the mean and maximum values were compared (Appendix E1 and E2).

The levels of BLa prior to the first round were similar and accumulation of BLa increased progressively during the exercise activity (Figure 5-6). Although not significant, lower BLa was observed whilst performing with MG6 compared to wearing no mouthguard or MG1. Following the third round, BLa during No MG condition was 24.0% higher than BLa recorded with MG1 (p = 0.036).

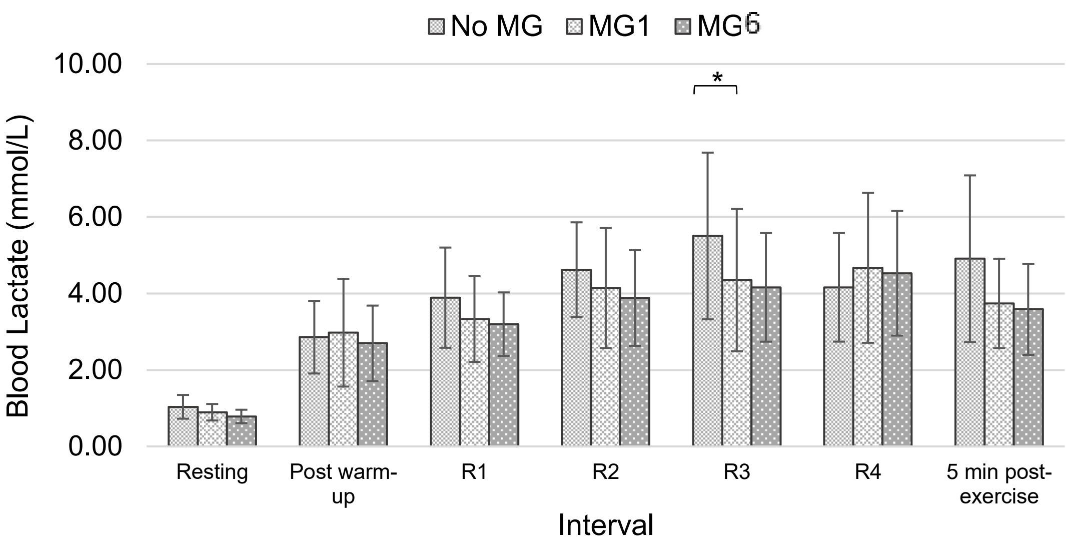

Figure 5-6: Differences in blood lactate levels at rest, post warm-up, rounds 1-4 (R1, R2, R3, R4) and 5 min post-exercise without a mouthguard (No MG), MG1 and MG6 under hypoxic condition (N = 7). Error bars represent standard deviation. Statistical differences ($p < 0.05$) are highlighted by: * No MG and MG6.

The perceived rate of exertion recorded following each boxing round did not differ significantly (from 11.90 ± 1.76 'Light' to 16.57 ± 2.60 'Very hard', 6 - 20 scale; $p > 0.059$).

5.3.2 Effects of Mouthguard Designs on Physiological Parameters under Normoxic and Hypoxic Conditions

Although no statistical differences were present, there was a 4.0% increase of the peak VO_2 (ml/min/kg) and HR recorded under altitude compared to normal conditions when no mouthguard was used (Table 5-4). Wearing MG1 did not lead to a general change in the exercise intensity, whereas MG6 showed higher VO_2 (ml/kg/min) and HR at hypoxic condition (2.0% and 3.0% respectively).

Table 5-4: Intensity of the boxing exercise protocol at normoxic and hypoxic conditions expressed as a percentage of the maximum oxygen uptake (VO_{2max} ml/kg/min) and maximum heart rate (HR_{max} bpm) recorded during participants' maximal exertion tests ($N = 7$).

		No MG			MG1			MG6		
		Mean		SD	Mean		SD	Mean		SD
%VO_{2max}	**Normoxia**	84%	±	0.1	86%	±	0.1	85%	±	0.1
	Hypoxia	88%	±	0.1	86%	±	0.1	87%	±	0.1
%HR_{max}	**Normoxia**	86%	±	0.8	85%	±	0.1	85%	±	0.1
	Hypoxia	90%	±	0.1	85%	±	0.1	88%	±	0.1

Although VO_2 (L/min and ml/kg/min), VE and HR followed the same pattern of changes throughout the exercise protocol regardless of the mouthguard design, greater measurements were observed whilst performing at simulated altitude conditions. For example, a significant increase in VO_2 (L/min and ml/kg/min) and VE were reported after the second boxing round when tests were performed with the same mouthguard but at different environmental condition (Table 5-5 and 5-6; Appendix E3 and E4). The effect of performing at altitude conditions was demonstrated by the increase of airflow parameters and HR. The average percentage increase between No MG(n) and No MG(h) within VO_2 (L/min) was 11.5% compared to 3.6% increase of MG1(n)-MG1(h) and 7.5% increase of MG6(n)-MG6(h). Additionally, the rest of the respiratory parameters also followed this trend.

Similarly, there was a 4.4% increase of HR at No MG(h) compared to No MG(h), which was significant prior to the second boxing round ($p \leq 0.05$). There was no statistical difference in HR when MG1 and MG6 were used in both environmental conditions. The percentage increase of HR between MG1(n)-MG1(h) and MG6(n)-MG6(h) was 0.3% and 2.5% respectively.

Table 5-5: Mean ± SD of VO_2 (L/min) and VE (L/min) and the absolute maximum of HR (bpm) for each round (R1-R4) under normoxic and hypoxic conditions whilst wearing no mouthguard (No MG(n) and No MG(h)) or the two selected designs (MG1(n) and MG1(h), MG6(n) and MG6(h)) (N = 7).

		No MG(n)			No MG(h)				MG1(n)			MG1(h)				MG6(n)			MG6(h)			
Parameter		**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***
VO_2	**R1**	2.63	±	0.70	2.87	±	0.28	0.106	2.85	±	0.57	2.75	±	0.75	0.600	2.72	±	0.71	2.91	±	0.79	0.504
	R2	2.77	±	0.77	3.12	±	0.55	0.067	2.93	±	0.32	2.95	±	0.49	0.846	2.75	±	0.59	2.93	±	0.73	0.175
	R3	**2.68**	**±**	**0.73***	**3.11**	**±**	**0.35***	**0.008**	**2.85**	**±**	**0.39#**	**2.93**	**±**	**0.44#**	**0.019**	2.70	±	0.57	2.99	±	0.53	0.108
	R4	**2.68**	**±**	**0.66***	**2.99**	**±**	**0.35***	**0.026**	2.92	±	0.47	2.95	±	0.40	0.711	2.72	±	0.53	3.05	±	0.53	0.166
VE	**R1**	82.0	±	22.3	94.3	±	28.8	0.058	78.9	±	18.7	91.3	±	28.1	0.148	77.6	±	24.2	94.3	±	34.2	0.087
	R2	92.1	±	29.9	108.1	±	26.1	0.068	87.9	±	15.7	101.8	±	26.4	0.074	**86.5**	**±**	**24.9†**	**102.3**	**±**	**44.7†**	**0.012**
	R3	**88.8**	**±**	**28.5***	**108.1**	**±**	**23.6***	**0.022**	86.9	±	14.4	98.7	±	20.3	0.085	**83.2**	**±**	**24.5†**	**104.0**	**±**	**27.2†**	**0.004**
	R4	90.2	±	30.2	106.5	±	16.7	0.053	93.1	±	21.5	116.7	±	22.1	0.135	**85.4**	**±**	**25.6†**	**109.4**	**±**	**22.0†**	**0.006**
HR	**R1**	**167**	**±**	**19***	**174**	**±**	**13***	**0.037**	168	±	14	169	±	21	0.969	165	±	14	168	±	14	0.305
	R2	173	±	19	180	±	12	0.099	174	±	13	176	±	17	0.678	172	±	13	175	±	13	0.073
	R3	173	±	19	181	±	12	0.081	178	±	13	178	±	16	0.882	173	±	16	178	±	13	0.171
	R4	176	±	20	184	±	10	0.290	180	±	14	180	±	13	0.873	174	±	14	183	±	17	0.091

Significant main effects are highlighted in bold. Statistical group differences between parameters recorded at normoxic and hypoxic conditions ($p < 0.05$) are highlighted by: * = No MG(n) and No MG(h); # = MG1(n) and MG1(h); † = MG6(n) and MG6(h).

Table 5-6: Mean ± SD of VO_2 (L/min) and VE (L/min) and the absolute maximum of HR (bpm) for each resting interval under normoxic and hypoxic conditions whilst wearing no mouthguard (No MG(n) and No MG(h)) or the two selected designs (MG1(n) and MG1(h), MG6(n) and MG6(h)) (*N* = 7).

	No MG(n)			**No MG(h)**				**MG1(n)**			**MG1(h)**				**MG6(n)**			**MG6(h)**			
Parameter	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***
VO_2																					
Pre-test	0.68	±	0.10	0.79	±	0.15	0.060	0.71	±	0.11	0.79	±	0.17	0.086	0.66	±	0.12	0.77	±	0.20	0.081
Rest 1	1.89	±	0.36	2.17	±	0.27	0.083	1.98	±	0.28	1.97	±	0.30	0.940	1.85	±	0.30	1.95	±	0.35	0.439
Rest 2	**1.87**	**±**	**0.35***	**2.29**	**±**	**0.33***	**0.004**	2.03	±	0.22	2.08	±	0.19	0.555	1.92	±	0.32	1.94	±	0.29	0.713
Rest 3	**1.76**	**±**	**0.32***	**2.26**	**±**	**0.25***	**0.000**	2.02	±	0.25	2.06	±	0.22	0.728	1.89	±	0.29	2.06	±	0.22	0.166
Rest 4	1.76	±	0.40	2.00	±	0.32	0.051	1.90	±	0.29	1.98	±	0.19	0.379	**1.81**	**±**	**0.34†**	**2.03**	**±**	**0.28†**	**0.008**
Post-test	1.08	±	0.19	1.16	±	0.23	0.458	1.08	±	0.20	1.29	±	0.32	0.145	1.15	±	0.12	1.19	±	0.18	0.468
VE																					
Pre-test	24.2	±	4.3	27.5	±	6.3	0.159	23.0	±	4.9	26.3	±	5.3	0.106	**22.5**	**±**	**5.5†**	**26.7**	**±**	**6.5†**	**0.029**
Rest 1	53.0	±	12.7	66.8	±	16.1	0.055	44.9	±	17.8	59.1	±	12.5	0.223	52.5	±	11.0	60.0	±	14.5	0.109
Rest 2	**56.4**	**±**	**13.4***	**75.9**	**±**	**18.6***	**0.015**	59.2	±	10.1	57.7	±	10.5	0.836	54.6	±	11.2	59.9	±	10.3	0.109
Rest 3	**55.1**	**±**	**11.7**	**76.3**	**±**	**16.6**	**0.006**	59.5	±	10.7	65.0	±	10.2	0.139	56.2	±	11.4	67.4	±	12.7	0.083
Rest 4	**52.8**	**±**	**12.3***	**68.0**	**±**	**12.2**	**0.018**	**58.1**	**±**	**11.2#**	**65.9**	**±**	**8.2#**	**0.007**	**52.1**	**±**	**13.0†**	**65.5**	**±**	**13.9†**	**0.005**
Post-test	37.3	±	4.2	41.5	±	11.4	0.089	40.0	±	7.1	42.8	±	10.9	0.212	39.0	±	6.8	42.8	±	8.4	0.091

	No MG(n)			No MG(h)				MG1(n)			MG1(h)				MG6(n)			MG6(h)			
Parameter	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***
HR																					
Pre-test	105	±	18	107	±	15	0.497	102	±	16	105	±	18	0.644	103	±	13	102	±	12	0.901
Rest 1	**160**	**±**	**17***	**169**	**±**	**15***	**0.018**	161	±	23	164	±	22	0.754	161	±	13	161	±	13	0.814
Rest 2	**167**	**±**	**18***	**175**	**±**	**14***	**0.050**	171	±	16	173	±	18	0.720	167	±	13	167	±	11	0.915
Rest 3	169	±	18	180	±	11	0.326	172	±	17	174	±	16	0.597	168	±	14	170	±	11	0.520
Rest 4	170	±	18	180	±	11	0.330	175	±	16	175	±	15	0.943	170	±	13	176	±	14	0.324
Post-test	141	±	18	145	±	18	0.357	147	±	23	141	±	27	0.635	143	±	27	148	±	18	0.354

Significant main effects are highlighted in bold. Statistical group differences between parameters recorded at normoxic and hypoxic conditions ($p < 0.05$) are highlighted by: * = No MG(n) and No MG(h); # = MG1(n) and MG1(h); † = MG6(n) and MG6(h).

Performing at hypoxic conditions increased the levels of BLa regardless of wearing a mouthguard or the design of the device (Figure 5-7 to 5-9). However, a statistical difference was recorded after the second boxing round only when participants performed without a mouthguard (increase of 4.6%, $p = 0.040$) (Figure 5-7). Similar to the findings reported earlier, performing without a mouthguard led to a higher percentage difference of BLa levels between normoxic and hypoxic condition (4.0%). However, wearing MG6 showed the lowest change in BLa accumulation of 3.1% between MG6(n)-MG6(h), although not significant.

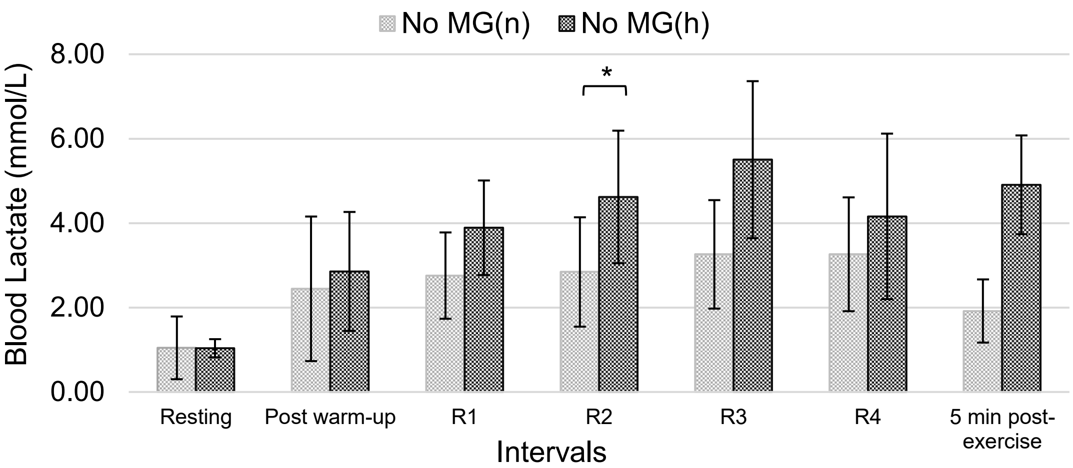

Figure 5-7: Differences in blood lactate levels at rest, post warm-up, rounds 1-4 (R1, R2, R3, R4) and 5 min post-exercise without a mouthguard under normoxic (No MG(n)) and hypoxic conditions (No MG(h)) ($N = 7$). Error bars represent the standard deviation. Statistical group differences ($p < 0.05$) are highlighted by: * = No MG(n) and No MG(h).

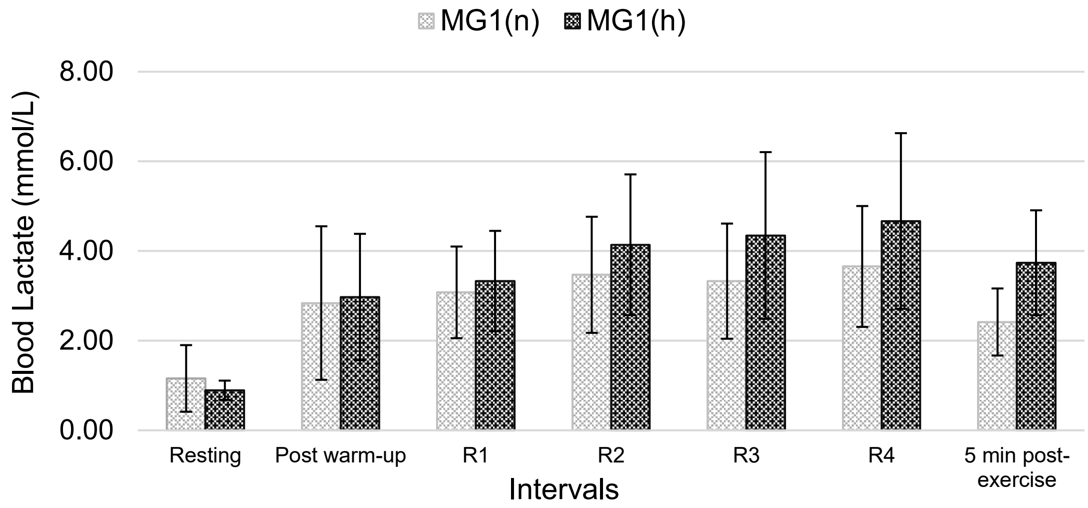

Figure 5-8: Differences in blood lactate levels at rest, post warm-up, rounds 1-4 (R1, R2, R3, R4) and 5 min post-exercise with MG1 at normoxic (MG1(n)) and hypoxic conditions (MG1(h)) (*N* = 7). Error bars represent the standard deviation.

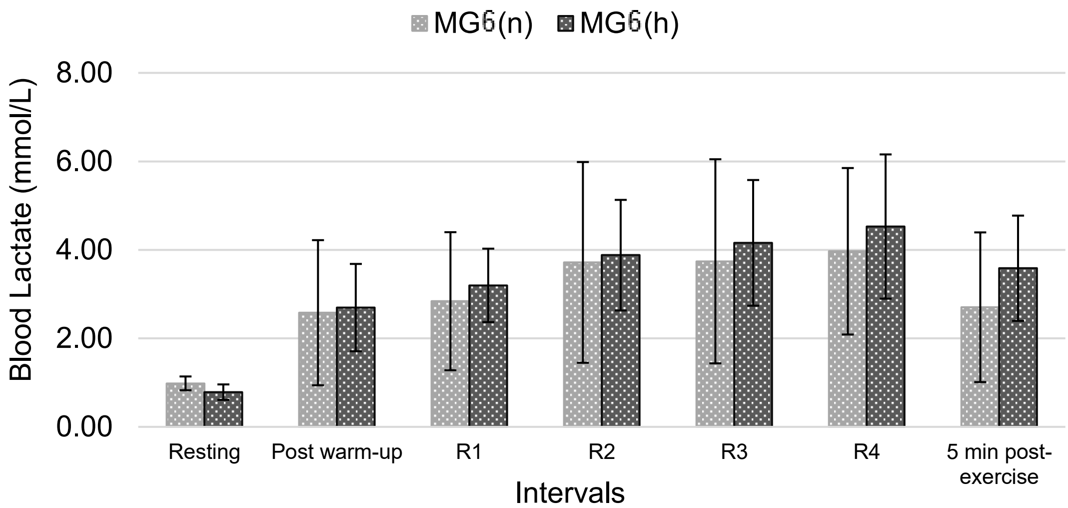

Figure 5-9: Differences in blood lactate levels at rest, post warm-up, rounds 1-4 (R1, R2, R3, R4) and 5 min post-exercise with MG6 at normoxic (MG6(n)) and hypoxic conditions (MG6(h)) (*N* = 7). Error bars represent the standard deviation.

