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Neurophysiological Mechanisms of Fatigue Résistance during Repeated Sprints in Hot and Hypoxic Environments: Application to Team Sports

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Thèse de doctorat ès sciences de la vie (PhD)

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par

Franck BROCHERIE

Master (DESS) en sciences des sports de l'Université de Bourgogne (Dijon, France)

Jury

Prof. Lluis Fajas Coll, Président
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Dr. Olivier Girard, Co-directeur
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NEUROPHYSIOLOGICAL MECHANISMS OF FATIGUE RESISTANCE DURING REPEATED SPRINTS IN HOT AND HYPOXIC ENVIRONMENTS: APPLICATION TO TEAM SPORTS

Lausanne, le 4 juillet 2016

pour le Doyen
de la Faculté de biologie et de médecine

Prof. Lluis Fajas Coll

UNIL | Université de Lausanne
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“Ignore any idea that is not initiated during outdoor activity, while also the muscles are filled with blood.”
Friedrich Nietzche.

« *Nous ne savons pas ce dont le corps humain est capable.* »
Baruch Spinoza.

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"That is the way to learn the most, that when you are doing something with such enjoyment that you don't notice that the time passes."
Albert Einstein.

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List of publications

Peer-reviewed articles [*]

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2. Brocherie F, Girard O, Forchino F, Al Haddad H, Dos Santos G, Millet GP. Relationships between anthropometric measures and athletic performance, with special reference to repeated sprint ability, in the Qatar national soccer team. *J Sports Sci.* 2014; 32(13): 1243-54.
3. **Brocherie F, Girard O, Faiss R, Millet GP. High-intensity intermittent training in hypoxia: a double-blinded, placebo-controlled field study in youth football players. *J Strength Cond Res.* 2015 Jan; 29(1): 226-37.**
4. **Brocherie F, Girard O, Farooq A, Millet GP. Influence of weather, rank, and home advantage on football outcomes in the gulf region *Med Sci Sports Exerc.* 2015 Feb; 47(2): 401-10.**
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[*] Specific contributions to the present thesis are highlighted in bold.

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Table of contents

Abstract

In team sports, characterising fatigue is complex, with the underlying processes (*e.g.*, metabolic energy supply, intramuscular accumulation of metabolic by-products, hyperthermia, dehydration) developing as match proceeds to ultimately manifest as a decline in physical performance. Using acute and chronic manipulations of environmental stress (*i.e.*, heat or hypoxia) and unique tools (*i.e.*, instrumented sprint treadmill, 45-m long hypoxic marquee), the main intention of this work was to better understand the neurophysiological and biomechanical manifestations of fatigue during repeated-sprint exercise. Our work first demonstrated the effect of heat stress on football matches outcomes. Next, we demonstrated during repeated treadmill sprints that (i) hot and hypoxic stresses do not accentuate the extent of fatigue-induced changes in running mechanics, (ii) alterations in RSA and associated neuro-mechanical responses with increase in hypoxia severity do not follow a monotonic (*i.e.*, linear) pattern, and (iii) the rate of force development is not modified by hypoxia exposure. Lastly, we verified the putative benefit of the 'repeated sprint training in hypoxia' in a team-sport context and established the usefulness of the combination with 'traditional' hypoxic method, namely 'live high-train low and high', for inducing concomitant 'aerobic' and 'anaerobic' adaptive mechanisms via blood oxygen carrying-capacity improvement (*i.e.*, haemoglobin mass gains) and muscle molecular up-regulations (*i.e.*, hypoxia inducible factor-1α subunit pathway and its target genes). In summary, our work provides deeper insights in the understanding of the neurophysiological mechanisms of fatigue resistance during repeated sprinting in the face of challenging environmental conditions and opens new frontiers in performance optimisation.