Participants' perception of exertion was higher during all testing sessions under simulated altitude conditions compared to normal conditions, although the increase was significant at the first round only when MG6 was used (p = 0.047) (Figure 5-8). Participants reported that performing under hypoxic conditions was more challenging. At the end of the boxing protocol the level of exertion was rated as 'Very Hard' (16.57 ± 2.60), whereas at normoxic conditions it was rated as 'Somewhat Hard' (14.40 ± 2.50).

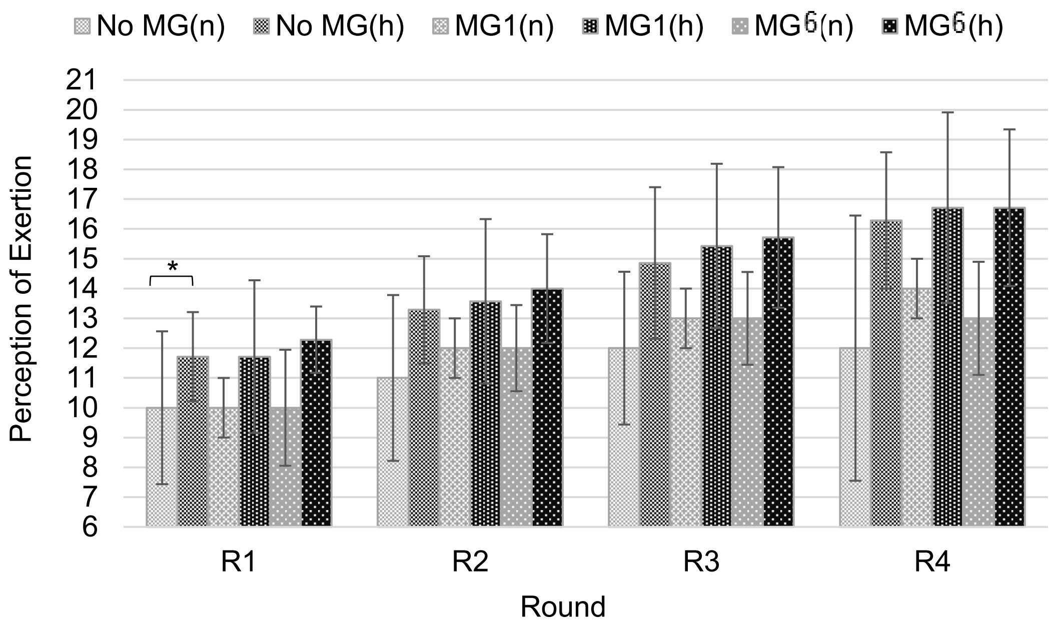

Figure 5-10: Perception of exertion following each round (R1, R2, R3, R4) without a mouthguard and with two mouthguards during normoxic and hypoxic conditions (No MG(n), MG1(n), MG6(n) and No MG(h), MG1(h), MG6(h)) (N = 7). Error bars represent the standard deviation. Statistical group differences (p < 0.05) are highlighted by: * = MG1(n) and No MG1(h).

There was a general increase of all examined parameters recorded under simulated altitude conditions, including measurements of respiratory flow, BLa accumulation and RPE.

5.3.3 Punch Velocity

Six participants successfully used the punch trackers whilst performing without a mouthguard. Three participants managed to complete No MG and MG6 conditions and only one participant completed all three laboratory visits using the tracker. There were no significant differences between the velocities recorded during No MG and MG6 conditions within three of the participants. However, there was a 35.9 - 42.4% increase in velocity of the right hand with MG6 (Table 5-7), which was due to higher exercise effort from one of the participants. Nevertheless, there was a consistency in terms of punch type thrown, 94.0% were reported ‘straight’.

Table 5-7: Velocity of punches (km/h) recorded with the left and right hand whilst using no mouthguard (No MG) and MG6 design (N = 3).

Round	**Left Hand Velocity (km/h)**		***p***	**Right Hand Velocity (km/h)**		***p***
	No MG	**MG6**		**No MG**	**MG6**	
1	17.02 ± 1.01	16.73 ± 1.22	0.825	16.73 ± 1.22	23.38 ± 6.84	0.211
2	17.73 ± 1.12	17.15 ± 2.53	0.626	17.15 ± 1.12	23.30 ± 4.69	0.193
3	18.14 ± 1.95	16.19 ± 1.95	0.427	16.19 ± 1.95	23.05 ± 7.47	0.194
4	16.82 ± 1.20	16.82 ± 1.28	0.998	16.82 ± 1.20	23.43 ± 7.74	0.281

5.4 Discussion

To the authors' knowledge, the present study is the first one to examine the effects of moderate altitude conditions and custom-made mouthguard designs using a sport-specific protocol. Seven boxers, based at sea level, performed under hypoxic conditions (16.77%) with two selected mouthguards and without a mouthguard. It was found that wearing a customised device did not affect respiratory flow under altitude. Initially, it was assumed that performing without a mouthguard was more beneficial due to a trend of a greater VO_2 (L/min and ml/kg/min) and VE compared to MG1 and MG6 throughout the protocol. However, it was noted that the percentage increase of airflow was no more than 8.7% during all boxing rounds, which is less than what Lovell et al. (2013) classified as a substantial and meaningful change in relation to boxing (15.0% increase in VO_2 L/min) (discussed in Chapter 4, Section 4.4). The findings reported lower HR and BLa whilst using the two mouthguards compared to having no mouthguard (2.0 - 5.5% and 10 - 24% respectively). Although this decrease was not significant, it could be considered as a positive outcome supporting the use of MG1 and MG6 under altitude. Additionally, there was an observed trend showing that MG1 design caused the least airflow impairments and lower BLa accumulation compared to MG6 or having no mouthguard. Hence, the belief that 4 mm palatal flange hinders these parameters is not supported by the present and previous study (Chapter 4) assessing performance under both normoxic and hypoxic conditions.

A significant increase of 7.6 – 24.0% in VO_2 (L/min and ml/kg/min) and VE was recorded during the recovery intervals after the first and second round without a mouthguard. Such a substantial increase of airflow whilst wearing no device could propose that during the short breaks between rounds boxers should remove their mouthguards to allow for greater oxygen uptake. It is worth mentioning that in reality it is a common practice for coaches to remove boxers' mouthguards as soon as a round is finished. Additionally, at the time of testing under simulated altitude, on a number of visits participants mentioned that they were having more difficulties to breathe during the resting intervals rather than whilst performing the exercise.

Regardless of the mouthguard condition, a general increase of all physiological parameters and perception of exertion whilst participants performed under hypoxic conditions were recorded compared to normal conditions. This could be explained by the effects of simulated altitude or the lack of participants' experience and previous training under such environment, which often lead to less oxygen transported to the muscles, higher VE, HR and BLa (McSharry, 2007; Gore et al., 2008). The findings demonstrated a significant increase in VO_2 (L/min and ml/kg/min) and VE during the second half of the protocol (R3 and R4), mainly during No MG(h) or MG6(h). In addition, significantly higher BLa (4.0%) was reported only during No MG(h) compared to No MG(n). This is a result of a lower PO_2 in the air, which hinders aerobic metabolic process and leads to greater build-up of BLa (Brutsaert et al., 2000; Girard et al., 2017). It was concluded that the lowest percentage change in parameters between normoxic and hypoxic conditions occurred when MG1 was used (3.6%), whereas the highest significant percentage change was recorded without a mouthguard (11.5%). These findings could suggest that using a custom-made mouthguard whilst performing at simulated moderate altitude may potentially decrease the initial environmental effect by reducing the changes in airflow and HR.

In the current work, the type and tempo of punches was controlled in order to control exercise intensity within participants, which was found as a limitation in a previous study by Kravitz et al. (2003), who made an attempt to validate a boxing protocol. The punch tracker sensors demonstrated reasonably high consistency of punches thrown by reporting 94.0% as cross (straight) punches. Additionally, the Hykso® punch trackers were recently validated against a VICON 3D motion capture system by measuring peak punch velocity (Hawkey and Finch, 2018). The authors found a strong correlation between the findings of Hykso® and VICON when a professional mixed martial art participant performed a combination of punches (R^2 = 0.868 – 1.000). Hence, demonstrating the effectiveness of using the tracker sensors under controlled laboratory conditions. In the present study, not all participants were able to use the punch tracker sensors during each session due to a prolonged waiting period of a required device replacement. Therefore, more data is still required to make conclusions as to whether any of the two mouthguard designs had an influence on punching velocity within participants. However, it was noted that the live feedback of punching velocity encouraged participants to keep up the same effort whilst performing.

A number of previous studies investigated the effects of mouthguard usage on physiological parameters within combat sports such as taekwondo, martial arts, boxing and karate (Kececi et al., 2005; Cetin et al., 2009; Rexhepi and Brestovci, 2013; Yarar et al., 2013). As shown in Figure 5-1, the locations of these studies were between 740 m – 1032 m, which corresponds to approximately 18.5% oxygen concentration. Due to the participants being residents at such altitudes, previous authors did not consider the effects of hypoxic conditions. However, the environmental factor should be taken into account during evaluation of the reported measurements. The studies' main findings were discussed in Chapter 4 (Section 4.1 and 4.4) and they all reported no significant differences in cardiopulmonary parameters whilst performing with and without a mouthguard. Only Cetin et al. (2009) reported a positive influence of wearing a custom-made mouthguard on hamstring torque power and peak power during a 30-s Wingate test. The authors claimed that their proposed mouthguard opened the jaw bite by 2-3 mm, which allowed an increase of airflow. VO_{2max} tests were performed by all of the cited investigations and only Rexhepi and Brestovci (2013) demonstrated around an 8.0% increase in VO_{2max} and HR_{max} whilst using a custom-made mouthguard, whereas the rest of the authors did not report more than a 2.0% change. The present study did not pre and post-tested participants' maximal exertion, which would have provided further information about changes in aerobic fitness following the three hypoxic testing sessions, as proposed by Galvin et al. (2013). However, the current work reported an 8.7% significant increase in VE, which was comparable to the results reported by Rexhepi and Brestovci (2013).

The present research reported that participants showed no signs of iron deficiency. In addition, no significant changes in Hb and Hct levels between testing sessions were recorded, which identified no physiological adaptation in terms of haematological responses (increase of red blood cells). However, performing the chosen boxing protocol under moderate altitude increased respiratory parameters and BLa levels, which indicated interference with metabolic responses. In contrast, Friedmann et al. (1999) who tested the effects of iron-supplementation in 16 boxers (9 treated and 6 controlled) did not agree that exercising under hypoxic conditions has an effect on aerobic metabolism in male boxers (pre vs post 18-day altitude camp VO_{2max} ml/kg/min: control 62.1 ± 3.6 vs 61.6 ± 5.7; iron-treated 61.0 ± 4.9 vs 63.1 ± 3.6). Although previous literature has evaluated some of the benefits of certain hypoxic training methods on haematological capacity (Ploszczyca et al., 2018), ventilatory adaptation (Townsend et al., 2016) and muscle buffering capacity (Gore et al., 2001), there is a lack of studies evaluating how to incorporate such methods in a normal training programme (Millet et al., 2010). This also includes a lack of research assessing the effects of altitude conditioning in boxing, which has been recommended previously; especially within simulated altitude conditions (Ruddock, 2015). Future work could consider similar exercise protocols to the current study in an attempt to examine the effects of hypoxic training in boxers. McLean et al. (2014) and Millet et al. (2010) proposed that sub-maximal training sessions (≥ 20 min) under hypoxic conditions could be used to improve endurance performance in athletes. Therefore, the time of the proposed exercising protocol could be extended, especially when examining professional boxers.

The present study was able to test a relatively small sample size as only seven participants agreed to continue with the follow up study. Additionally, testing under simulated conditions was completed eight months after the sessions under normal conditions and any possible changes in the fitness level of the participants were not examined. However, to the authors' knowledge, no previous published studies proposing boxing exercise performed with custom-made devices under simulated hypoxic conditions were found. Furthermore, the current research proposed possible effects on physiological parameters within the same participant, whereas previous studies examining hypoxic training have reported their findings based on two or more groups of participants (e.g. controlled and experimental) (Friedmann et al., 1999; Hamlin et al., 2008; Galvin et al., 2013; Singh et al., 2014). In addition, a great advantage of the current work was the use of an environmental chamber to precisely control and simulate consistent hypoxic conditions between testing sessions. The environmental chamber was ideal for participants to perform the proposed exercise protocol due to its size, which allowed testing equipment to be fitted without having an impact on the exercise activities. In comparison, previous studies have used hypoxicator machines for participants to breathe in, which did not provide the same level of controlled environment (Hamlin et al., 2008; Taralov et al., 2015). Finally, the present study examined punching velocity, which is an important metric that could be used to evaluate how physiological values could be compared against real time performance values. However, more data was required to further assess this parameter and make conclusions about the influence of using mouthguards on punching velocity.

5.5 Conclusions

The present study demonstrated that using custom-made mouthguards whilst performing a boxing protocol under hypoxic environment did not influence physiological parameters such as oxygen uptake, minute ventilation, heart rate and blood lactate. However, the findings recommended that participants should remove their protective devices during resting intervals in order to facilitate consumption of oxygen and ventilation. When cardiopulmonary parameters recorded under normal and altitude conditions were compared, it was found that wearing either of the mouthguard designs decreased the initial negative effects of hypoxia, especially during boxing rounds. This was demonstrated by a decrease in heart rate and blood lactate accumulation throughout the protocol, which was a positive outcome. The findings of the current study may contribute to future research within hypoxic training with the emphasis of using participants' full protective gear. Additionally, the study findings could be useful to coaches aiming to improve athletes' aerobic capacity and performance.

The final chapter will highlight the main conclusions based on the findings of this thesis. Additionally, it will evaluate the advantages and novel findings of the experimental procedures used in each study and propose further work within the area of mouthguards.

Chapter 6 : General Discussion and Conclusion

The overall aim of the present thesis was to investigate how variations in custom-made mouthguard designs influence retention and physiological parameters during both normoxic and hypoxic conditions and how these factors could affect users' compliance.

This chapter will highlight the main findings of each chapter/ study. It will also identify the strengths of the methods used and the novelty of the main findings, enhancing the knowledge within mouthguard research. In addition, it will evaluate possible limitations in order to propose improvements for future work.

6.1 Chapter 1: Previous Literature within the Area of Mouthguard Research.

Previous literature has identified the role of mouthguards as a primary preventative measure to reduce the rate of dental injuries in sports (FDI, 1990; ADA, 2004). In the UK, boxing is the only sport that requires the use of mouth protection at all times. Although high rates of dental injuries have been reported in other high-risk sports (e.g. rugby, field and ice-hockey and combat sports), regulatory bodies still have not made mouthguards compulsory at most competition levels. Previous studies have demonstrated that prevalence of orofacial injuries has decreased in sports where mouth protection has been regulated (Quarrie et al., 2005; Cohenca et al., 2007; Gould et al., 2016; Aman et al., 2018; Galic et al., 2018). Hence, in sports where the use of mouthguards is only recommended, additional guidelines or advisory materials should be provided to educate players, coaches and parents of young participants about the possible long-term benefits of using such a device.

Studies have agreed that custom-made devices have superior properties in terms of fit, protection and comfort compared to other types of devices such as stock and 'boil-and-bite' (Newsome et al., 2001; Parker et al., 2017; Gawlak et al., 2014, 2015). In addition, self-reported questionnaires have provided a greater understanding of mouthguard use in different countries within various levels of sport (Brionnet et al., 2001; Maeda et al., 2006; Duarte-Pereira et al., 2008; Boffano et al., 2012; Emerich and Nadolska-Gazda, 2013; Nozaki et al., 2013; Gawlak et al., 2014; Ilia et al., 2014; Liew et al., 2014; Galic et al., 2018; Gomez-Gimeno et al., 2019). It was noted that besides improvements in technologies and fabricating techniques, participants still experience comfort-related issues regardless of their location and sport. For example, Berry et al. (2005) reported that although 82.0% of their participants used customised mouthguards, 74.7% of the cohort still experienced comfort-related issues.

A factor that could relate to discomfort and possibly have a negative impact on speech and breathing is a lack of sufficient retention. Previously, two studies have examined the importance of retention within customised mouthguards. Del Rossi et al. (2008) has shown that using EVA blanks with darker colours lead to better device adaptability due to higher absorption of infrared energy during the thermoforming process. In contrast, Maeda et al. (2009) examined the influence of mouthguard design in terms of palatal flange and buccal extension. They also highlighted that the fabrication technique (pressure or vacuum thermoforming) was an influential factor on retention. A mouthguard without a palatal flange was more retentive than a mouthguard with a 4 mm palatal flange, but only when the devices were pressure-formed and not vacuum-formed. Whilst the two studies had different aims and experimental methods, they both highlighted that retention is an essential key factor, which had not been fully addressed previously. Therefore, Chapter 2 – Study 1 of this thesis proposed a further investigation evolving from the already published literature. An overview of the main findings and novelty of the work are reported in Section 6.2 of this chapter.

Within the literature, it was also found that participants at both recreational and elite level often refer to breathing issues due to wearing a mouthguard, which leads to an increase in non-compliance (Boffano et al., 2012; Emerich and Nadolska-Gazda, 2013; Lee et al., 2013; Queiroz et al., 2013; Tiwari et al., 2014; Miller et al., 2016). It has also been reported that customised devices cause least interference to respiratory flow and power output in comparison to ‘boil-and-bite’ devices (Kececi et al., 2005; Bourdin et al., 2006; Duarte-Pereira et al., 2008; Cetin et al., 2009; Gebauer et al., 2011; Collares et al., 2014; El-Ashker and El-Ashker, 2015; Piero et al., 2015). A variation in studies has often been associated with sample size, mouthguard type, exercise protocols and methods used to assess their chosen physiological parameters. To the authors' knowledge, Gebauer et al. (2011) were the only investigators to examine the use of two custom-made devices (with and without palatal flange) during exercise, whereas the remaining literature compared the effects between commercially available and customised mouthguards. Although the benefits of custom-made devices have been demonstrated, further work is still required to establish design characteristics that lead to minimal impedance of performance. In addition, many studies have not addressed the importance of replicating the physiological and aerobic demands of the athletes when engaging in their sport. Hence, Chapters 3 and 4 described novel sports-specific protocols in order to advance insight into the influence of mouthguards on respiratory performance. An overview of the main findings and novelty of these studies are reported in Sections 6.3 and 6.4 of this chapter.

Another important factor that has not been examined within the published literature relates to a change in physiological parameters experienced by participants wearing a mouthguard and performing in normoxic and hypoxic conditions. Previous research has demonstrated that training and competing under altitude conditions leads to increase in ventilation, heart rate and blood lactate accumulation (Péronnet et al., 1991; Gore et al., 1997; Wehrlin and Hallén, 2006; Clark et al., 2007; Gore et al., 2008; Hamlin et al., 2008; Chapman et al., 2011; Nassis, 2013; McArdle et al., 2015; Girard et al., 2017). Since changes in these physiological parameters have been associated with the use of mouthguards in controlled laboratory conditions, it would be beneficial to investigate whether wearing a device under different altitude conditions could have an effect on respiratory flow. Such findings would be useful for athletes who aim to improve their performance whilst training and competing at moderate / high altitudes, especially in sports where mouthguards are required. Therefore, a novel study (Chapter 5) was developed to examine the use of different mouthguards during performance under normoxic and hypoxic conditions within the same participant. An overview of the main findings and novelty of the work are reported in Section 6.5 of this chapter.

6.2 Chapter 2 – Study 1: The Effects of Custom-Made Mouthguard Designs on Retention.

Retention level of mouthguards relates to the superior fit of mouthguards. Frequently, issues with poor retention are associated with ‘boil-and-bite’ type devices, which are self-adapted causing loose fit and variations in thickness. By improving the retentive properties of the mouthguards, the levels of discomfort and interference with speech and breathing during performance should be minimised. The latter improvements would lead to an increase in device usage and participant compliance. Hence, it is important to examine how different fabrication techniques and design features of custom-made devices could possibly affect retention. Therefore, the aim of Chapter 2 was to measure the retentive properties of customised mouthguards in relation to their design features and thickness, whilst also investigating the effect of saliva on retention whilst wetting the dental model in artificial saliva.

As far as the author is aware, this is the first study to examine the retention of six different maxillary mouthguards. They were all pressure-formed over the same standard anatomical teaching model but varied in terms of design. Thickness was measured ten times at seven different points located in the anterior and posterior regions. The retention levels of each device were recorded at the same regions via six loading scenarios; with total retention being the mean of all measurements. Additionally, a novel experimental rig and procedure was used to assess retention. In brief, orthodontic brackets were attached onto the mouthguard at five specific points across the dental arch. Then, hard stainless steel wires (0.35 mm Ø and 120 mm length) were attached in order to connect the mouthguards to a newly designed rig, which was positioned in a Hounsfield testing machine. The maximum force required to fully displace the device from the dental model was considered to be an indication of the retention level of a mouthguard. The tests were performed under dry and wet (artificial saliva solution) conditions.

The findings of this study highlighted significant differences in retention of different mouthguard designs fabricated over the same model. Devices made from two layers of EVA (often referred to as dual laminate mouthguards) were more retentive than devices made from a single layer. This could partly be explained by the fact that 64.0% of the variability in retention was related to the mouthguard thickness under dry conditions and 55.0% under wet conditions. Previous studies have mainly assessed the importance of thickness in relation to energy absorption (Westerman et al., 2002; Yamada, 2006; Knapik et al., 2007; Maeda et al., 2008; Verissimo et al., 2016). However, the current work is believed to be the first to highlight the influence of thickness on mouthguard retention levels. Based on the findings of the present study, dental technicians and mouthguard suppliers should fabricate mouthguards that are thicker at specific regions (e.g. labial surfaces and/ or occlusal surfaces). For example, the most retentive device was made from a 2 mm layer that covered the labial surfaces of the anterior teeth and the occlusal surfaces of the posterior teeth, and a second 4 mm layer thermoformed over the whole dental arch. In addition, it is worth mentioning that this design had no palatal flange, which has been associated previously with improvements in comfort, speech and breathing (Gebauer et al., 2011; Nozaki et al., 2013; Gomez-Gimeno et al., 2019).

In comparison to previous methods used by Del Rossi et al. (2008) and Maeda et al. (2009), the current study demonstrated key improvements in terms of measuring retention at different regions of the mouthguards (behind the central incisors and the two canines, and over the two first molars). The newly designed rig allowed recording the retention at more than one site at the same time. For instance, in order to record the retention at the posterior region of a mouthguard, the wires attached to both first molars could be connected simultaneously to the testing machine. Furthermore, the present study also tried to replicate the oral environment, which has not been reported previously. The tests were repeated with the mouthguard and the dental model being soaked in artificial saliva solution prior to measuring retention. However, a limitation of this work was that the saliva solution did not contain mucin. The glycoprotein could have increased the viscosity of the saliva solution, which would have reflected the oral conditions more accurately and consequently influenced the retention results.

In order to improve the work investigating retention of mouthguards, future studies should include mucin in the artificial saliva solution and examine custom-made mouthguards thermoformed over dental models with various dental anatomy. Due to a variation and individuality of participants' dental anatomy, it could be useful to identify areas that should be considered when fabricating a custom-made device. For example, areas such as deep undercuts, teeth irregularities or missing teeth should be assessed.

6.3 Chapter 3 – Study 2: The Effects of Various Customised Mouthguard Designs on Physiological Parameters and Comfort in Male Rugby Players

Chapter 3 examined the influence of three customised mouthguards on breath-by-breath gas exchange and blood lactate within rugby union players who performed a newly designed rugby-specific protocol. Additionally, a short questionnaire was designed to assess participants' perception of comfort-related issues.

Fourteen rugby union players were provided with three different customised mouthguard devices (MG1, MG4 and MG6). The selected mouthguards were those that demonstrated the highest retention levels in Study 1 (Chapter 2). Each participant followed a sports specific protocol, which consisted of ten repetitions of the following: 1 min walk on a treadmill (5 km/h), acceleration from 5 km/h to 20 km/h, then a 15 s sprint at 20 km/h and deceleration down to 5 km/h, whilst their gas exchange and blood lactate were measured. The participants were also asked to rate their level of exertion after each sprint. Finally, the perception of comfort level for each device was assessed through a specifically designed questionnaire (1-10 scale, with 10 having the greatest merit).

The present study found no statistically significant influence on physiological parameters whilst wearing any of the three customised devices during exercise. However, despite the lack of statistical differences, a trend indicating an increase in oxygen consumption and ventilation with a decrease in blood lactate was observed when using MG1 compared to MG4 or MG6. Hence, having a device that was made from a 5 mm EVA blank that extends 4 mm in the palate (MG1) showed improvements in the parameters, although not statistically significant, in comparison to mouthguards with no palatal flange, made from two EVA layers (MG4 and MG6). However, participants reported that they were least likely to wear MG1 and favoured MG4 instead due to perceived minimal interferences with comfort-related issues. This was anticipated as MG4 design was the thinnest, did not extend into the palate and only covered the posterior teeth up to the first molars. According to the participants, the most retentive mouthguard was MG6, which confirmed the outcome presented in Chapter 2.

The new rugby-specific protocol probed physical activities that were previously reported as key elements in a rugby union game. Respiratory flow was examined over four different intervals such as walking, accelerating, sprinting and decelerating, which had not been included previously in mouthguard research. Additionally, the speed of the treadmill was informed by published data that reported the mean running speeds during a typical rugby union game (Cunniffe et al., 2009). The current research featured more accurate assessment of measurements by reporting breath-by-breath gas exchange and accumulation of blood lactate, which had not been addressed previously in male rugby players performing with and without a mouthguard. The findings reported should be disseminated amongst coaches and athletes in order to increase the use of custom-made devices that do not cause impairment in performance.

The current study was limited to three mouthguard designs and the method of breath-by-breath analysis to examine changes in respiratory flow, with and without a mouthguard. Future work should examine more variations of custom-made devices in terms of fabrication technique and materials. Additionally, the use of a portable breath-by-breath gas analyser is recommended in order to minimise any restrictions in movement during performance.

6.4 Chapter 4 – Study 3: The Effects of Various Customised Mouthguard Designs on Physiological Parameters and Comfort in Male Boxers

Chapter 4 examined the influence of different custom-made mouthguards on breath-by-breath gas exchange, blood lactate and levels of comfort within a cohort of boxers.

Fourteen participants were provided with the same three customised mouthguard devices as used by rugby players in Chapter 3. Participants performed a novel boxing protocol with and without each mouthguard. The main exercise consisted of 4 x 3 min boxing rounds with 1 min recovery in-between. During each round, the participants performed a combination of four straight maximum punches every 6 seconds against a punch bag. Breath-by-breath respiratory flow and accumulation of blood lactate were recorded throughout. Participants' perception in terms of comfort-related issues was also assessed with the same questionnaire as in Chapter 3.

The study reported here is believed to be the first to examine the use of three custom-made mouthguards in male boxers. No statistically significant effects in terms of using mouthguards on respiratory flow, heart rate and blood lactate were reported during the boxing rounds. However, additional data analyses was performed and a substantial increase of 8.3% - 15.0% in blood lactate concentration was reported when MG1 was used compared to No MG, MG4 and MG6. It was also demonstrated that participants should remove their devices during the resting intervals due to 0.2 – 18.2% greater oxygen consumption and 1.4 – 5.8% lower heart rate when no mouthguard was worn.

Participants were least likely to wear the mouthguard (MG4, thicker posterior region and no palatal extension) which demonstrated the most beneficial changes in physiological parameters. Instead, the design with a 4 mm palatal extension (MG1) was significantly more comfortable. The same mouthguard was rated more retentive than the other two, which did not agree with the findings from Chapter 2 where MG1 was recorded with the lowest retention level compared to MG4 and MG6. Based on these findings and the fact that none of the mouthguards caused any negative effects on physiological parameters, dental technicians should consider providing the participants' most favoured mouthguard design to increase compliance. Future work should investigate whether the level of compliance and usage during training would increase if participants were given the opportunity to choose their own mouthguard, knowing its benefits and limitations. It could be proposed that having the mind-set that they wear a custom device according to their own preferences could encourage them to wear mouth protection at all times.

Previously, one study El-Ashker and El-Ashker (2015) investigated the use of two different types of mouthguards (stock and custom-made) in boxers, whereas the present study examined three different custom-made mouthguard designs. The current research also attempted to replicate the physiological demands during a boxing match by using a new protocol. Initially, it was proposed to conduct all data collection at the boxing academy where participants trained. This would have been beneficial in terms of recruiting more participants and completion of the testing in their training environment. However, it was difficult to accommodate the testing sessions within the academy timetable and transport the necessary equipment whenever required.

To improve the methods of this study and enhance the validity of the findings, VICON motion capture system could be used to assess kinematic data and demonstrate the reliability of the exercise protocol. This was previously proposed by Maurer et al. (2015), who examined running patterns in recreational runners that used four different occlusion and jaw-repositioning dental splints.

6.5 Chapter 5 – Study 4: The Effects of Customised Mouthguard Designs on Physiological Parameters during Exercise under Hypoxic Conditions in Male Boxers

Chapter 5 examined the effects of wearing a mouthguard whilst training or competing under moderate / high altitude conditions, which is important especially in boxing where mouthguards are mandatory. The objective was to investigate the influence of custom-made devices on physiological parameters within boxers during hypoxic conditions. Findings were compared to those recorded under normoxic conditions (Study 3, Chapter 4) in order to evaluate the effects of altitude.

This study involved seven participants who took part in Study 3 (Chapter 4), though due to laboratory access and participants' time constraints only two mouthguards from Chapter 4 were tested. The two designs were selected based on the participants' perception of comfort that was reported in Section 4.3.4, Chapter 4. MG1 and MG6 were rated with the highest total score and participants were most likely to wear them again. In the present study, the chosen MG6 design was re-named to MG4. In order to compare performance under both normal and hypoxic conditions, the same experimental protocol as described in Chapter 4 (Section 4.2.4) was followed. Participants exercised in an environmental chamber that was programmed to FO_2 = 16.77%, 20˚C temperature and 50% humidity. In addition, as an improvement to the previous boxing protocol, punch tracker sensors were used to monitor punch velocity and types of punches thrown.

To the authors' knowledge, this is the first study to examine the influence of two custom-made mouthguards on airflow and blood lactate during simulated altitude in boxing. Using MG1 or MG4 did not have a statistically significant impact on oxygen consumption whilst performing under hypoxic conditions. However, similar to the findings reported under normal condition, statistically greater oxygen uptake and ventilation were recorded during the first two recovery intervals without a mouthguard. This suggested again that participants should remove their devices during the one-minute rest between boxing rounds. A beneficial effect of using the two mouthguards compared to no mouthguard under altitude was related to a substantial decrease in blood lactate accumulation during the boxing protocol; which was significant following the third round. This is a positive outcome for participants as lower levels of blood lactate lead to an increase in participants' endurance.

Overall, a greater oxygen consumption, minute ventilation, heart rate and blood lactate concentration were reported whilst participants performed under simulated altitude, which were significant following the second round. Using MG1 under hypoxic conditions led to the lowest increase of 3.6% in cardiopulmonary parameters compared to No MG and MG4, whereas training without a mouthguard led to an 11.5% increase. Hence, the initial environmental effect was reduced by the use of MG1. These findings suggested that wearing custom-made mouthguards had a positive effect on the examined parameters whilst exercising under altitude. However, it is recommended that participants remove their devices during recovery between rounds.

The present study proposed a boxing protocol that could be adapted by future research to monitor improvements in participants' performance following altitude training. Additionally, it demonstrated that using a facility such as an environmental chamber could achieve controlled conditions and its size was big enough to accommodate all equipment without restricting participants' movements. The use of punch tracker sensors in the present study further contributed to confirm the consistency in participants' performance in terms of number and type of punches thrown during each test session. For example, it was identified that 94.0% of the time participants performed a cross (straight) punch as they were initially instructed to do.

Further work is also required to examine a larger population in order to support the current findings. Currently, there is a lack of studies addressing the influence of using custom-made mouthguards in boxing whilst performing under altitude conditions, therefore research within that area should be promoted. More variations in mouthguard design and type should be investigated and coaches and athletes should also be informed of the findings. Such information may lead to improvements or considerations when designing a training programme, which is essential for participants' preparation prior to competitions.

6.6 Overall Conclusion

This thesis examined how variations in the design of customised mouthguards influenced retentive properties and affected physiological parameters during exercise. Additionally, it assessed participants' perception of comfort-related issues whilst wearing different devices. In terms of retention, a novel rig was designed to examine six specific mouthguard designs and their retentive properties. It was found that a design with a 4 mm palatal flange was more retentive than a design without one, which contradicted previous literature (Maeda et al., 2006). In addition, devices made from two EVA layers had a greater thickness and demonstrated superior retentive properties. These findings should be considered during mouthguard fabrication to enhance comfort. Wearing custom-made mouthguards did not have a statistically significant impact on oxygen uptake, minute ventilation, heart rate and blood lactate accumulation during sport-specific exercise protocols within rugby and boxing. This supported the outcome of previous research, which compared the use of 'boil-an-bite' to customised mouthguards. However, the current research reported observations, which should be considered, although no statistical significance was recorded. A trend in the rugby players' cohort showed that wearing MG1 design could lead to a lower percentage change in the examined physiological parameters compared to using MG4 and MG6. This is a positive finding as it shows some of the benefits of the most commonly fabricated mouthguard design. An important finding related to the use of mouthguards in boxing was that participants should remove their devices during the recovery intervals to allow for higher oxygen uptake. Nevertheless, the effects of hypoxic environment (increase in ventilation, oxygen uptake, heart rate and blood lactate) were minimised when a custom mouthguard was used during exercise in comparison to having no mouthguard. The benefits of wearing MG1 and MG6 designs were demonstrated by the substantial decrease in blood lactate accumulation whilst participants were performing a boxing protocol under simulated altitude conditions. This is essential as it relates to a lower rate of muscle fatigue and it could lead to an increase in endurance. It was highlighted that wearing custom-made mouthguards had no influence on physiological parameters and punching velocity within boxing participants who performed under moderate altitude conditions. The novelty of the present research arises from the assessment of different customised mouthguard designs and the proposed sports-specific exercise protocols, which reflect on participants' physiological demands. To the authors' knowledge, this is the first study that has examined mouthguard usage on physiological parameters within the same individual during normoxic and hypoxic conditions.

Overall, the reported findings demonstrated that custom-made mouthguards had no negative effects on physiological parameters within both rugby and boxing, which should encourage participants to wear mouth protection at all times. Further work should focus on disseminating this positive outcome and educating sports participants about the benefits of using customised devices, which may lead to a reduction in the rate of dental injuries.

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Appendix

A

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FACULTY OF SCIENCE AND ENGINEERING

SCHOOL OF HEALTHCARE SCIENCE

The effects of various designs of custom-made mouthguard on physiological parameters during exercise

PhD Study

Medical and dental assessment sheet

Participant's Information		
Name:		
Ref. No:		
Age:		**Gender:** male / female
E-Mail address:		
Mobile:		
Sport:		
Years of training: **Years of competition:**		**Years of using a mouthguard:**
Smoking Habits	Are you currently a smoker?	Yes / No
	If yes, how many do you smoke per day?	………..
	Are you a previous smoker?	Yes / No
	If yes, how long is it since you stopped?	………..
Do you consume alcohol?		Yes / No
If you answered **Yes**, do you usually have: an occasional drink / a drink every day / more than one drink a day?		
Participant's Medical Health		

1. **As far as you are aware, do you suffer or have you ever suffered from:**
   a) Any allergies? Yes / No
   b) High / Low Blood Pressure? Yes / No
   c) Diabetes? Yes / No
   d) Epilepsy? Yes / No
   e) Any form of heart complaint? Yes / No
   f) Anaemia? Yes / No
   g) Asthma? Yes / No
   h) Bronchitis? Yes / No
   i) Sinus problems? Yes / No
   j) Aneurysm / embolism? Yes / No
   k) Any other medical condition or illness? Yes / No

   If you answered **Yes** to any of the above, please give details……………………………………..

   ………

   ………

2. **Have you ever had dizziness, blackouts, giddiness or fainting?** Yes / No

   If you answered **Yes**, please give details …………………………………………………………

   ………

   ………

3. **Do you currently have any form of muscle or joint injury?** Yes / No

   If you answered **Yes**, please give details …………………………………………………………

   ………

   ………

4. **Have you had to consult a doctor within the last 6 months?** Yes / No

   If you answered **Yes**, please give details…………………………………………………………

   ………

   ………

5. **Are you presently taking any form of medication?** Yes / No

   If you answered **Yes**, please give details…………………………………………………………..