Résumé

En sports collectifs, la fatigue est un phénomène complexe, dont les processus sous-jacents (fourniture énergétique, accumulation de métabolites, hyperthermie, déshydratation) se développant au cours d'un match se manifestent par une baisse de performance physique. En manipulant le stress environnemental (chaleur ou hypoxie) de façon aigue ou chronique, à l'aide d'outils uniques (tapis de course instrumentée, tunnel hypoxique de 45 m de long), le but de ce travail est de mieux comprendre les manifestations neurophysiologiques et biomécaniques de la fatigue au cours de répétition de sprints. Notre travail a d'abord démontré l'effet de la chaleur sur les résultats de matchs de football. Ensuite, nous avons constaté qu'au cours de sprints répétés (i) l'ampleur des altérations mécaniques de la course ne diffère pas en condition chaude ou hypoxique, (ii) la baisse de performance et les réponses biomécaniques et neurophysiologiques associées à une augmentation du stress hypoxique ne suivent pas un pattern linéaire, et (iii) la capacité à développer la force rapidement n'est pas modifiée par la sévérité du stress hypoxique. Finalement, après avoir vérifié l'avantage de « l'entraînement de sprints répétés en hypoxie » dans les sports collectifs, nous avons développé et validé une nouvelle méthode d'entraînement – « vivre en altitude-s'entrainer au niveau de la mer et en altitude » – pour induire des gains aérobie et anaérobie concomitants via l'amélioration du transport d'oxygène dans le sang (augmentation de la masse en hémoglobine) et des adaptations moléculaires au niveau musculaire (activation du facteur inductible par hipoxie-1α et ses gènes cibles). En conclusion, notre travail a permis d'approfondir les connaissances des mécanismes neurophysiologiques de la résistance à la fatigue au cours de sprint répétés en référence à un stress environnemental et ouvre de nouvelles perspectives dans l'optimisation de la performance physique.

Index of abbreviations

ATP
Adenosine triphosphate

CNS
central nervous system

CS
citrate synthase

D_{RF}
force application technique

EMG
Surface electromyography

F_H
horizontal ground reaction force

F_{Tot}
net total ground reaction force

F_V
vertical ground reaction force

FI
fatigue index

FiO_2
inspired fraction of oxygen

Hb_{mass}
haemoglobin mass

HIF-1α
hypoxia inducible factor - 1α

HR
heart rate

K_{leg}
leg stiffness

K_{vert}
vertical stiffness

LHTH
'live high-train high'

LHTL
'live high-train low'

LHTLH
'live high-train low and high'

LLTH
'live low-train high'

Mb
myoglobin

MH
moderate hypoxia (FiO_2 = 0.17)

MVC
maximal voluntary contraction

O_2
oxygen

PCr
phosphocreatine

PGC-1α
: peroxisome proliferator-activated receptor gamma coactivator 1-alpha

Post-
: after intervention

Pre-
: before intervention

RF
: ratio of support-averaged net horizontal to total force

RFD
: rate of force development

RMS
: Root mean square

RSA
: repeated-sprint ability

RSH
: repeated-sprint training in hypoxia

RSN
: repeated-sprint training in normoxia

S_{dec}
: sprint decrement

S_F
: stride frequency

SL
: sea level ($FiO_2 = 0.21$)

SH
: severe hypoxia ($FiO_2 = 0.13$)

SpO_2
: arterial oxygen saturation

t_c
: contact time

t_{swing}
: swing time

TFAM
: mitochondrial transcription factor A

VEGF
: vascular endothelial growth factor

VO_{2max}
: maximal oxygen uptake

YYIR1 and 2
: Yo-Yo intermittent recovery test level 1 and 2

Chapter 1

Introduction

1. Introduction

1.1 Defining fatigue in team sports

Although it is not difficult to recognise an individual or an athlete in a fatigued state, identifying the neurophysiological mechanisms responsible for this condition is more challenging. Since the seminal work of Mosso (1906), fatigue generally refers to *'an inability to maintain the required or expected force/power output'* (Edwards, 1981) or *'an exercise-induced reduction in the ability of muscle to produce force or power whether or not the task can be sustained'* (Bigland-Ritchie *et al.*, 1983; Bigland-Ritchie & Woods, 1984; Sogaard *et al.*, 2006). Further, in order to consider the performance fatigability as well as the perceived fatigability (Jones, 1993; Enoka & Duchateau, 2016), fatigue definition was extended as *'an acute impairment of performance that includes both an increase in the perceived effort necessary to exert a desired force and an eventual inability to produce this force'* (Enoka & Stuart, 1992; Enoka & Duchateau, 2008).