   ………

   ………

6. **Have you been in hospital for observation or had any serious illness or major operation?** Yes / No

   If you answered **Yes**, please give details …………………………………………………………

   ………

   ………

Participant's Dental Health

1. **Have you previously had any damage, knocks or bangs to your teeth?** Yes / No
   If you answered **Yes**, please give details …………………………………………………………
   ..………………………………………………………………………………………………
   …………………………………………………………………………………………………
2. **Do you suffer from headache, migraine or pains in your face, jaw joints around your head and neck?** Yes / No
   If you answered **Yes**, please give details …………………………………………………..…
   ……..……………………………………………………………………………………………
   ..…………………………………………………………………………………………………
3. **Do your jaw joints ever click/pop when you open and close?** Yes / No
   If you answered **Yes**, please give details (e.g. do both of them click or just one)…………
   ……..……………………………………………………………………………………………
   ………………………………………………………………………………………………..
4. **Do you ever have difficulty opening wide or moving the jaw to one side?** Yes / No
   If you answered **Yes**, please give details …………………………………………………..…
   ……..……………………………………………………………………………………………
   ..…………………………………………………………………………………………………

To be completed by the clinician:

Dental impressions

- ☐ **Upper and Lower Alginate Dental Impressions**
- ☐ **Wax Bite**
- ☐ **Appropriate disinfection of all devices**

Any considerations in relation to fabricating a mouthguard for this participant: ………………………………………………………………………………………………………

To be completed by the researcher:

Haemoglobin level (g/l): Measure 1 ………. Measure 2 ………. Mean ……….

As far as I am aware, the information I have given is accurate.

Signature of the clinician:

Signature of the participant:

Date:

B

[redacted]

FACULTY OF SCIENCE AND ENGINEERING

SCHOOL OF HEALTHCARE SCIENCE

The effects of various designs of custom-made mouthguard on physiological parameters during exercise

PhD Study

Participant Questionnaire

Participant's reference number:

Type of mouthguard (completed by the researcher):

Date:

How do you perceive this type of mouthguard design?

Total Mouthguard use: Less than 2h 3 – 5h 6h and more

Uncomfortable	1	2	3	4	5	6	7	8	9	10	**Comfortable**
Too Thick	1	2	3	4	5	6	7	8	9	10	**Reasonably thick**
Difficult to keep it in place	1	2	3	4	5	6	7	8	9	10	**Easy to keep it in place**
Difficult to breath	1	2	3	4	5	6	7	8	9	10	**Easy to breath**
Difficult to speak	1	2	3	4	5	6	7	8	9	10	**Easy to speak**
Negative effect on concentration	1	2	3	4	5	6	7	8	9	10	**No effect on concentration**
Poor Protection	1	2	3	4	5	6	7	8	9	10	**High Protection**
Not likely to wear it again	1	2	3	4	5	6	7	8	9	10	**Likely to wear it again**

Did you wear the mouthguard during your usual training and/ or competition matches for the period of one week? Yes / No

Please give details……………………………………………………………………

C

C1) Mean ± SD of all cardiopulmonary parameters and Max ± SD of heart rate during walking intervals (1 – 11) whilst wearing no mouthguard (No MG) or three selected designs (MG1, MG4 or MG6) (*N* = 14).

	No MG			MG1			MG4			MG6			
	Mean		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
VO_2 (L/min)													
1	**1.49**	**±**	**0.38†**	1.39	±	0.29	1.44	±	0.31	**1.33**	**±**	**0.20†**	**0.048**
2	2.94	±	0.51	3.00	±	0.67	3.00	±	0.55	2.83	±	0.42	0.973
3	2.98	±	0.49	3.10	±	0.64	3.03	±	0.52	2.94	±	0.44	0.590
4	2.86	±	0.49	2.93	±	0.65	2.91	±	0.35	2.83	±	0.45	0.924
5	2.98	±	0.53	3.06	±	0.73	3.08	±	0.42	2.89	±	0.50	0.650
6	3.01	±	0.54	3.01	±	0.64	3.02	±	0.46	2.91	±	0.45	0.702
7	2.83	±	0.38	2.97	±	0.56	2.96	±	0.45	2.85	±	0.44	0.402
8	2.97	±	0.49	3.10	±	0.61	3.08	±	0.52	2.94	±	0.39	0.258
9	2.99	±	0.47	3.10	±	0.56	3.08	±	0.44	2.86	±	0.51	0.115
10	3.01	±	0.47	3.02	±	0.64	3.03	±	0.43	2.91	±	0.47	0.692
11	2.74	±	0.41	2.86	±	0.52	2.83	±	0.47	2.83	±	0.42	0.347
VO_2 (ml/kg/min)													
1	**16**	**±**	**4†**	15	±	2	**15**	**±**	**2§**	**15**	**±**	**1†§**	**0.001**
2	32	±	3	32	±	3	32	±	3	31	±	3	1.000
3	32	±	3	34	±	3	32	±	2	32	±	2	0.499
4	31	±	3	32	±	4	31	±	2	31	±	2	1.000
5	32	±	4	33	±	7	33	±	2	32	±	3	0.828
6	33	±	3	33	±	4	32	±	2	32	±	2	0.894
7	31	±	2	32	±	3	32	±	2	31	±	2	0.554
8	32	±	2	33	±	4	33	±	2	32	±	2	0.709
9	33	±	3	34	±	3	33	±	2	31	±	3	0.104
10	33	±	3	33	±	4	33	±	1	32	±	2	0.907
11	30	±	3	32	±	3	31	±	2	31	±	2	0.276
VE (L/min)													
1	48.1	±	11.4	41.5	±	8.4	41.7	±	10.6	40.6	±	6.6	0.170
2	77.1	±	12.0	75.9	±	15.8	74.9	±	23.0	76.1	±	9.9	1.000
3	83.2	±	13.4	81.4	±	16.7	78.5	±	25.0	83.7	±	14.3	1.000
4	84.7	±	17.9	82.1	±	20.4	81.8	±	26.0	85.9	±	17.9	0.777
5	89.4	±	19.4	88.1	±	31.1	86.7	±	29.2	88.0	±	21.1	1.000
6	91.4	±	20.7	89.8	±	21.9	87.2	±	28.7	88.4	±	20.9	0.807
7	90.2	±	19.0	88.7	±	19.7	85.6	±	27.3	90.0	±	19.2	0.272
8	94.7	±	21.5	93.5	±	19.3	88.8	±	28.0	93.0	±	19.1	0.300
9	94.6	±	19.6	92.7	±	20.0	89.5	±	27.3	91.0	±	18.4	0.473

	No MG			MG1			MG4			MG6			
	Mean		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
10	92.8	±	16.3	90.2	±	18.1	87.2	±	24.2	92.0	±	19.2	0.480
11	85.32	±	15.33	86.93	±	14.31	81.98	±	22.26	90.65	±	13.87	0.523
RER													
1	1.02	±	0.11	1.00	±	0.08	1.00	±	0.08	0.98	±	0.07	0.836
2	0.94	±	0.06	0.98	±	0.07	0.99	±	0.06	1.00	±	0.08	0.082
3	0.98	±	0.05	1.00	±	0.06	1.01	±	0.05	1.03	±	0.07	0.081
4	**0.99**	**±**	**0.05*†**	**1.00**	**±**	**0.07*¥**	1.02	±	0.05	**1.04**	**±**	**0.05†¥**	**0.001**
5	0.99	±	0.06	1.00	±	0.09	1.02	±	0.06	1.02	±	0.05	0.579
6	0.99	±	0.04	1.01	±	0.06	1.02	±	0.04	1.01	±	0.05	0.343
7	0.99	±	0.04	0.99	±	0.05	1.01	±	0.05	1.01	±	0.04	1.000
8	0.99	±	0.03	1.00	±	0.06	0.99	±	0.03	1.00	±	0.04	1.000
9	0.98	±	0.04	0.98	±	0.05	0.98	±	0.02	1.00	±	0.03	0.077
10	0.97	±	0.04	0.98	±	0.06	0.99	±	0.03	0.99	±	0.03	1.000
11	0.97	±	0.04	0.98	±	0.04	0.98	±	0.04	0.99	±	0.04	1.000
HR (bpm)													
1	**117**	**±**	**14*#†**	**111**	**±**	**14***	**110**	**±**	**12#**	**108**	**±**	**11†**	**0.006**
2	**147**	**±**	**13*#†**	**141**	**±**	**13***	**140**	**±**	**14#**	**140**	**±**	**11†**	**0.013**
3	**153**	**±**	**13#†**	149	±	12	**148**	**±**	**11#**	**148**	**±**	**13†**	**0.041**
4	156	±	13	151	±	13	151	±	15	150	±	13	0.194
5	157	±	13	154	±	20	155	±	13	153	±	14	0.221
6	160	±	12	156	±	14	156	±	12	155	±	12	0.106
7	160	±	12	157	±	11	157	±	12	157	±	12	0.179
8	161	±	11	160	±	12	159	±	11	160	±	11	1.000
9	163	±	12	160	±	11	154	±	24	160	±	10	0.753
10	164	±	10	160	±	13	156	±	15	161	±	10	0.811
11	158	±	13	158	±	12	150	±	27	160	±	9	1.000
HRmax (bpm)													
1	**120**	**±**	**13*†**	**114**	**±**	**13***	114	±	13	**112**	**±**	**12†**	**0.018**
2	**169**	**±**	**11#†**	165	±	12	**163**	**±**	**12#**	**163**	**±**	**12†**	**0.007**
3	174	±	10	172	±	11	171	±	11	170	±	11	0.080
4	176	±	10	173	±	11	173	±	11	174	±	11	0.288
5	176	±	10	175	±	20	175	±	10	175	±	10	1.000
6	178	±	9	177	±	10	176	±	9	176	±	10	0.555
7	178	±	9	178	±	9	177	±	9	177	±	9	0.869
8	179	±	9	178	±	9	178	±	8	178	±	9	1.000
9	180	±	9	179	±	9	178	±	8	179	±	8	1.000
10	180	±	9	179	±	9	178	±	9	179	±	8	1.000
11	179	±	9	179	±	9	175	±	14	179	±	8	1.000

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * = No MG and MG1; # = No MG and MG4; † = No MG and MG6; ¥ = MG1 and MG6; § = MG4 and MG6.

C2) Mean ± SD of all cardiopulmonary parameters and Max ± SD of heart rate during acceleration intervals (1 – 10) whilst wearing no mouthguard (No MG) or three selected designs (MG1, MG4 or MG6) (*N* = 14).

	No MG			MG1			MG4			MG6			
	Mean		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
VO_2 (L/min)													
1	1.95	±	0.39	1.86	±	0.39	2.08	±	0.38	1.88	±	0.31	0.071
2	2.68	±	0.43	2.68	±	0.57	2.71	±	0.50	2.52	±	0.42	0.070
3	2.75	±	0.49	2.81	±	0.67	2.78	±	0.48	2.60	±	0.44	0.220
4	2.75	±	0.46	2.83	±	0.60	2.92	±	0.53	2.63	±	0.44	0.356
5	2.73	±	0.43	3.02	±	0.77	2.80	±	0.40	2.71	±	0.45	0.507
6	2.76	±	0.47	2.83	±	0.68	2.84	±	0.49	2.67	±	0.48	0.282
7	2.83	±	0.52	2.85	±	0.63	2.86	±	0.45	2.78	±	0.47	1.000
8	2.76	±	0.49	2.94	±	0.54	2.91	±	0.54	2.68	±	0.46	0.284
9	2.84	±	0.46	2.80	±	0.56	2.84	±	0.39	2.68	±	0.49	0.289
10	2.87	±	0.64	2.89	±	0.64	2.83	±	0.42	2.71	±	0.47	0.492
VO_2 (ml/kg/min)													
1	21	±	3	20	±	3	22	±	2	21	±	2	0.550
2	29	±	2	29	±	3	29	±	3	28	±	3	0.080
3	30	±	3	30	±	5	30	±	3	28	±	3	0.482
4	30	±	3	31	±	4	31	±	3	29	±	3	0.464
5	30	±	3	33	±	6	30	±	2	30	±	2	0.562
6	30	±	3	31	±	5	30	±	3	29	±	3	0.735
7	31	±	4	31	±	4	31	±	2	30	±	2	1.000
8	30	±	3	32	±	4	31	±	3	29	±	3	0.592
9	31	±	3	31	±	3	31	±	2	30	±	3	1.000
10	32	±	5	32	±	4	31	±	2	30	±	2	0.423
VE (L/min)													
1	80.1	±	17.1	67.1	±	16.3	74.3	±	21.1	68.0	±	13.7	0.101
2	99.5	±	18.6	95.94	±	25.1	92.0	±	27.7	96.8	±	19.1	1.000
3	104.7	±	20.1	100.7	±	22.1	110.3	±	20.9	101.1	±	21.7	0.531
4	107.7	±	23.6	101.6	±	21.8	107.9	±	33.7	105.9	±	21.0	0.823
5	108.5	±	21.8	114.4	±	25.1	108.7	±	30.2	109.5	±	25.1	1.000
6	110.0	±	27.5	106.4	±	29.1	105.8	±	33.4	110.4	±	24.0	1.000

	No MG			MG1			MG4			MG6			
	Mean		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
7	113.6	±	24.6	110.9	±	22.3	108.7	±	33.1	111.9	±	23.1	1.000
8	111.9	±	26.0	114.8	±	24.0	110.5	±	33.4	110.7	±	21.7	1.000
9	115.3	±	23.2	111.1	±	22.0	107.8	±	30.1	108.7	±	25.4	1.000
10	112.4	±	25.0	110.2	±	21.4	105.7	±	28.3	111.4	±	20.9	1.000
RER													
1	1.08	±	0.11	1.03	±	0.11	**1.06**	**±**	**0.05§**	**1.01**	**±**	**0.07§**	**0.029**
2	1.08	±	0.08	1.12	±	0.09	1.12	±	0.09	1.14	±	0.09	0.097
3	1.11	±	0.07	1.13	±	0.09	1.16	±	0.08	1.15	±	0.08	0.133
4	1.12	±	0.05	1.12	±	0.07	1.14	±	0.07	1.17	±	0.09	0.142
5	**1.12**	**±**	**0.05#**	1.12	±	0.09	**1.16**	**±**	**0.08#**	1.13	±	0.06	**0.045**
6	1.10	±	0.05	1.11	±	0.08	1.13	±	0.06	1.15	±	0.06	0.075
7	1.09	±	0.07	1.11	±	0.07	1.12	±	0.08	1.12	±	0.07	1.000
8	1.08	±	0.06	1.09	±	0.10	1.12	±	0.07	1.12	±	0.06	0.899
9	1.08	±	0.06	1.11	±	0.08	1.10	±	0.06	1.12	±	0.05	0.285
10	1.07	±	0.07	1.09	±	0.10	1.10	±	0.06	1.11	±	0.07	0.895
HR (bpm)													
1	129	±	16	122	±	14	122	±	14	121	±	13	0.056
2	140	±	13	134	±	14	133	±	14	132	±	14	0.120
3	144	±	14	138	±	15	139	±	13	137	±	14	0.062
4	144	±	16	139	±	15	142	±	15	140	±	13	0.158
5	148	±	15	147	±	17	145	±	13	144	±	14	0.379
6	149	±	15	146	±	14	147	±	13	146	±	12	0.573
7	151	±	16	147	±	13	147	±	12	146	±	12	0.358
8	152	±	15	149	±	13	149	±	12	150	±	11	0.245
9	152	±	15	149	±	12	147	±	9	149	±	9	0.725
10	153	±	15	149	±	12	147	±	11	149	±	10	0.463
HRmax (bpm)													
1	136	±	15	129	±	14	130	±	15	128	±	14	0.102
2	145	±	13	141	±	13	140	±	14	139	±	14	0.152
3	**148**	**±**	**14†**	142	±	14	144	±	12	**142**	**±**	**14†**	0.044
4	148	±	15	145	±	14	147	±	14	145	±	13	0.245

	No MG		MG1		MG4		MG6		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p***
5	151	± 14	151	± 16	149	± 12	149	± 14	0.557
6	153	± 14	150	± 13	150	± 13	150	± 11	0.550
7	154	± 15	151	± 12	151	± 11	150	± 11	0.417
8	156	± 15	155	± 13	152	± 11	153	± 11	0.798
9	156	± 14	152	± 11	156	± 16	152	± 8	1.000
10	156	± 14	153	± 11	152	± 8	153	± 9	0.621

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: # = No MG and MG4; † = No MG and MG6; § = MG4 and MG6.

C3) Mean ± SD of VO_2 (ml/min/kg), RER, heart rate and Max ± SD heart rate during sprint intervals (1 – 10) whilst wearing no mouthguard (No MG) or three selected designs (MG1, MG4 or MG6) (*N* = 14).

	No MG			MG1			MG4			MG6			
	Mean		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
VO_2 (ml/kg/min)													
1	30	±	4	28	±	4	29	±	3	28	±	4	0.522
2	35	±	3	36	±	4	35	±	2	34	±	3	0.931
3	35	±	4	36	±	5	36	±	2	34	±	3	1.000
4	35	±	3	36	±	5	36	±	3	35	±	3	0.586
5	35	±	3	36	±	6	36	±	2	35	±	3	0.741
6	35	±	3	36	±	5	36	±	2	35	±	2	1.000
7	35	±	4	37	±	4	36	±	2	35	±	3	0.886
8	35	±	4	37	±	4	35	±	3	35	±	3	1.000
9	36	±	2	36	±	4	36	±	3	35	±	2	1.000
10	35	±	3	36	±	4	35	±	3	34	±	2	1.000
RER													
1	0.96	±	0.06	0.96	±	0.07	0.96	±	0.05	0.96	±	0.06	1.000
2	0.99	±	0.06	1.03	±	0.05	1.01	±	0.05	1.04	±	0.06	0.442
3	1.03	±	0.05	1.05	±	0.05	1.04	±	0.06	1.05	±	0.06	1.000
4	1.04	±	0.05	1.05	±	0.06	1.05	±	0.04	1.05	±	0.05	1.000
5	1.05	±	0.05	1.04	±	0.07	1.04	±	0.06	1.05	±	0.05	1.000
6	1.04	±	0.05	1.04	±	0.06	1.04	±	0.04	1.04	±	0.04	1.000
7	1.02	±	0.07	1.02	±	0.05	1.03	±	0.06	1.04	±	0.04	1.000
8	1.03	±	0.07	1.03	±	0.07	1.05	±	0.05	1.03	±	0.06	0.793
9	1.02	±	0.06	1.04	±	0.07	1.04	±	0.03	1.03	±	0.03	1.000
10	1.04	±	0.05	1.03	±	0.07	1.03	±	0.02	1.04	±	0.06	1.000
HR (bpm)													
1	152	±	14	148	±	13	147	±	13	145	±	14	0.104
2	160	±	11	156	±	13	155	±	12	153	±	14	0.148
3	160	±	12	157	±	13	157	±	13	156	±	14	0.184
4	160	±	13	159	±	12	160	±	12	159	±	12	0.422
5	162	±	12	162	±	14	161	±	11	160	±	13	0.502
6	164	±	12	162	±	12	162	±	11	162	±	11	0.673
7	165	±	13	163	±	11	162	±	9	162	±	11	0.431

	No MG			MG1			MG4			MG6			
	Mean		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
8	166	±	13	165	±	11	163	±	10	165	±	11	0.741
9	166	±	13	163	±	10	165	±	11	163	±	8	0.997
10	166	±	13	164	±	10	163	±	9	164	±	8	0.923

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by:* = No MG and MG1; † = No MG and MG6.

C4) Mean ± SD of all cardiopulmonary parameters and Max ± SD of heart rate during deceleration intervals (1 – 10) whilst wearing no mouthguard (No MG) or three selected designs (MG1, MG4 or MG6) (*N* = 14).

	No MG			MG1			MG4			MG6			
	Mean		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
VO_2 (L/min)													
1	3.49	±	0.48	3.63	±	0.69	2.45	±	0.56	3.47	±	0.49	0.653
2	3.71	±	0.49	3.88	±	0.73	3.85	±	0.55	3.64	±	0.48	0.228
3	3.54	±	0.59	3.79	±	0.72	3.78	±	0.54	3.67	±	0.47	0.054
4	3.61	±	0.52	3.82	±	0.70	3.79	±	0.45	3.62	±	0.46	0.438
5	3.65	±	0.49	3.78	±	0.71	3.66	±	0.47	3.61	±	0.48	1.000
6	3.53	±	0.57	3.76	±	0.64	3.74	±	0.38	3.61	±	0.48	0.291
7	3.63	±	0.49	3.76	±	0.69	3.72	±	0.58	3.62	±	0.54	0.571
8	3.62	±	0.43	3.74	±	0.59	3.75	±	0.52	3.57	±	0.51	1.000
9	3.59	±	0.47	3.76	±	0.66	3.69	±	0.50	3.55	±	0.52	0.648
10	**3.39**	**±**	**0.44***	**3.70**	**±**	**0.61***	3.63	±	0.36	3.46	±	0.43	**0.030**
VO_2 (ml/kg/min)													
1	38	±	4	39	±	4	26	±	3	38	±	3	1.000
2	41	±	3	42	±	5	41	±	3	40	±	3	0.463
3	39	±	4	41	±	5	41	±	3	40	±	2	0.055
4	39	±	4	42	±	5	41	±	3	40	±	3	0.468
5	40	±	4	41	±	5	39	±	2	40	±	3	0.895
6	39	±	4	41	±	4	40	±	3	40	±	3	0.270
7	40	±	3	41	±	4	40	±	4	40	±	4	0.868
8	40	±	4	41	±	4	40	±	2	39	±	4	1.000
9	40	±	2	42	±	4	40	±	3	39	±	2	0.527
10	**38**	**±**	**4***	**41**	**±**	**4***	39	±	3	38	±	3	**0.021**
VE (L/min)													
1	99.7	±	19.2	93.9	±	17.4	73.5	±	27.3	92.6	±	14.5	0.507
2	111.8	±	21.9	111.4	±	20.6	102.9	±	29.5	107.0	±	19.3	0.633
3	113.2	±	24.6	111.3	±	19.8	113.3	±	22.3	112.9	±	18.2	1.000
4	115.5	±	23.3	116.6	±	20.1	112.4	±	34.5	115.0	±	21.4	1.000
5	120.7	±	18.9	115.7	±	25.1	109.2	±	32.1	119.3	±	20.6	0.713
6	117.8	±	21.0	120.1	±	24.3	116.8	±	35.1	118.6	±	21.1	1.000
7	119.9	±	21.5	119.3	±	23.1	116.3	±	35.2	121.6	±	18.7	1.000
8	121.6	±	21.5	120.5	±	19.6	114.2	±	35.8	122.2	±	21.0	1.000
9	120.4	±	20.0	122.5	±	22.3	114.7	±	34.1	117.5	±	21.5	0.069
10	113.4	±	18.2	117.3	±	19.1	112.0	±	32.7	117.1	±	18.8	1.000

	No MG		MG1		MG4		MG6		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*p*
RER									
1	0.87	± 0.05	0.89	± 0.05	0.98	± 0.05	0.89	± 0.04	0.153
2	0.92	± 0.04	0.96	± 0.06	0.95	± 0.03	0.96	± 0.05	0.081
3	0.94	± 0.04	0.95	± 0.05	0.95	± 0.04	0.96	± 0.05	0.436
4	0.93	± 0.05	0.96	± 0.05	0.96	± 0.04	0.96	± 0.05	0.134
5	0.94	± 0.04	0.94	± 0.05	0.95	± 0.04	0.96	± 0.04	1.000
6	**0.94**	**± 0.04*†**	**0.94**	**± 0.05***	0.95	± 0.03	**0.95**	**± 0.04†**	**0.001**
7	0.93	± 0.04	0.94	± 0.04	0.94	± 0.02	0.95	± 0.04	0.791
8	0.93	± 0.05	0.94	± 0.05	**0.93**	**± 0.02§**	**0.95**	**± 0.03§**	**0.018§**
9	0.93	± 0.04	0.94	± 0.03	0.94	± 0.02	0.93	± 0.03	1.000
10	0.93	± 0.04	0.92	± 0.05	0.93	± 0.02	0.94	± 0.03	0.181
HR (bpm)									
1	**168**	**± 11†**	165	± 12	135	± 12	**163**	**± 12†**	**0.037**
2	173	± 10	171	± 11	169	± 11	169	± 12	0.148
3	174	± 11	171	± 12	170	± 12	172	± 11	0.239
4	174	± 11	173	± 11	174	± 11	173	± 11	1.000
5	176	± 10	175	± 11	175	± 10	175	± 11	0.526
6	176	± 10	175	± 10	174	± 10	175	± 10	0.969
7	177	± 11	176	± 9	175	± 9	176	± 10	1.000
8	177	± 10	178	± 10	176	± 10	177	± 9	1.000
9	178	± 10	176	± 9	176	± 8	177	± 9	0.764
10	177	± 9	176	± 10	175	± 9	177	± 8	0.786
HRmax (bpm)									
1	**169**	**± 11#†**	167	± 12	**141**	**± 11#**	**164**	**± 12†**	**0.005**
2	**174**	**± 10†**	172	± 11	171	± 11	**170**	**± 11†**	**0.036**
3	176	± 10	173	± 11	172	± 12	174	± 11	0.410
4	176	± 11	175	± 11	175	± 10	175	± 11	1.000
5	177	± 10	176	± 11	176	± 9	176	± 10	0.490
6	178	± 9	177	± 10	176	± 9	177	± 10	0.832
7	179	± 10	178	± 9	177	± 8	177	± 10	1.000
8	179	± 9	179	± 10	178	± 9	179	± 9	1.000
9	180	± 10	178	± 9	179	± 9	179	± 9	0.833
10	179	± 9	178	± 10	179	± 9	179	± 8	1.000

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * = No MG and MG1; † = No MG and MG6; § = MG4 and MG6.

D

D1) Mean ± SD of cardiopulmonary parameters for each round (R1-R4) and rest interval during three trials without a mouthguard (N = 5).

Parameter	Trial 1	Trial 2	Trial 3	*p*
VO_2 (L/min)				
Pre-test/ Rest	0.55 ± 0.08	0.74 ± 0.27	0.60 ± 0.12	0.602
R1	2.56 ± 0.56	2.11 ± 0.71	2.29 ± 0.61	0.503
Rest 1	1.71 ± 0.35	1.54 ± 0.34	1.58 ± 0.39	0.100
R2	2.50 ± 0.61	2.34 ± 0.77	2.35 ± 0.50	0.981
Rest 2	1.77 ± 0.41	1.70 ± 0.35	1.75 ± 0.48	1.000
R3	2.50 ± 0.59	2.41 ± 0.83	2.44 ± 0.42	1.000
Rest 3	1.62 ± 0.32	1.69 ± 0.35	1.74 ± 0.27	0.503
R4	2.55 ± 0.65	2.39 ± 0.63	2.40 ± 0.83	0.579
Rest 4	1.56 ± 0.43	1.52 ± 0.49	1.57 ± 0.36	1.000
Post-test/ Rest	0.91 ± 0.21	0.96 ± 0.21	0.88 ± 0.14	0.621
VO_2 (ml/kg/min)				
Pre-test/ Rest	7 ± 1	8 ± 1	8 ± 2	0.298
R1	32 ± 8	28 ± 7	29 ± 7	0.174
Rest 1	23 ± 8	19 ± 4	20 ± 5	0.490
R2	31 ± 8	30 ± 8	29 ± 6	0.768
Rest 2	22 ± 5	21 ± 5	22 ± 5	1.000
R3	30 ± 11	31 ± 9	31 ± 6	1.000
Rest 3	20 ± 4	21 ± 5	22 ± 4	0.225
R4	32 ± 8	30 ± 6	32 ± 8	0.533
Rest 4	19 ± 6	19 ± 6	20 ± 6	1.000
Post-test/ Rest	11 ± 3	12 ± 3	11 ± 2	1.000
VE (L/min)				
Pre-test/ Rest	21.4 ± 3.4	22.0 ± 5.6	22.8 ± 6.3	1.000
R1	72.2 ± 17.3	60.9 ± 18.1	62.1 ± 22.8	0.269
Rest 1	50.4 ± 9.8	42.7 ± 10.9	46.7 ± 17.1	0.068
R2	78.6 ± 21.1	69.7 ± 23.3	69.1 ± 22.7	0.189
Rest 2	54.7 ± 16.5	48.5 ± 15.2	51.9 ± 18.1	0.135
R3	80.7 ± 26.3	72.2 ± 27.5	70.8 ± 17.2	0.224
Rest 3	54.4 ± 14.0	48.0 ± 13.2	56.4 ± 14.9	0.334
R4	84.2 ± 29.3	73.8 ± 29.4	77.3 ± 21.6	0.115
Rest 4	51.7 ± 18.6	41.1 ± 17.8	50.5 ± 15.9	0.235
Post-test/ Rest	34.8 ± 8.6	34.1 ± 9.8	34.1 ± 8.8	1.000

VCO_2 (L/min)				
Pre-test/ Rest	0.58 ± 0.10	0.62 ± 0.17	0.58 ± 0.11	1.000
R1	2.30 ± 0.52	1.92 ± 0.50	2.01 ± 0.56	0.119
Rest 1	1.61 ± 0.36	1.37 ± 0.32	1.51 ± 0.43	0.103
R2	2.38 ± 0.64	2.19 ± 0.60	2.16 ± 0.45	0.281
Rest 2	1.71 ± 0.49	1.58 ± 0.41	1.66 ± 0.52	0.555
R3	2.36 ± 0.63	2.31 ± 0.71	2.25 ± 0.40	1.000
Rest 3	1.57 ± 0.38	1.55 ± 0.38	1.70 ± 0.35	0.551
R4	2.40 ± 0.73	2.21 ± 0.64	2.34 ± 0.54	0.390
Rest 4	1.48 ± 0.49	1.45 ± 0.49	1.50 ± 0.35	1.000
Post-test/ Rest	0.94 ± 0.27	2.97 ± 0.23	2.96 ± 0.20	1.000
RER				
Pre-test/ Rest	1.00 ± 0.06	0.97 ± 0.11	0.95 ± 0.07	0.201
R1	0.89 ± 0.04	0.88 ± 0.02	0.88 ± 0.03	1.000
Rest 1	0.94 ± 0.68	0.90 ± 0.04	0.96 ± 0.10	0.283
R2	0.95 ± 0.04	0.91 ± 0.03	0.92 ± 0.04	0.118
Rest 2	0.96 ± 0.83	0.93 ± 0.05	0.96 ± 0.10	0.736
R3	0.93 ± 0.05	0.93 ± 0.03	0.92 ± 0.05	1.000
Rest 3	0.96 ± 0.78	0.92 ± 0.05	0.98 ± 0.11	0.296
R4	0.93 ± 0.07	0.94 ± 0.04	0.94 ± 0.07	1.000
Rest 4	0.94 ± 0.08	0.95 ± 0.04	0.96 ± 0.09	1.000
Post-test/ Rest	1.02 ± 0.06	1.01 ± 0.32	1.08 ± 0.12	0.313
HR (bpm)				
Pre-test/ Rest	106 ± 17	117 ± 31	105 ± 20	1.000
R1	166 ± 18	161 ± 21	151 ± 23	0.067
Rest 1	151 ± 22	145 ± 34	131 ± 27	0.095
R2	**174 ± 18#**	168 ± 19	**158 ± 21#**	**0.035**
Rest 2	**159 ± 24#**	154 ± 33	**139 ± 26#**	**0.011**
R3	**175 ± 20#**	171 ± 16	**164 ± 21#**	**0.044**
Rest 3	159 ± 23	156 ± 29	147 ± 27	0.072
R4	**178 ± 20#**	**175 ± 12†**	**167 ± 21#†**	**0.039**
Rest 4	**159 ± 25#**	156 ± 31	**144 ± 26#**	**0.024**
Post-test/ Rest	136 ± 18	141 ± 32	122 ± 22	0.084

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: # = Trial 1 and Trial 3; † = Trial 2 and Trial 3.

D2) Max ± SD of cardiopulmonary parameters for each round (R1-R4) and rest interval during three trials without a mouthguard (*N* = 5).

Parameter	Trial 1	Trial 2	Trial 3	*p*
VO_2 (L/min)				
Pre-test/ Rest	0.85 ± 0.08	1.14 ± 0.27	0.83 ± 0.13	0.179
R1	3.20 ± 0.55	2.72 ± 1.05	2.77 ± 0.66	0.079
Rest 1	2.45 ± 0.53	2.18 ± 0.52	2.97 ± 0.61	0.393
R2	3.18 ± 0.84	2.96 ± 0.98	3.09 ± 0.85	0.541
Rest 2	2.63 ± 0.87	2.58 ± 0.61	2.41 ± 0.54	0.721
R3	3.45 ± 0.91	2.90 ± 0.98	2.94 ± 0.54	0.114
Rest 3	2.49 ± 0.52	2.38 ± 0.64	2.48 ± 0.55	0.813
R4	**3.25 ± 0.92***	**2.92 ± 0.82***	3.25 ± 0.92	**0.024**
Rest 4	2.58 ± 0.78	2.21 ± 0.57	2.42 ± 0.69	0.145
Post-test/ Rest	1.28 ± 0.22	1.38 ± 0.32	1.30 ± 0.34	1.000
VO_2 (ml/kg/min)				
Pre-test/ Rest	11 ± 2	12 ± 3	11 ± 2	0.361
R1	40 ± 8	36 ± 12	35 ± 8	0.154
Rest 1	29 ± 11	27 ± 7	29 ± 7	0.736
R2	38 ± 8	37 ± 9	38 ± 7	1.000
Rest 2	33 ± 12	32 ± 9	30 ± 7	0.871
R3	43 ± 10	37 ± 11	37 ± 9	0.072
Rest 3	31 ± 8	29 ± 8	31 ± 6	0.411
R4	**41 ± 10***	**36 ± 9***	40 ± 12	**0.006**
Rest 4	32 ± 11	28 ± 8	30 ± 10	0.188
Post-test/ Rest	16 ± 4	17 ± 5	17 ± 5	1.000
VE (L/min)				
Pre-test/ Rest	28.6 ± 3.1	48.8 ± 34.6	32.0 ± 8.8	0.722
R1	93.4 ± 19.4	75.9 ± 26.0	77.6 ± 26.4	0.098
Rest 1	**71.5 ± 18.2***	**58.8 ± 15.5***	66.5 ± 20.9	**0.023**
R2	**100.5 ± 29.4***	**87.3 ± 28.1***	87.3 ± 26.4	**0.003**
Rest 2	79.7 ± 29.6	69.1 ± 21.4	71.5 ± 23.1	0.476
R3	**103.2 ± 28.5***	**85.1 ± 31.4***	88.7 ± 23.3	**0.041**
Rest 3	76.8 ± 20.5	71.1 ± 26.6	78.5 ± 24.1	0.388
R4	**102.7 ± 35.0***	**87.1 ± 30.8***	100.3 ± 33.4	**0.011**
Rest 4	83.5 ± 35.1	63.7 ± 27.2	75.7 ± 31.3	0.058
Post-test/ Rest	46.5 ± 14.3	47.8 ± 16.3	47.1 ± 15.2	1.000

VCO_2 (L/min)				
Pre-test/ Rest	0.86 ± 0.10	**0.95 ± 0.17†**	**0.84 ± 0.18†**	**0.044**
R1	2.95 ± 0.54	2.48 ± 0.75	2.46 ± 0.75	0.053
Rest 1	2.24 ± 0.49	1.97 ± 0.53	2.10 ± 0.56	0.336
R2	3.00 ± 0.89	2.73 ± 0.77	2.77 ± 0.69	0.259
Rest 2	2.53 ± 0.92	2.28 ± 0.65	2.33 ± 0.63	0.428
R3	3.03 ± 0.77	2.74 ± 0.87	2.75 ± 0.57	0.514
Rest 3	2.31 ± 0.60	2.17 ± 0.63	2.33 ± 0.55	0.312
R4	3.08 ± 0.95	2.62 ± 0.75	3.03 ± 0.92	0.128
Rest 4	2.47 ± 0.95	2.00 ± 0.56	2.27 ± 0.65	0.200
Post-test/ Rest	1.24 ± 0.26	1.37 ± 0.40	1.37 ± 0.36	1.000
RER				
Pre-test/ Rest	1.04 ± 0.05	1.07 ± 0.69	1.04 ± 0.12	0.937
R1	0.94 ± 0.04	0.93 ± 0.02	0.92 ± 0.04	0.828
Rest 1	1.02 ± 0.07	0.98 ± 0.03	1.03 ± 0.08	1.456
R2	1.00 ± 0.05	0.97 ± 0.02	0.95 ± 0.06	0.230
Rest 2	1.02 ± 0.07	1.02 ± 0.05	1.04 ± 0.11	1.000
R3	0.99 ± 0.06	0.99 ± 0.04	0.97 ± 0.08	1.000
Rest 3	1.04 ± 0.08	1.00 ± 0.03	1.05 ± 0.09	0.662
R4	0.98 ± 0.09	0.99 ± 0.06	0.99 ± 0.08	1.000
Rest 4	0.99 ± 0.09	1.00 ± 0.05	1.03 ± 0.12	1.000
Post-test/ Rest	1.07 ± 0.07	1.04 ± 0.06	1.12 ± 0.10	0.615
HR (bpm)				
Pre-test/ Rest	112 ± 16	125 ± 34	115 ± 22	1.000
R1	167 ± 18	164 ± 21	155 ± 22	0.210
Rest 1	**168 ± 18#**	164 ± 21	**147 ± 22#**	**0.017**
R2	174 ± 18	168 ± 19	159 ± 21	0.055
Rest 2	**174 ± 18#**	168 ± 22	**157 ± 18#**	**0.002**
R3	175 ± 20	173 ± 16	166 ± 21	0.086
Rest 3	**175 ± 19#**	173 ± 17	**161 ± 19#**	**0.014**
R4	179 ± 20	176 ± 12	170 ± 20	0.174
Rest 4	**178 ± 20#**	172 ± 16	**166 ± 20#**	**0.042**
Post-test/ Rest	136 ± 18	141 ± 32	122 ± 22	0.084

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * = Trial 1 and Trial 2; # = Trial 1 and Trial 3; † = Trial 2 and Trial 3.