In team sports[*], characterising fatigue is complex, with the underlying processes (*e.g.*, metabolic energy supply, intramuscular accumulation of metabolic by-products, hyperthermia, dehydration) developing as match proceeds to ultimately manifest as a decline in physical performance (*i.e.*, running patterns, involvement with the ball) (Knicker *et al.*, 2011). For instance, a decrease in work rate may occur toward match-end (*i.e.*, sustained fatigue) (Mohr *et al.*, 2003; 2005; Spencer *et al.*, 2005; Reilly *et al.*, 2008) and less high-velocity running and/or peak/mean sprint distances could arise transiently after intense running (*i.e.*, temporary fatigue) (Mohr *et al.*, 2003; Girard *et al.*, 2011a). In addition to cumulative/residual fatigue (*i.e.*, over several days of a tournament) (Spencer *et al.*, 2005;

[*] Team sports share a common exercise pattern which is intermittent in nature, requiring players to repeatedly produce skilful actions and maximal or near-maximal efforts (*e.g.*, accelerations, changes in pace and direction, sprints, jumps and kicks), in a semi-stochastic fashion, interspersed with low- to moderate-intensity activity periods or brief recovery intervals, with and without the ball/puck, over an extended period of time (*e.g.*, 2 × 45 min in football, 2 × 40 min in rugby union, 4 × 10-12 min in basketball or 3 × 20 min in ice hockey).

Reilly *et al.*, 2008), this is likely to indirectly influence the match outcome (Faude *et al.*, 2012).

Although the importance of repeated-sprint ability (RSA)[*] as a crucial physical component of team-sport performance (Rampinini *et al.*, 2007) is constantly challenged (Carling, 2013; Schimpchen *et al.*, 2016), fatigue development in team sports has been linked with the ability to reproduce sprints (Krustrup *et al.*, 2010). Moreover, RSA deteriorates substantially from before to after elite football matches (Mohr *et al.*, 2004; Krustrup *et al.*, 2006). Referring to the aforementioned definitions of fatigue, during repeated-sprint exercise, fatigue would denote a transient reduction in the maximal power output (*i.e.*, cycling) or velocity (*i.e.*, running) over sprint repetitions (*i.e.*, typically developing rapidly after the first sprint), even though the task can be sustained (Mendez-Villanueva *et al.*, 2008; Girard *et al.*, 2011a). The inability to produce and maintain the required force/power or velocity can encompass several potential mechanisms occurring within cortical regions (*i.e.*, neural factors; central fatigue resulting from a failure to activate the muscle voluntarily) to muscular contractile elements (*i.e.*, muscular level; peripheral fatigue occurring at or distal to the neuromuscular junction), with each of these stages as a possible limiting factor (Gandevia, 2001; Enoka & Duchateau, 2008).

The magnitude and the aetiology of fatigue are dependent on the exercise characteristics (the so-called *'task dependency'*) (Enoka & Stuart, 1992; Enoka & Duchateau, 2008). As such, a better understanding of the factors [*i.e.*, metabolic (*e.g.*, oxidative capacity, phosphocreatine (PCr) recovery and H^+ buffering) and neural factors (*e.g.*, muscle activation and recruitment strategies) among others] underlying fatigue during RSA tests (Girard *et al.*, 2011a) is arguably the first step in order to design interventions (*i.e.*, training programmes, ergogenic aids) that could delay the onset of fatigue, enhance RSA and eventually improve in-game physical performance in team sports (Fig. 1).

[*] RSA is defined as short-duration sprints (≤10 s) interspersed with brief recovery periods (usually ≤60 s) (Girard *et al.*, 2011a).