D3) Mean ± SD of cardiopulmonary parameters for each round (R1-R4) and rest interval while wearing no mouthguard (No MG) or three selected designs (MG1, MG4 or MG6) (N = 14).

Parameter	No MG	MG1	MG4	MG6	p
VO_2 (L/min)					
Pre-test/ Rest	0.63 ± 0.12	0.64 ± 0.13	0.60 ± 0.14	0.59 ± 0.17	0.157
R1	2.60 ± 0.40	2.65 ± 0.42	2.61 ± 0.47	2.69 ± 0.42	1.000
Rest 1	1.77 ± 0.31	1.83 ± 0.34	1.79 ± 0.28	1.80 ± 0.28	0.100
R2	2.60 ± 0.50	2.71 ± 0.40	2.75 ± 0.37	2.72 ± 0.51	0.623
Rest 2	1.76 ± 0.33	1.88 ± 0.28	1.79 ± 0.26	1.84 ± 0.35	1.000
R3	2.57 ± 0.47	2.72 ± 0.37	2.64 ± 0.45	2.64 ± 0.51	0.901
Rest 3	**1.69 ± 0.27***	**1.85 ± 0.30***	1.89 ± 0.48	1.89 ± 0.48	**0.046**
R4	2.62 ± 0.45	2.67 ± 0.41	2.69 ± 0.36	2.68 ± 0.56	1.000
Rest 4	1.70 ± 0.34	1.78 ± 0.32	1.82 ± 0.35	1.85 ± 0.58	0.145
Post-test/ Rest	0.96 ± 0.22	0.97 ± 0.20	1.00 ± 0.17	1.11 ± 0.37	0.124
VO_2 (ml/kg/min)					
Pre-test/ Rest	8 ± 2	8 ± 2	8 ± 2	7 ± 2	0.121
R1	33 ± 6	34 ± 6	33 ± 7	34 ± 5	1.000
Rest 1	23 ± 6	24 ± 6	23 ± 5	23 ± 4	0.349
R2	33 ± 6	35 ± 5	35 ± 6	35 ± 6	0.473
Rest 2	22 ± 5	24 ± 11	23 ± 5	24 ± 6	0.114
R3	33 ± 6	34 ± 5	33 ± 6	33 ± 6	1.000
Rest 3	**21 ± 3***	**23 ± 4***	22 ± 4	23 ± 5	**0.050**
R4	33 ± 5	34 ± 5	34 ± 5	34 ± 6	1.000
Rest 4	21 ± 4	22 ± 4	23 ± 5	23 ± 6	0.083
Post-test/ Rest	11 ± 4	12 ± 3	13 ± 3	14 ± 3	0.430
VE (L/min)					
Pre-test/ Rest	**23.2 ± 3.9#†**	22.7 ± 5.0	**19.7 ± 3.9#**	**20.5 ± 6.2†**	**0.018**
R1	**73.7 ± 16.4†**	73.4 ± 15.7	**71.7 ± 13.2¥**	**86.2 ± 17.5†¥**	**0.002**
Rest 1	51.3 ± 10.8	46.1 ± 14.2	49.4 ± 9.1	51.8 ± 11.2	0.363
R2	79.3 ± 20.8	78.9 ± 15.9	78.9 ± 14.1	81.3 ± 23.5	1.000
Rest 2	54.1 ± 13.7	55.7 ± 9.9	52.7 ± 10.5	54.5 ± 12.9	1.000
R3	79.5 ± 20.5	81.3 ± 18.2	79.2 ± 15.3	79.7 ± 22.3	1.000
Rest 3	52.8 ± 11.7	55.9 ± 10.5	52.2 ± 11.0	57.0 ± 16.2	1.000
R4	83.3 ± 21.5	83.3 ± 18.1	82.1 ± 14.7	82.9 ± 24.1	1.000
Rest 4	51.5 ± 12.9	54.9 ± 11.7	53.3 ± 11.8	54.6 ± 19.6	0.815
Post-test/ Rest	34.7 ± 5.9	36.1 ± 7.6	33.8 ± 5.9	36.5 ± 10.3	1.000

VCO_2 (L/min)					
Pre-test/ Rest	0.64 ± 0.11	0.64 ± 0.14	0.58 ± 0.14	0.56 ± 0.17	0.078
R1	2.31 ± 0.37	2.40 ± 0.41	2.38 ± 0.45	2.38 ± 0.36	1.000
Rest 1	1.64 ± 0.31	1.73 ± 0.33	1.69 ± 0.34	1.68 ± 0.28	1.000
R2	2.42 ± 0.48	2.55 ± 0.39	2.58 ± 0.38	2.55 ± 0.49	0.602
Rest 2	1.68 ± 0.38	1.83 ± 0.28	1.72 ± 0.31	1.75 ± 0.36	1.000
R3	2.40 ± 0.48	2.53 ± 0.36	2.49 ± 0.44	2.44 ± 0.48	1.000
Rest 3	1.59 ± 0.31	1.81 ± 0.28	1.69 ± 0.33	1.78 ± 0.49	0.062
R4	2.43 ± 0.46	2.53 ± 0.43	2.47 ± 0.43	2.52 ± 0.51	1.000
Rest 4	1.58 ± 0.36	1.70 ± 0.33	1.74 ± 0.37	1.72 ± 0.11	0.061
Post-test/ Rest	0.97 ± 0.20	1.02 ± 0.22	1.04 ± 0.18	1.10 ± 0.38	0.184
RER					
Pre-test/ Rest	0.98 ± 0.08	0.99 ± 0.08	0.97 ± 0.05	0.94 ± 0.06	0.698
R1	0.91 ± 0.04	0.90 ± 0.05	0.92 ± 0.03	0.89 ± 0.05	0.681
Rest 1	0.93 ± 0.05	0.94 ± 0.05	0.94 ± 0.06	0.93 ± 0.04	1.000
R2	0.95 ± 0.05	0.95 ± 0.03	0.94 ± 0.04	0.94 ± 0.06	1.000
Rest 2	0.95 ± 0.07	0.96 ± 0.04	0.96 ± 0.06	0.95 ± 0.05	1.000
R3	0.96 ± 0.05	0.97 ± 0.04	0.98 ± 0.04	0.96 ± 0.04	0.773
Rest 3	0.94 ± 0.07	0.96 ± 0.03	0.96 ± 0.05	0.94 ± 0.05	0.706
R4	**1.03 ± 0.28***	**0.97 ± 0.03***	0.98 ± 0.04	0.95 ± 0.06	**0.003**
Rest 4	0.93 ± 0.06	0.96 ± 0.04	0.96 ± 0.04	0.93 ± 0.06	0.287
Post-test/ Rest	1.02 ± 0.09	**1.05 ± 0.07¢**	1.04 ± 0.07	**0.99 ± 0.06¢**	**0.024**
HR (bpm)					
Pre-test/ Rest	97 ± 25	97 ± 23	101 ± 27	96 ± 16	0.807
R1	155 ± 23	158 ± 18	153 ± 20	157 ± 16	1.000
Rest 1	137 ± 25	141 ± 23	135 ± 21	138 ± 22	1.000
R2	161 ± 22	165 ± 17	160 ± 20	162 ± 16	1.000
Rest 2	146 ± 27	148 ± 18	142 ± 21	146 ± 20	1.000
R3	163 ± 24	168 ± 14	162 ± 21	164 ± 16	1.000
Rest 3	149 ± 24	153 ± 18	148 ± 22	149 ± 20	1.000
R4	166 ± 22	171 ± 16	166 ± 19	167 ± 16	1.000
Rest 4	146 ± 23	153 ± 19	153 ± 21	153 ± 19	1.000
Post-test/ Rest	122 ± 20	128 ± 18	125 ± 17	124 ± 18	0.865

Significant main effects are highlighted in bold. Statistical group differences (p <0.05) are highlighted by: * = No MG and MG1; # = No MG and MG4; † = No MG and MG6; ¢ = MG1 and MG6; ¥ = MG4 and MG6.

D4) Max ± SD of cardiopulmonary parameters for each round (R1-R4) and rest interval while wearing no mouthguard (No MG) or three selected designs (MG1, MG4 or MG6) (*N* = 14).

Parameter	No MG	MG1	MG4	MG6	*p*
VO_2 (L/min)					
Pre-test/ Rest	**1.02 ± 0.25#**	1.04 ± 0.33	**0.86 ± 0.22#**	0.92 ± 0.15	**0.003**
R1	3.35 ± 0.59	3.30 ± 0.50	3.37 ± 0.66	3.37 ± 0.66	1.000
Rest 1	2.71 ± 0.69	2.69 ± 0.39	2.80 ± 0.61	2.67 ± 0.46	1.000
R2	3.32 ± 0.74	3.43 ± 0.50	3.61 ± 0.61	3.52 ± 0.63	0.313
Rest 2	2.82 ± 0.65	2.92 ± 0.55	2.84 ± 0.54	2.97 ± 0.67	1.000
R3	3.49 ± 0.67	3.43 ± 0.50	3.46 ± 0.65	3.41 ± 0.61	0.706
Rest 3	2.66 ± 0.47	2.84 ± 0.57	2.66 ± 0.56	2.96 ± 0.86	0.883
R4	3.35 ± 0.60	3.34 ± 0.56	3.49 ± 0.55	3.57 ± 0.55	0.174
Rest 4	2.72 ± 0.53	2.95 ± 0.51	2.77± 0.56	2.78 ± 0.66	1.000
Post-test/ Rest	1.45 ± 0.35	1.67 ± 0.53	1.61 ± 0.60	1.75 ± 0.88	0.152
VO_2 (ml/kg/min)					
Pre-test/ Rest	**13 ± 4#**	13 ± 5	**11 ± 4#**	12 ± 3	**0.006**
R1	41 ± 8	41 ± 8	41 ± 9	42 ± 9	1.000
Rest 1	34 ± 9	33 ± 6	36 ± 8	34 ± 7	1.000
R2	42 ± 9	43 ± 6	44 ± 6	44 ± 8	0.690
Rest 2	36 ± 9	36 ± 8	36 ± 9	37 ± 6	1.000
R3	44 ± 8	42 ± 6	44 ± 9	43 ± 7	1.000
Rest 3	34 ± 7	36 ± 8	34 ± 9	37 ± 8	0.953
R4	42 ± 7	42 ± 8	43 ± 7	44 ± 8	0.311
Rest 4	34 ± 8	37 ± 7	35 ± 8	34 ± 7	0.915
Post-test/ Rest	13 ± 1	13 ± 1	11 ± 1	12 ± 1	0.073
VE (L/min)					
Pre-test/ Rest	**35.5 ± 10.8#**	37.8 ± 14.5	**28.6 ± 7.9#**	33.0 ± 8.6	**0.007**
R1	90.8 ± 20.3	87.5 ± 18.1	89.6 ± 18.5	93.7 ± 23.0	0.880
Rest 1	79.3 ± 24.7	74.2 ± 16.1	72.1 ± 19.1	73.9 ± 19.4	0.196
R2	100.0 ± 20.4	100.1 ± 20.5	95.8 ± 20.5	101.4 ± 22.2	1.000
Rest 2	83.7 ± 25.7	83.1 ± 21.5	78.0 ± 17.9	90.1 ± 23.1	0.217
R3	101.6 ± 24.6	100.5 ± 20.5	97.2 ± 21.0	99.8 ± 25.7	1.000
Rest 3	82.1 ± 23.1	81.8 ± 22.5	75.7 ± 18.6	90.4 ± 29.6	0.312
R4	104.5 ± 26.8	104.0 ± 24.3	98.5 ± 18.8	105.3 ± 26.8	1.000
Rest 4	82.5 ± 24.5	90.2 ± 23.2	78.3 ± 19.8	81.8 ± 25.4	0.319
Post-test/ Rest	48.2 ± 11.0	52.6 ± 18.4	51.0 ± 23.1	57.4 ± 29.4	0.152

VCO_2 (L/min)					
Pre-test/ Rest	**1.01 ± 0.22#**	1.05 ± 0.33	**0.83 ± 0.22#**	0.91 ± 0.17	**0.005**
R1	2.94 ± 0.50	2.92 ± 0.49	2.97 ± 0.59	2.93 ± 0.57	1.000
Rest 1	2.46 ± 0.62	2.48 ± 0.43	2.55 ± 0.54	2.39 ± 0.44	1.000
R2	3.01 ± 0.70	3.13 ± 0.47	3.30 ± 0.50	3.14 ± 0.61	0.284
Rest 2	2.64 ± 0.69	2.72 ± 0.56	2.69 ± 0.57	2.78 ± 0.67	1.000
R3	3.14 ± 0.65	3.15 ± 0.45	3.22 ± 0.60	3.09 ± 0.58	1.000
Rest 3	2.46 ± 0.52	2.61 ± 0.50	2.51 ± 0.57	2.75 ± 0.81	0.643
R4	3.07 ± 0.59	3.10 ± 0.56	3.24 ± 0.51	3.20 ± 0.55	0.802
Rest 4	2.49 ± 0.60	2.73 ± 0.50	2.58 ± 0.55	2.53 ± 0.65	0.184
Post-test/ Rest	1.37 ± 0.28	1.62 ± 0.21	1.62 ± 0.63	1.70 ± 0.83	0.084
RER					
Pre-test/ Rest	1.04 ± 0.08	1.05 ± 0.08	1.02 ± 0.06	1.03 ± 0.04	0.238
R1	0.99 ± 0.08	1.04 ± 0.12	1.02 ± 0.07	0.99 ± 0.08	0.735
Rest 1	1.01 ± 0.07	1.05 ± 0.17	1.00 ± 0.07	1.03 ± 0.04	0.238
R2	1.05 ± 0.09	1.05 ± 0.06	1.06 ± 0.07	1.07 ± 0.07	1.000
Rest 2	1.02 ± 0.08	1.04 ± 0.06	1.04 ± 0.08	1.02 ± 0.07	1.000
R3	1.07 ± 0.06	1.07 ± 0.05	1.07 ± 0.05	1.09 ± 0.08	0.648
Rest 3	1.02 ± 0.07	1.04 ± 0.06	1.02 ± 0.08	1.00 ± 0.07	1.000
R4	1.07 ± 0.08	1.08 ± 0.07	1.08 ± 0.08	1.06 ± 0.06	0.123
Rest 4	0.99 ± 0.07	1.02 ± 0.06	1.02 ± 0.06	0.99 ± 0.07	0.319
Post-test/ Rest	1.05 ± 0.09	1.09 ± 0.07	1.08 ± 0.07	1.07 ± 0.09	0.310
HR (bpm)					
Pre-test/ Rest	105 ± 20	103 ± 16	108 ± 33	102 ± 17	0.701
R1	159 ± 16	160 ± 18	156 ± 22	165 ± 22	0.096
Rest 1	155 ± 23	157 ± 21	153 ± 21	158 ± 16	1.000
R2	164 ± 23	166 ± 17	164 ± 22	165 ± 16	1.000
Rest 2	163 ± 24	166 ± 17	161 ± 16	163 ± 23	1.000
R3	166 ± 23	173 ± 18	167 ± 22	166 ± 17	0.074
Rest 3	164 ± 23	168 ± 16	165 ± 22	166 ± 16	1.000
R4	166 ± 22	**165 ± 16¢**	171 ± 19	**169 ± 16¢**	**0.041**
Rest 4	166 ± 23	168 ± 19	168 ± 16	171 ± 16	1.000
Post-test/ Rest	135 ± 21	143 ± 23	140 ± 24	139 ± 25	0.276

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: # = No MG and MG4; ¢ = MG1 and MG6.

E

E1) Mean ± SD of cardiopulmonary parameters for each round (R1-R4) and rest interval at hypoxic condition whilst wearing no mouthguard (No MG), MG1 and MG6 (*N* = 7).

	No MG			**MG1**			**MG6**			
Parameter	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
VO_2 (L/min)										
Pre-test	0.79	±	0.15	0.79	±	0.17	0.77	±	0.20	1.000
R1	2.87	±	0.28	2.75	±	0.75	2.91	±	0.79	0.575
Rest 1	**2.17**	**±**	**0.27***	**1.97**	**±**	**0.30***	1.95	±	0.35	**0.035**
R2	3.12	±	0.55	2.95	±	0.49	2.93	±	0.73	0.339
Rest 2	**2.29**	**±**	**0.33*#**	**2.08**	**±**	**0.19***	**1.94**	**±**	**0.29#**	**0.018**
R3	3.11	±	0.35	2.93	±	0.44	2.99	±	0.53	0.063
Rest 3	2.26	±	0.25	2.06	±	0.22	2.06	±	0.22	0.076
R4	2.99	±	0.35	2.95	±	0.40	3.05	±	0.53	0.209
Rest 4	2.00	±	0.32	1.98	±	0.19	2.03	±	0.28	1.000
Post-test	1.16	±	0.23	1.29	±	0.32	1.19	±	0.18	0.782
VO_2 (ml/kg/min)										
Pre-test	10	±	2	10	±	2	9	±	3	0.448
R1	35	±	5	34	±	8	36	±	11	1.000
Rest 1	**27**	**±**	**4*#**	**25**	**±**	**4***	**24**	**±**	**5#**	**0.015**
R2	38	±	6	36	±	6	36	±	12	0.563
Rest 2	**28**	**±**	**5*#**	**26**	**±**	**4***	**25**	**±**	**5#**	**0.005**
R3	38	±	6	36	±	4	37	±	8	0.466
Rest 3	28	±	5	26	±	3	26	±	4	0.078
R4	37	±	5	37	±	6	38	±	8	0.206
Rest 4	25	±	4	25	±	3	26	±	5	0.278
Post-test	14	±	3	15	±	4	15	±	3	0.328
VE (L/min)										
Pre-test	27.5	±	6.3	26.3	±	5.3	26.7	±	6.5	1.000
R1	94.3	±	28.8	91.3	±	28.1	94.3	±	34.2	1.000
Rest 1	**66.8**	**±**	**16.1*#**	**59.1**	**±**	**12.5***	**60.0**	**±**	**14.5#**	**0.011**
R2	108.1	±	26.1	101.8	±	26.4	102.3	±	44.7	0.234
Rest 2	**75.9**	**±**	**18.6*#**	**57.7**	**±**	**10.5***	**59.9**	**±**	**10.3#**	**0.024**
R3	**108.1**	**±**	**23.6***	**98.7**	**±**	**20.3***	104.0	±	27.2	**0.036**

	No MG			MG1			MG6			
Parameter	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
Rest 3	76.3	±	16.6	65.0	±	10.2	67.4	±	12.7	0.053
R4	106.5	±	16.7	116.7	±	22.1	109.4	±	22.0	0.236
Rest 4	68.0	±	12.2	65.9	±	8.2	65.5	±	13.9	1.000
Post-test	41.5	±	11.4	42.8	±	10.9	42.8	±	8.41	1.000
VCO_2 (L/min)										
Pre-test	0.75	±	0.17	0.76	±	0.19	0.73	±	0.18	0.498
R1	94.3	±	28.8	91.3	±	28.1	94.3	±	34.2	0.511
Rest 1	2.07	±	0.42	1.85	±	0.30	1.80	±	0.36	0.055
R2	2.97	±	0.71	2.82	±	0.42	2.69	±	0.75	0.330
Rest 2	**2.23**	**±**	**0.45#**	1.99	±	0.14	**1.81**	**±**	**0.28#**	**0.043**
R3	2.84	±	0.48	2.76	±	0.27	2.74	±	0.37	0.236
Rest 3	2.18	±	0.38	1.96	±	0.13	1.92	±	0.27	0.115
R4	2.74	±	0.29	2.79	±	0.27	2.80	±	0.34	1.000
Rest 4	1.89	±	0.33	1.89	±	0.08	1.88	±	0.31	1.000
Post-test	1.14	±	0.27	1.15	±	0.27	1.15	±	0.21	1.000
RER										
Pre-test	0.95	±	0.08	0.96	±	0.08	0.96	±	0.06	1.000
R1	0.93	±	0.11	0.96	±	0.09	0.92	±	0.09	0.843
Rest 1	0.95	±	0.10	0.94	±	0.06	0.93	±	0.07	1.000
R2	0.95	±	0.06	0.98	±	0.09	0.95	±	0.07	0.459
Rest 2	0.97	±	0.09	0.96	±	0.06	0.93	±	0.07	0.584
R3	0.94	±	0.07	0.97	±	0.08	0.95	±	0.11	1.000
Rest 3	0.97	±	0.10	0.96	±	0.08	0.94	±	0.08	0.700
R4	0.93	±	0.07	0.97	±	0.10	0.95	±	0.10	0.332
Rest 4	0.95	±	0.12	0.97	±	0.08	0.93	±	0.08	0.605
Post-test	0.98	±	0.07	0.99	±	0.10	0.97	±	0.10	1.000
HR (bpm)										
Pre-test	103	±	16	100	±	17	94	±	14	0.147
R1	**173**	**±**	**13#**	167	±	21	**165**	**±**	**14#**	**0.018**
Rest 1	152	±	17	144	±	25	143	±	16	0.102
R2	179	±	12	175	±	17	171	±	13	0.156
Rest 2	159	±	13	153	±	23	148	±	13	0.073
R3	180	±	12	177	±	16	173	±	13	0.249

	No MG			MG1			MG6			
Parameter	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
Rest 3	162	±	16	156	±	20	154	±	15	0.833
R4	157	±	70	179	±	13	178	±	17	0.176
Rest 4	183	±	10	152	±	18	160	±	17	0.110
Post-test	134	±	17	131	±	23	131	±	17	0.189

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * = No MG and MG1; # = No MG and MG6.

E2) Max ± SD of cardiopulmonary parameters for each round (R1-R4) and rest interval at hypoxic condition whilst wearing no mouthguard (No MG), MG1 and MG6 (N = 7).

	No MG			MG1			MG6			
Parameter	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
VO_2 (L/min)										
Pre-test	1.13	±	0.22	1.15	±	0.33	1.24	±	0.33	0.811
R1	3.50	±	0.28	3.77	±	0.75	3.48	±	0.79	0.781
Rest 1	3.00	±	0.52	2.86	±	0.61	2.75	±	0.68	1.000
R2	3.79	±	0.55	3.60	±	0.49	3.56	±	0.73	0.236
Rest 2	3.14	±	0.42	3.08	±	0.56	2.90	±	0.57	0.838
R3	3.74	±	0.35	3.63	±	0.44	3.54	±	0.53	0.590
Rest 3	3.24	±	0.40	2.90	±	0.37	3.08	±	0.53	0.028
R4	3.66	±	0.35	3.49	±	0.40	3.61	±	0.53	0.347
Rest 4	3.16	±	0.58	2.84	±	0.39	3.20	±	0.56	0.171
Post-test	1.79	±	0.36	1.71	±	0.25	1.78	±	0.32	1.000
VO_2 (ml/kg/min)										
Pre-test	14	±	4	15	±	5	16	±	5	0.89
R1	43	±	5	44	±	8	43	±	11	1.000
Rest 1	37	±	3	36	±	8	35	±	10	1.000
R2	46	±	6	44	±	6	45	±	12	0.202
Rest 2	37	±	8	39	±	9	36	±	8	0.976
R3	46	±	6	45	±	4	44	±	8	0.301
Rest 3	**40**	±	**6***	**36**	±	**5***	37	±	11	**0.048**
R4	45	±	5	43	±	6	45	±	8	0.570
Rest 4	40	±	6	36	±	6	41	±	8	0.198
Post-test	22	±	5	21	±	4	23	±	4	1.000
VE (L/min)										
Pre-test	38.0	±	8.5	38.9	±	10.9	43.0	±	11.6	0.629
R1	113.9	±	28.8	123.3	±	28.1	112.5	±	34.2	0.696
Rest 1	95.4	±	21.0	87.3	±	19.6	85.3	±	24.4	0.455
R2	128.0	±	26.1	126.1	±	26.4	130.7	±	44.7	1.000
Rest 2	102.4	±	22.6	99.5	±	19.6	97.9	±	17.1	1.000
R3	**132.1**	±	**23.6***	**122.6**	±	**20.3***	124.6	±	27.2	**0.012**
Rest 3	109.5	±	20.0	95.1	±	13.3	104.5	±	20.7	0.098

	No MG			MG1			MG6			
Parameter	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
R4	132.9	±	16.7	126.3	±	22.1	128.4	±	22.0	0.767
Rest 4	**108.5**	**±**	**12.3***	**90.6**	**±**	**10.3***	111.1	±	29.5	**0.028**
Post-test	63.3	±	17.7	62.9	±	12.0	61.8	±	11.7	1.000
VCO_2 (L/min)										
Pre-test	1.07	±	0.22	1.07	±	0.24	1.19	±	0.29	0.731
R1	3.23	±	0.60	3.53	±	0.60	3.07	±	0.67	0.253
Rest 1	2.86	±	0.63	2.64	±	0.46	2.50	±	0.63	0.462
R2	3.55	±	0.71	3.42	±	0.42	3.38	±	0.75	0.236
Rest 2	3.00	±	0.64	2.93	±	0.47	2.71	±	0.42	0.731
R3	3.47	±	0.48	3.36	±	0.27	3.24	±	0.37	0.121
Rest 3	3.04	±	0.58	2.72	±	0.27	2.83	±	0.40	0.471
R4	3.38	±	0.29	3.23	±	0.27	3.25	±	0.34	0.344
Rest 4	2.97	±	0.34	2.59	±	0.27	2.99	±	0.51	0.052
Post-test	1.72	±	0.40	1.70	±	0.25	1.72	±	0.29	1.000
RER										
Pre-test	1.03	±	0.11	1.01	±	0.09	1.02	±	0.05	1.000
R1	1.04	±	0.11	1.04	±	0.09	1.03	±	0.09	0.735
Rest 1	1.01	±	0.10	0.99	±	0.05	0.99	±	0.09	1.000
R2	1.03	±	0.06	1.07	±	0.09	1.03	±	0.07	0.941
Rest 2	1.03	±	0.11	1.03	±	0.07	1.00	±	0.08	0.259
R3	1.04	±	0.07	1.07	±	0.08	1.05	±	0.11	1.000
Rest 3	1.04	±	0.14	1.02	±	0.08	1.01	±	0.09	1.000
R4	1.05	±	0.07	1.08	±	0.10	1.04	±	0.10	0.446
Rest 4	1.00	±	0.14	1.03	±	0.12	0.98	±	0.08	0.853
Post-test	1.03	±	0.08	1.05	±	0.11	1.02	±	0.09	0.769
HR (bpm)										
Pre-test	107	±	15	105	±	18	102	±	12	0.379
R1	**174**	±	**13#**	169	±	21	**168**	±	**14#**	**0.018**
Rest 1	**169**	±	**15#**	164	±	22	**161**	±	**13#**	**0.023**
R2	180	±	12	176	±	17	175	±	13	0.068
Rest 2	175	±	14	173	±	17	167	±	11	0.176
R3	181	±	12	178	±	16	178	±	13	0.394

	No MG			MG1			MG6			
Parameter	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	***p***
Rest 3	180	±	11	174	±	16	170	±	11	0.889
R4	184	±	10	180	±	13	183	±	17	1.000
Rest 4	180	±	10	175	±	15	176	±	14	1.000
Post-test	145	±	18	141	±	27	148	±	18	1.000

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * = No MG and MG1; # = No MG and MG6.

E3) Mean ± SD of cardiopulmonary parameters for each round (R1-R4) and resting interval at both normoxia and hypoxia whilst wearing no mouthguard (No MG(n) and No MG(h)) or two selected designs (MG1(n) and MG1(h), MG6(n) and MG6(h)). *P*-values indicate significant differences between parameters recorded at normoxic and hypoxic conditions, whereas *p*-*Int* indicates the possibility of significant interaction between the main factors of environmental conditions and mouthguard type (*N* = 7).

	No MG(n)			**No MG(h)**				**MG1(n)**			**MG1(h)**				**MG6(n)**			**MG6(h)**				***p***
Parameter	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	***Int***
VO_2 (L/min)																						
Pre-test	0.68	±	0.10	0.79	±	0.15	0.060	0.71	±	0.11	0.79	±	0.17	0.086	0.66	±	0.12	0.77	±	0.20	0.081	0.884
R1	2.63	±	0.70	2.87	±	0.28	0.106	2.85	±	0.57	2.75	±	0.75	0.600	2.72	±	0.71	2.91	±	0.79	0.504	0.503
Rest 1	1.89	±	0.36	2.17	±	0.27	0.083	1.98	±	0.28	1.97	±	0.30	0.940	1.85	±	0.30	1.95	±	0.35	0.439	0.226
R2	2.77	±	0.77	3.12	±	0.55	0.067	2.93	±	0.32	2.95	±	0.49	0.846	2.75	±	0.59	2.93	±	0.73	0.175	0.135
Rest 2	**1.87**	**±**	**0.35***	**2.29**	**±**	**0.33***	**0.004**	2.03	±	0.22	2.08	±	0.19	0.555	1.92	±	0.32	1.94	±	0.29	0.713	**0.006**
R3	**2.68**	**±**	**0.73***	**3.11**	**±**	**0.35***	**0.008**	**2.85**	**±**	**0.39#**	**2.93**	**±**	**0.44#**	**0.019**	2.70	±	0.57	2.99	±	0.53	0.108	0.060
Rest 3	**1.76**	**±**	**0.32***	**2.26**	**±**	**0.25***	**0.000**	2.02	±	0.25	2.06	±	0.22	0.728	1.89	±	0.29	2.06	±	0.22	0.166	**0.026**
R4	**2.68**	**±**	**0.66***	**2.99**	**±**	**0.35***	**0.026**	2.92	±	0.47	2.95	±	0.40	0.711	2.72	±	0.53	3.05	±	0.53	0.166	0.080
Rest 4	1.76	±	0.40	2.00	±	0.32	0.051	1.90	±	0.29	1.98	±	0.19	0.379	**1.81**	**±**	**0.34†**	**2.03**	**±**	**0.28†**	**0.008**	0.064
Post-test	1.08	±	0.19	1.16	±	0.23	0.458	1.08	±	0.20	1.29	±	0.32	0.145	1.15	±	0.12	1.19	±	0.18	0.468	0.296
VO_2 (ml/kg/min)																						
Pre-test	8	±	2	10	±	2	0.058	9	±	2	10	±	2	0.220	8	±	2	9	±	3	0.078	0.611

	No MG(n)			No MG(h)				MG1(n)			MG1(h)				MG6(n)			MG6(h)				*p*
Parameter	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	***Int***
R1	32	±	10	35	±	5	1.000	35	±	9	34	±	8	0.694	33	±	9	36	±	11	0.421	0.530
Rest 1	25	±	7	27	±	4	0.445	24	±	5	25	±	4	0.834	23	±	5	24	±	5	0.371	0.729
R2	34	±	10	38	±	6	0.078	35	±	5	36	±	6	0.455	34	±	7	36	±	12	0.138	0.251
Rest 2	**23**	**±**	**5***	**28**	**±**	**5***	**0.018**	**25**	**±**	**4#**	**26**	**±**	**4#**	**0.017**	**25**	**±**	**7†**	**25**	**±**	**5†**	**0.000**	**0.000**
R3	**33**	**±**	**9***	**38**	**±**	**6***	**0.009**	**35**	**±**	**5#**	**36**	**±**	**4#**	**0.003**	33	±	7	37	±	8	0.062	0.109
Rest 3	**22**	**±**	**4***	**28**	**±**	**5***	**0.025**	**25**	**±**	**4#**	**26**	**±**	**4#**	**0.009**	**23**	**±**	**4†**	**26**	**±**	**4†**	**0.003**	**0.635**
R4	**33**	**±**	**7***	**37**	**±**	**5***	**0.035**	35	±	7	37	±	6	0.301	33	±	6	38	±	8	0.108	**0.045**
Rest 4	**21**	**±**	**5***	**25**	**±**	**4***	**0.060**	23	±	5	25	±	4	0.263	**22**	**±**	**5†**	**26**	**±**	**5†**	**0.004**	**0.017**
Post-test	13	±	3	14	±	3	0.503	13	±	3	15	±	4	0.604	14	±	2	15	±	3	0.482	0.954
VE (L/min)																						
Pre-test	24.2	±	4.3	27.5	±	6.3	0.159	23.0	±	4.9	26.3	±	5.3	0.106	**22.5**	**±**	**5.5†**	**26.7**	**±**	**6.5†**	**0.029**	0.879
R1	82.0	±	22.3	94.3	±	28.8	0.058	78.9	±	18.7	91.3	±	28.1	0.148	77.6	±	24.2	94.3	±	34.2	0.087	0.832
Rest 1	53.0	±	12.7	66.8	±	16.1	0.055	44.9	±	17.8	59.1	±	12.5	0.223	52.5	±	11.0	60.0	±	14.5	0.109	0.582
R2	92.1	±	29.9	108.1	±	26.1	0.068	87.9	±	15.7	101.8	±	26.4	0.074	**86.5**	**±**	**24.9†**	**102.3**	**±**	**44.7†**	**0.012**	0.960
Rest 2	**56.4**	**±**	**13.4***	**75.9**	**±**	**18.6***	**0.015**	59.2	±	10.1	57.7	±	10.5	0.836	54.6	±	11.2	59.9	±	10.3	0.109	0.093
R3	**88.8**	**±**	**28.5***	**108.1**	**±**	**23.6***	**0.022**	86.9	±	14.4	98.7	±	20.3	0.085	**83.2**	**±**	**24.5†**	**104.0**	**±**	**27.2†**	**0.004**	0.232
Rest 3	**55.1**	**±**	**11.7**	**76.3**	**±**	**16.6**	**0.006**	59.5	±	10.7	65.0	±	10.2	0.139	56.2	±	11.4	67.4	±	12.7	0.083	0.095
R4	90.2	±	30.2	106.5	±	16.7	0.053	93.1	±	21.5	116.7	±	22.1	0.135	**85.4**	**±**	**25.6†**	**109.4**	**±**	**22.0†**	**0.006**	0.813
Rest 4	**52.8**	**±**	**12.3***	**68.0**	**±**	**12.2**	**0.018**	**58.1**	**±**	**11.2#**	**65.9**	**±**	**8.2#**	**0.007**	**52.1**	**±**	**13.0†**	**65.5**	**±**	**13.9**	**0.005**	0.111
Post-test	37.3	±	4.2	41.5	±	11.4	0.089	40.0	±	7.1	42.8	±	10.9	0.212	39.0	±	6.8	42.8	±	8.4	0.091	0.831

	No MG(n)	No MG(h)		MG1(n)	MG1(h)		MG6(n)	MG6(h)		*p*
Parameter	**Mean SD**	**Mean SD**	***p***	**Mean SD**	**Mean SD**	***p***	**Mean SD**	**Mean SD**	***p***	***Int***
VCO_2 (L/min)										
Pre-test	**0.67 ± 0.11***	**0.75 ± 0.17***	**0.004**	**0.68 ± 0.13#**	**0.76 ± 0.19#**	**0.049**	**0.62 ± 0.14†**	**0.73 ± 0.18†**	**0.046**	0.420
R1	**2.37 ± 0.55***	**2.68 ± 0.06***	**0.041**	2.52 ± 0.59	2.60 ± 0.60	0.676	2.41 ± 0.54	2.62 ± 0.67	0.261	0.549
Rest 1	1.74 ± 0.36	2.07 ± 0.42	0.075	**1.87 ± 0.34#**	**1.85 ± 0.30#**	**0.000**	**1.72 ± 0.30†**	**1.80 ± 0.36†**	**0.034**	0.533
R2	2.58 ± 0.70	2.97 ± 0.71	0.073	2.73 ± 0.33	2.82 ± 0.42	0.550	2.54 ± 0.48	2.69 ± 0.75	0.080	0.279
Rest 2	1.76 ± 0.37	2.23 ± 0.45	0.101	**1.96 ± 0.25#**	**1.99 ± 0.14#**	**0.003**	**1.78 ± 0.32†**	**1.81 ± 0.28†**	**0.004**	0.107
R3	**2.43 ± 0.61***	**2.84 ± 0.48***	**0.007**	2.62 ± 0.34	2.76 ± 0.27	0.153	**2.48 ± 0.49†**	**2.74 ± 0.37†**	**0.046**	**0.033**
Rest 3	1.64 ± 0.34	2.18 ± 0.38	0.052	**1.94 ± 0.27#**	**1.96 ± 0.13#**	**0.019**	**1.74 ± 0.30†**	**1.92 ± 0.27†**	**0.025**	0.134
R4	2.44 ± 0.52	2.74 ± 0.29	0.088	2.73 ± 0.46	2.79 ± 0.27	0.633	2.47 ± 0.49	2.80 ± 0.34	0.064	**0.007**
Rest 4	**1.61 ± 0.39***	**1.89 ± 0.33***	**0.009**	**1.81 ± 0.32#**	**1.89 ± 0.08#**	**0.004**	**1.66 ± 0.33†**	**1.88 ± 0.31†**	**0.012**	0.125
Post-test	1.08 ± 0.17	1.14 ± 0.27	0.161	**1.13 ± 0.23#**	**1.15 ± 0.27#**	**0.040**	1.16 ± 0.15	1.15 ± 0.21	0.078	0.706
RER										
Pre-test	0.99 ± 0.08	0.95 ± 0.08	0.434	0.96 ± 0.07	0.96 ± 0.08	0.884	0.94 ± 0.07	0.96 ± 0.06	0.615	0.600
R1	0.91 ± 0.07	0.93 ± 0.11	0.554	0.91 ± 0.14	0.96 ± 0.09	0.223	0.91 ± 0.08	0.92 ± 0.09	0.692	0.833
Rest 1	0.92 ± 0.05	0.95 ± 0.10	0.397	0.94 ± 0.05	0.94 ± 0.06	1.000	0.93 ± 0.05	0.93 ± 0.07	0.969	0.769
R2	0.95 ± 0.08	0.95 ± 0.06	0.827	0.95 ± 0.09	0.98 ± 0.09	0.162	0.95 ± 0.09	0.95 ± 0.07	0.931	0.684
Rest 2	0.94 ± 0.06	0.97 ± 0.09	0.189	0.96 ± 0.04	0.96 ± 0.06	1.000	0.93 ± 0.06	0.93 ± 0.07	0.922	0.587
R3	0.95 ± 0.07	0.94 ± 0.07	0.597	0.95 ± 0.06	0.97 ± 0.08	0.407	0.96 ± 0.06	0.95 ± 0.11	0.910	0.775
Rest 3	0.93 ± 0.06	0.97 ± 0.10	0.511	0.96 ± 0.04	0.96 ± 0.08	0.930	0.93 ± 0.06	0.94 ± 0.08	0.828	0.932