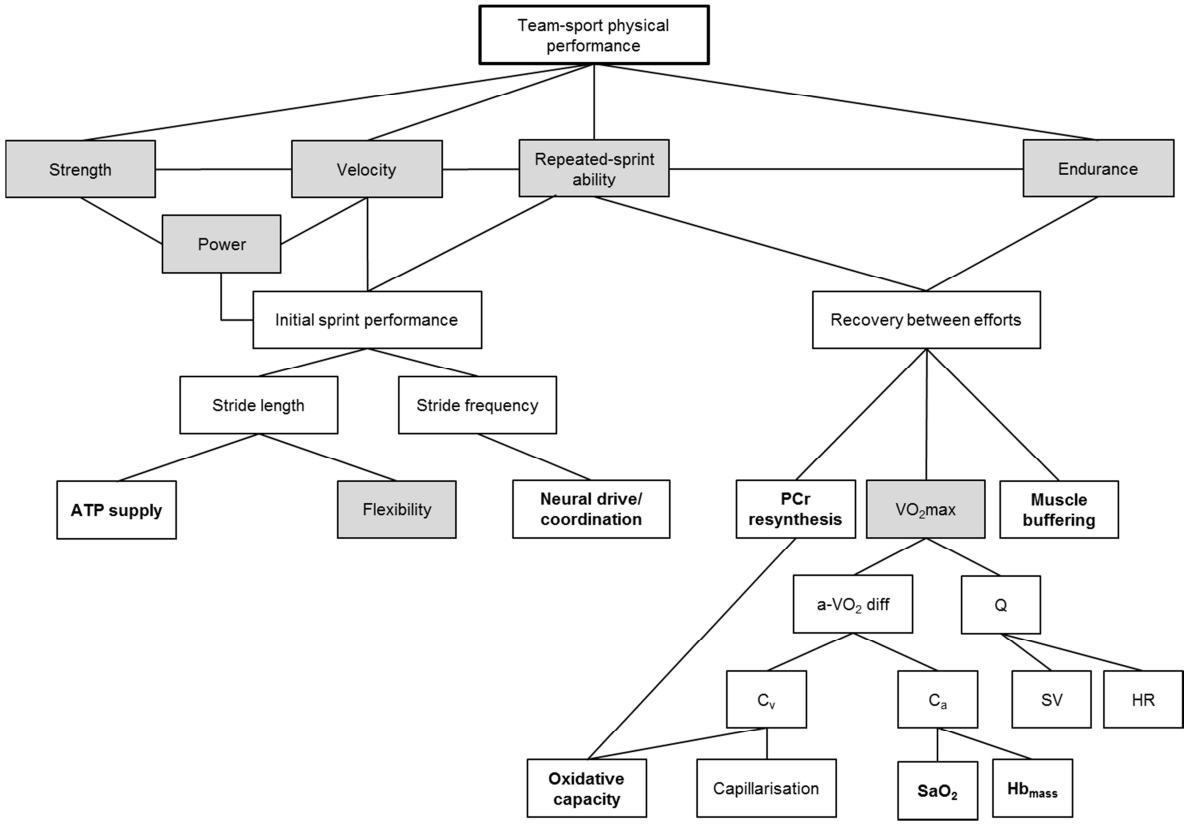

Figure 1. Summary of the main neurophysiological factors that affect team-sport physical performance [adapted from (Bishop & Girard 2013)]. *Grey cases* refer to training interventions. ATP, adenosine triphosphate, PCr, Phosphocreatine; VO_2max, maximal oxygen uptake; a-VO_2 diff, arterio-venous oxygen difference; Q, cardiac output; C_v, veinous content; C_a, arterial content; SV, stroke volume; HR, heart rate; SaO_2, arterial oxygen saturation, Hb_{mass}, haemoglobin mass.

Besides, environmental stresses such as hot-dry/hot-humid (*e.g.*, 2014 or 2022 FIFA World Cup in Brazil and Qatar, respectively) or altitude (*e.g.*, 2010 FIFA World Cup in South Africa) is likely to exacerbate fatigue and compromise team-sport-related physical performance (*i.e.*, in-match high-intensity locomotor activities and/or RSA). Although exercising in challenging environment being an area of interest for many sporting organizations – *e.g.*, Fédération Internationale de Football Association (FIFA) symposium on playing football at altitude (Lundby *et al.*, 2012) or heat (Grantham *et al.*, 2010), conference on altitude training for team sports (Girard *et al.*, 2013a), and International Olympic Committee (IOC) consensus statement on thermoregulatory and altitude challenges for all high-level athletes (Bergeron *et al.*, 2012) – research on this area in team sports is still in its

infancy. In this view, the experimental approach of using an additional environmental stress [*i.e.*, warm to hot (25–45°C) *vs*. temperate to cool (15–25°C) ambient temperature; moderate to severe hypoxia [1800-4000 m or inspired fraction of oxygen (FiO_2) 0.17-0.12] *vs*. normoxia (sea level; 0 m or FiO_2 0.21)] to further perturb homeostasis is potentially valuable to gain knowledge regarding the nature of the fatigue-related mechanisms limiting RSA.