	No MG(n)			No MG(h)				MG1(n)			MG1(h)				MG6(n)			MG6(h)				*p*
Parameter	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	***Int***
R4	0.94	±	0.07	0.93	±	0.07	0.482	0.97	±	0.08	0.97	±	0.10	0.720	0.95	±	0.06	0.95	±	0.10	0.975	0.626
Rest 4	0.91	±	0.06	0.95	±	0.12	0.562	0.95	±	0.04	0.97	±	0.08	0.655	0.92	±	0.07	0.93	±	0.08	0.902	0.848
Post-test	1.01	±	0.08	0.98	±	0.07	0.179	1.05	±	0.08	0.99	±	0.10	0.547	1.01	±	0.07	0.97	±	0.10	0.471	0.991
HR (bpm)																						
Pre-test	98	±	17	103	±	16	0.068	98	±	15	100	±	17	0.544	96	±	14	94	±	14	0.433	**0.030**
R1	**164**	**±**	**19***	**173**	**±**	**13***	**0.018**	167	±	14	167	±	21	0.984	163	±	14	165	±	14	0.308	0.384
Rest 1	**139**	**±**	**21***	**152**	**±**	**17***	**0.013**	144	±	26	144	±	25	0.987	139	±	21	143	±	16	0.511	0.358
R2	**169**	**±**	**19***	**179**	**±**	**12***	**0.030**	173	±	13	175	±	17	0.732	169	±	13	171	±	13	0.492	0.312
Rest 2	150	±	24	159	±	13	0.081	151	±	20	153	±	23	0.735	147	±	18	148	±	13	0.949	0.503
R3	172	±	19	180	±	12	0.074	175	±	13	177	±	16	0.784	170	±	16	173	±	13	0.392	0.376
Rest 3	151	±	21	162	±	16	0.301	158	±	20	156	±	20	0.249	149	±	18	154	±	15	0.512	0.395
R4	172	±	20	183	±	10	0.328	179	±	14	179	±	13	1.000	173	±	14	178	±	17	0.059	0.374
Rest 4	145	±	17	156	±	15	0.399	156	±	17	152	±	18	0.393	145	±	14	160	±	17	0.071	0.413
Post-test	126	±	17	134	±	17	0.667	129	±	18	131	±	23	0.851	124	±	16	131	±	17	0.705	0.965

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * = No MG(n) and No MG(h); # = MG1(n) and MG1(h); † = MG6(n) and MG6(h).

E4) Max ± SD of cardiopulmonary parameters for each round (R1-R4) and resting interval at both normoxia and hypoxia whilst wearing no mouthguard (No MG(n) and No MG(h)) or two selected designs (MG1(n) and MG1(h), MG6(n) and MG6(h)). *P*-values indicate significant differences between parameters recorded at normoxic and hypoxic conditions, whereas *p-Int* indicates the possibility of significant interaction between the main factors of environmental conditions and mouthguard type (*N* = 7).

	No MG(n)			**No MG(h)**				**MG1(n)**			**MG1(h)**				**MG6(n)**			**MG6(h)**				***p***
Parameter	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	***Int***
VO_2 (L/min)																						
Pre-test	1.10	±	0.23	1.13	±	0.22	0.627	1.02	±	0.32	1.15	±	0.33	0.425	**0.93**	**±**	**0.15†**	**1.24**	**±**	**0.33†**	**0.048**	0.234
R1	3.42	±	0.70	3.50	±	0.28	0.725	3.54	±	0.57	3.77	±	0.75	0.506	3.43	±	0.71	3.48	±	0.79	0.833	0.855
Rest 1	2.90	±	0.77	3.00	±	0.52	0.789	2.84	±	0.42	2.86	±	0.61	0.939	2.66	±	0.58	2.75	±	0.68	0.715	0.970
R2	3.51	±	0.77	3.79	±	0.55	0.298	3.70	±	0.32	3.60	±	0.49	0.330	3.55	±	0.59	3.56	±	0.73	0.925	0.319
Rest 2	2.87	±	0.73	3.14	±	0.42	0.379	3.15	±	0.62	3.08	±	0.56	0.700	3.18	±	0.76	2.90	±	0.57	0.225	0.202
R3	3.59	±	0.73	3.74	±	0.35	0.376	3.58	±	0.39	3.63	±	0.44	0.547	3.54	±	0.57	3.54	±	0.53	0.975	0.771
Rest 3	**2.77**	**±**	**0.56***	**3.24**	**±**	**0.40***	**0.009**	2.82	±	0.61	2.90	±	0.37	0.743	3.31	±	0.93	3.08	±	0.53	0.458	0.257
R4	**3.38**	**±**	**0.66***	**3.66**	**±**	**0.35***	**0.039**	3.56	±	0.47	3.49	±	0.40	0.683	3.59	±	0.53	3.61	±	0.53	0.945	0.312
Rest 4	**2.62**	**±**	**0.47***	**3.16**	**±**	**0.58***	**0.082**	2.98	±	0.59	2.84	±	0.39	0.492	**2.75**	**±**	**0.49†**	**3.20**	**±**	**0.56†**	**0.041**	**0.085**
Post-test	1.57	±	0.39	1.79	±	0.36	0.502	1.94	±	0.49	1.71	±	0.25	0.152	1.88	±	0.80	1.78	±	0.32	0.678	0.511
VO_2 (ml/kg/min)																						
Pre-test	14	±	4	14	±	4	0.726	13	±	5	15	±	5	0.345	**12**	**±**	**2†**	**16**	**±**	**5**	**0.026**	0.183

	No MG(n)	No MG(h)		MG1(n)	MG1(h)		MG6(n)	MG6(h)		***p***
Parameter	**Mean ± SD**	**Mean ± SD**	***p***	**Mean ± SD**	**Mean ± SD**	***p***	**Mean ± SD**	**Mean ± SD**	***p***	***Int***
R1	42 ± 10	43 ± 5	0.813	43 ± 9	44 ± 8	0.634	42 ± 9	43 ± 11	0.862	0.999
Rest 1	36 ± 11	37 ± 3	0.833	35 ± 6	36 ± 8	0.801	33 ± 9	35 ± 10	0.626	0.981
R2	43 ± 10	46 ± 6	0.306	45 ± 5	44 ± 6	0.812	43 ± 7	45 ± 12	0.514	0.508
Rest 2	35 ± 10	37 ± 8	0.505	39 ± 10	39 ± 9	0.934	39 ± 7	36 ± 8	0.451	0.563
R3	44 ± 9	46 ± 6	0.448	43 ± 5	45 ± 4	0.063	43 ± 7	44 ± 8	0.699	0.976
Rest 3	34 ± 8	40 ± 6	0.007	34 ± 8	36 ± 5	0.593	40 ± 8	37 ± 11	0.580	0.319
R4	**41 ± 7***	**45 ± 5***	**0.071**	43 ± 7	43 ± 6	0.939	44 ± 6	45 ± 8	0.787	0.415
Rest 4	**31 ± 6***	**40 ± 6***	**0.017**	37 ± 9	36 ± 6	0.558	**34 ± 7†**	**41 ± 8†**	**0.020**	**0.041**
Post-test	20 ± 6	22 ± 5	0.606	24 ± 8	21 ± 4	0.371	23 ± 11	23 ± 4	0.780	0.599
VE (L/min)										
Pre-test	36.2 ± 9.12	38.0 ± 8.5	0.648	33.3 ± 11.3	38.9 ± 10.9	0.354	**31.5 ± 8.1†**	**43.0 ± 11.6†**	**0.041**	0.358
R1	**102.0 ± 22.3***	**113.9 ± 28.8***	**0.003**	**94.3 ± 18.7#**	**123.3 ± 28.1#**	**0.012**	**100.7 ± 24.2†**	**112.5 ± 34.2†**	**0.009**	**0.483**
Rest 1	86.5 ± 29.23	95.4 ± 21.0	0.453	82.3 ± 15.9	87.3 ± 19.6	0.451	74.2 ± 21.1	85.3 ± 24.4	0.213	0.834
R2	112.1 ± 29.9	128.0 ± 26.1	0.080	109.7 ± 15.7	126.1 ± 26.4	0.061	**105.6 ± 24.9†**	**130.7 ± 44.7†**	**0.045**	0.581
Rest 2	88.9 ± 28.3	102.4 ± 22.6	0.294	93.4 ± 23.2	99.5 ± 19.6	0.377	97.8 ± 25.5	97.9 ± 17.1	0.989	0.573
R3	**113.2 ± 28.5***	**132.1 ± 23.6***	**0.012**	107.7 ± 14.4	122.6 ± 20.3	0.098	**105.1 ± 24.5†**	**124.6 ± 27.2†**	**0.025**	0.745
Rest 3	91.0 ± 25.4	109.5 ± 20.0	0.101	87.4 ± 25.6	95.1 ± 13.3	0.455	100.8 ± 26.5	104.5 ± 20.7	0.683	0.570
R4	115.2 ± 30.2	132.9 ± 16.7	0.053	117.5 ± 21.5	126.3 ± 22.1	0.325	**109.6 ± 25.6†**	**128.4 ± 22.0†**	**0.036**	0.762
Rest 4	**83.6 ± 20.0***	**108.5 ± 12.3***	**0.001**	98.0 ± 27.2	90.6 ± 10.3	0.408	83.1 ± 18.8	111.1 ± 29.5	0.058	**0.025**

	No MG(n)			No MG(h)				MG1(n)			MG1(h)				MG6(n)			MG6(h)				*p*
Parameter	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	***Int***
Post-test	49.4	±	10.7	63.3	±	17.7	0.066	60.4	±	20.6	62.9	±	12.0	0.055	64.5	±	34.3	61.8	±	11.7	0.107	0.742
VCO_2 (L/min)																						
Pre-test	1.06	±	0.21	1.07	±	0.22	0.907	0.99	±	0.29	1.07	±	0.24	0.521	0.90	±	0.18	1.19	±	0.29	0.055	0.236
R1	3.03	±	0.55	3.23	±	0.60	0.409	3.10	±	0.59	3.53	±	0.60	0.213	3.05	±	0.54	3.07	±	0.67	0.864	0.395
Rest 1	2.64	±	0.73	2.86	±	0.63	0.524	2.69	±	0.45	2.64	±	0.46	0.767	2.40	±	0.59	2.50	±	0.63	0.649	0.665
R2	3.17	±	0.70	3.55	±	0.71	0.180	3.39	±	0.33	3.42	±	0.42	0.884	3.18	±	0.48	3.38	±	0.75	0.343	0.336
Rest 2	2.71	±	0.74	3.00	±	0.64	0.349	2.97	±	0.61	2.93	±	0.47	0.795	2.97	±	0.76	2.71	±	0.42	0.312	0.330
R3	3.17	±	0.61	3.47	±	0.48	0.096	3.24	±	0.34	3.36	±	0.27	0.216	3.16	±	0.49	3.24	±	0.37	0.619	0.336
Rest 3	**2.58**	**±**	**0.60***	**3.04**	**±**	**0.58***	**0.022**	2.61	±	0.55	2.72	±	0.27	0.630	3.07	±	0.89	2.83	±	0.40	0.440	0.209
R4	**3.08**	**±**	**0.52***	**3.38**	**±**	**0.29***	**0.042**	3.34	±	0.46	3.23	±	0.27	0.557	3.19	±	0.49	3.25	±	0.34	0.760	0.153
Rest 4	**2.42**	**±**	**0.48***	**2.97**	**±**	**0.34***	**0.006**	2.80	±	0.56	2.59	±	0.27	0.310	2.54	±	0.54	2.99	±	0.51	0.090	**0.021**
Post-test	1.46	±	0.28	1.72	±	0.40	0.207	1.89	±	0.47	1.70	±	0.25	0.587	1.83	±	0.77	1.72	±	0.29	0.179	0.723
RER																						
Pre-test	1.03	±	0.09	1.03	±	0.11	0.940	1.02	±	0.07	1.01	±	0.09	0.803	1.00	±	0.10	1.02	±	0.05	0.706	0.927
R1	0.99	±	0.07	1.04	±	0.11	0.178	1.02	±	0.14	1.04	±	0.09	0.704	1.00	±	0.08	1.03	±	0.09	0.314	0.922
Rest 1	1.01	±	0.06	1.01	±	0.10	0.856	1.08	±	0.21	0.99	±	0.05	0.356	1.03	±	0.05	0.99	±	0.09	0.374	0.441
R2	1.07	±	0.08	1.03	±	0.06	0.250	1.04	±	0.09	1.07	±	0.09	0.501	1.07	±	0.09	1.03	±	0.07	0.379	0.465
Rest 2	1.01	±	0.08	1.03	±	0.11	0.433	1.04	±	0.07	1.03	±	0.07	0.894	1.01	±	0.07	1.00	±	0.08	0.757	0.777

	No MG(n)			No MG(h)				MG1(n)			MG1(h)				MG6(n)			MG6(h)				*p*
Parameter	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	**Mean**		**SD**	**Mean**		**SD**	***p***	***Int***
R3	1.08	±	0.07	1.04	±	0.07	0.227	1.05	±	0.06	1.07	±	0.08	0.469	1.08	±	0.06	1.05	±	0.11	0.543	0.544
Rest 3	1.01	±	0.08	1.04	±	0.14	0.854	1.02	±	0.06	1.02	±	0.08	1.000	0.99	±	0.08	1.01	±	0.09	0.736	0.990
R4	1.04	±	0.07	1.05	±	0.07	0.833	1.10	±	0.08	1.08	±	0.10	0.559	1.03	±	0.06	1.04	±	0.10	0.957	0.968
Rest 4	0.97	±	0.07	1.00	±	0.14	0.775	1.00	±	0.04	1.03	±	0.12	0.554	0.99	±	0.09	0.98	±	0.08	0.736	0.788
Post-test	1.05	±	0.09	1.03	±	0.08	0.415	1.10	±	0.08	1.05	±	0.11	0.664	1.10	±	0.11	1.02	±	0.09	0.226	0.506
HR (bpm)																						
Pre-test	105	±	18	107	±	15	0.497	102	±	16	105	±	18	0.644	103	±	13	102	±	12	0.901	0.832
R1	**167**	**±**	**19***	**174**	**±**	**13***	**0.037**	168	±	14	169	±	21	0.969	165	±	14	168	±	14	0.305	0.514
Rest 1	**160**	**±**	**17***	**169**	**±**	**15***	**0.018**	161	±	23	164	±	22	0.754	161	±	13	161	±	13	0.814	0.427
R2	173	±	19	180	±	12	0.099	174	±	13	176	±	17	0.678	172	±	13	175	±	13	0.073	0.653
Rest 2	**167**	**±**	**18***	**175**	**±**	**14***	**0.050**	171	±	16	173	±	18	0.720	167	±	13	167	±	11	0.915	0.278
R3	173	±	19	181	±	12	0.081	178	±	13	178	±	16	0.882	173	±	16	178	±	13	0.171	0.357
Rest 3	169	±	18	180	±	11	0.326	172	±	17	174	±	16	0.597	168	±	14	170	±	11	0.520	0.367
R4	176	±	20	183	±	10	0.290	180	±	14	180	±	13	0.873	174	±	14	183	±	17	0.091	0.576
Rest 4	170	±	18	180	±	11	0.330	175	±	16	175	±	15	0.943	170	±	13	176	±	14	0.324	0.613
Post-test	141	±	18	145	±	18	0.357	147	±	23	141	±	27	0.635	143	±	27	148	±	18	0.354	0.390

Significant main effects are highlighted in bold. Statistical group differences ($p < 0.05$) are highlighted by: * = No MG(n) and No MG(h); # = MG1(n) and MG1(h); † = MG6(n) and MG6(h).

RER					
Pre-test/ Rest	1.04 ± 0.08	1.05 ± 0.08	1.02 ± 0.06	1.03 ± 0.04	0.238
R1	0.99 ± 0.08	1.04 ± 0.12	1.02 ± 0.07	0.99 ± 0.08	0.735
Rest 1	1.01 ± 0.07	1.05 ± 0.17	1.00 ± 0.07	1.03 ± 0.04	0.238
R2	1.05 ± 0.09	1.05 ± 0.06	1.06 ± 0.07	1.07 ± 0.07	1.000
Rest 2	1.02 ± 0.08	1.04 ± 0.06	1.04 ± 0.08	1.02 ± 0.07	1.000
R3	1.07 ± 0.06	1.07 ± 0.05	1.07 ± 0.05	1.09 ± 0.08	0.648
Rest 3	1.02 ± 0.07	1.04 ± 0.06	1.02 ± 0.08	1.00 ± 0.07	1.000
R4	1.07 ± 0.08	1.08 ± 0.07	1.08 ± 0.08	1.06 ± 0.06	0.123
Rest 4	0.99 ± 0.07	1.02 ± 0.06	1.02 ± 0.06	0.99 ± 0.07	0.319
Post-test/ Rest	1.05 ± 0.09	1.09 ± 0.07	1.08 ± 0.07	1.07 ± 0.09	0.310
HR (bpm)					
Pre-test/ Rest	105 ± 20	103 ± 16	108 ± 33	102 ± 17	0.701
R1	159 ± 16	160 ± 18	156 ± 22	165 ± 22	0.096
Rest 1	155 ± 23	157 ± 21	153 ± 21	158 ± 16	1.000
R2	164 ± 23	166 ± 17	164 ± 22	165 ± 16	1.000
Rest 2	163 ± 24	166 ± 17	161 ± 16	163 ± 23	1.000
R3	166 ± 23	173 ± 18	167 ± 22	166 ± 17	0.074
Rest 3	164 ± 23	168 ± 16	165 ± 22	166 ± 16	1.000
R4	166 ± 22	**165 ± 16¢**	171 ± 19	**169 ± 16¢**	**0.041**
Rest 4	166 ± 23	168 ± 19	168 ± 16	171 ± 16	1.000
Post-test/ Rest	135 ± 21	143 ± 23	140 ± 24	139 ± 25	0.276

Significant main effects are highlighted in bold. Group differences (p <0.05) are highlighted by: # = No MG and MG2; ¢ = MG1 and MG3.