1.2 Quantifying neuromuscular fatigue during and after repeated-sprint ability (RSA)

1.2.1 Conventional factors for RSA determination

During a RSA test, producing maximal power output or sprint velocity (*i.e.*, both initial and averaged performance) and the ability to maintain a high performance level throughout the test (resistance to fatigue) are the main derived performance outcomes. Conventionally, fatigue resistance during a repeated-sprint exercise is quantified by one of the two following indices, *i.e.*, the fatigue index (FI) or the percentage decrement score (S_{dec}). While the FI is generally calculated as the drop-off in performance from the best to worst sprint performance, the S_{dec} takes all sprints into consideration and compares actual performance to an 'ideal' one (*i.e.*, where the best sprint would be replicated at each repetition). Corresponding S_{dec} equations are as follows (Girard *et al.*, 2011a):

$$S_{dec}\ (\%) = \left[1 - \frac{(S_1+S_2+S_3+\cdots+S_{final})}{S_{best}\times \text{number of sprints}}\right] \times 100 \qquad (\textit{eq. 1}\text{; for cycling})$$

$$= \left[\frac{(S_1+S_2+S_3+\cdots+S_{final})}{S_{best}\times \text{number of sprints}} - 1\right] \times 100 \qquad (\textit{eq. 2}\text{; for running})$$

where S refers to sprint performance and can be calculated for either velocity, work or power scores.

Although S_{dec} calculation has been found to be the most valid and reliable method to quantify fatigue during RSA test (Glaister *et al.*, 2008), total mechanical work/sprint time (*i.e.*, sum of power outputs/times for all sprint repetitions) is also a relevant factor to consider and is

commonly used in conjunction to more accurately evaluate RSA performance (Pyne *et al.*, 2008). In practice, it is absolutely necessary to contextualize the calculated fatigue indices when RSA is evaluated because less/greater fatigue does not always equate to better/worse performance. This is mainly due by the positive relationship reported between initial sprint and fatigue indices (Hamilton *et al.*, 1991; Bishop *et al.*, 2003; Bishop & Edge, 2006; Mendez-Villanueva *et al.*, 2008).

1.2.2 Assessing central and peripheral fatigue

Traditionally, studies of neuromuscular fatigue during exercise have focused on alteration in the recruitment of motor units or muscles themselves (Vollestad, 1997). This is generally quantified as a decrease in surface electromyography (EMG) activity after the onset of an exercise or perturbations in muscle contractility (Enoka & Duchateau, 2008), which potentially reflects alterations at any levels from the brain to skeletal muscles (Gandevia, 2001). In addition, a superimposed percutaneous electrical or magnetic stimulation of motor nerve (*e.g.*, femoral nerve for the *vastus lateralis* and *rectus femoris* muscles) (Allen *et al.*, 1995; Cairns *et al.*, 2005) or transcranial magnetic stimulations is required to evaluate the level of central fatigue of an individual (Vollestad, 1997).

Briefly, the twitch superimposed to the peak force or power of an isometric contraction during a maximal voluntary contraction (MVC) is compared to the twitch evoked on the relaxed muscle in order to calculate the level of voluntary activation [twitch interpolation technique; (Merton, 1954; Allen *et al.*, 1995)]. Also, to explore peripheral fatigue (*i.e.*, amplitude of the mechanical response), electrical or magnetic stimulation can also be evoked on the relaxed muscle. By analysing the changes in the integrated electromyography signal (iEMG), or root mean square (RMS), and the compound action potential (M-wave) during voluntary and evoked contractions, respectively, it is therefore possible to clarify whether a decrease in

MVC force is completely attributable to the loss of muscle contractile properties, or whether central drive may contribute to this decrease (Vollestad, 1997). While such method has been mostly applied to isometric contractions [an easily measurable variable under relative standardized conditions (Cairns *et al.*, 2005)], it is also applicable to dynamic movements (Gandevia, 2001) (Fig. 2).

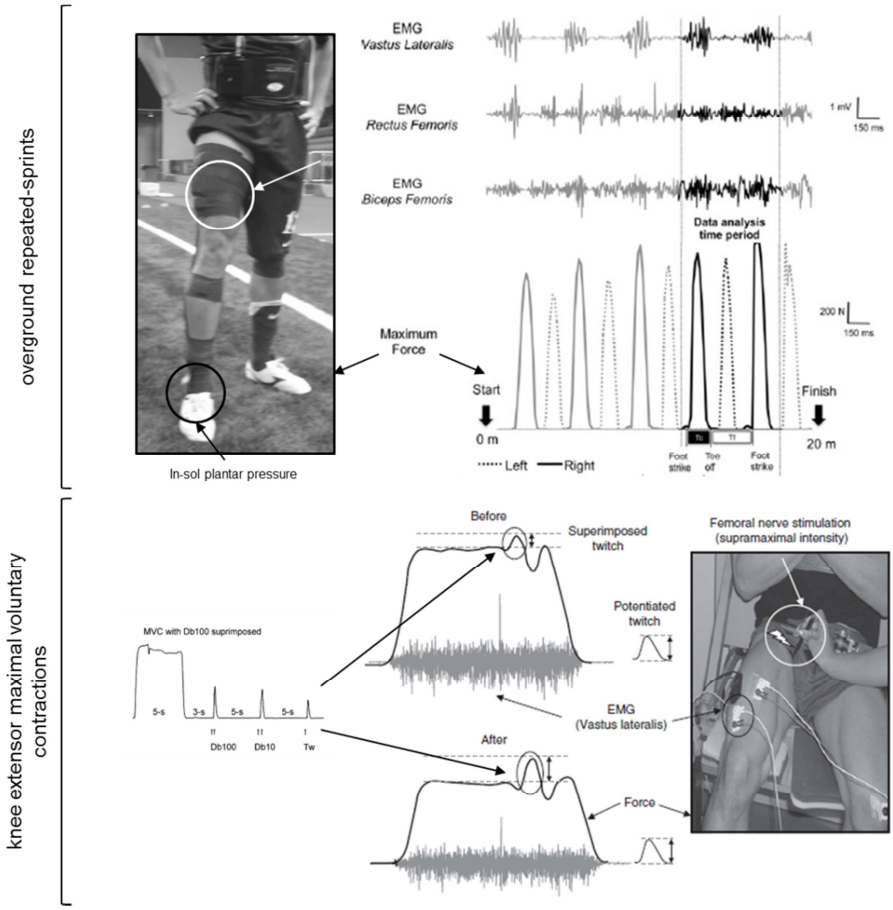

Figure 2. Typical examples of (a) raw EMG recording with stride kinematics during an overground repeated-sprint exercise (*i.e.*, 6 × 20 m – 20 s recovery). Maximum force was obtained from in-sole plantar pressure (Pedar device), while EMG signals were recorded from the *vastus lateralis*, *rectus femoris*, and *biceps femoris* muscles of the right leg. Tc contact time, Tf flight time; (b) force and electromyogram (EMG) [*vastus lateralis* muscle] traces during knee extensor maximal voluntary contractions (MVC) before and after a repeated-sprint cycling exercise (10 × 6 s – 30 s recovery) [adapted from (Girard *et al.*, 2011a; Girard *et al.*, 2011c)]. By comparing the twitch superimposed to a MVC and the twitch evoked on the relaxed muscle (*i.e.*, femoral nerve supra-maximal stimulation), the twitch interpolation technique in conjunction with surface EMG (*i.e.*, root mean square [RMS] value normalized by the maximal muscle compound action potential [M-wave]) can be a reliable non-invasive technique to characterize muscle activation. Voluntary activation level (%) was estimated according to the following formula: (1 – [superimposed twitch/potentiated twitch)] × 100). Note that both the voluntary activation level and the normalized RMS activity were depressed (-2.5% and -14.5%, respectively) compared to values obtained before exercise. Neuromuscular testing procedure (*Db*, paired electrical stimulation; *Tw*, single electrical stimulation). *Black line*, typical torque trace, MVC, maximum isometric knee extensors contraction, *Db100* doublet at 100 Hz (10-ms inter-stimulus interval), *Db10* doublet at 10 Hz (100-ms inter-stimulus interval), *Tw* single twitch.

Beside several metabolic energy supply-related factors (*i.e.*, PCr hydrolysis, anaerobic glycolysis, oxidative metabolism, intramuscular accumulation of metabolic by-products) implicated as potential fatigue agents at the muscle level [for review, see (Girard *et al.*, 2011a)], failure to fully activate the contracting musculature and/or changes in inter-muscle recruitment strategies (*i.e.*, neural factors) are also associated with fatigue outcomes during repeated sprints. Reportedly, as maximal sprint exercise demands high levels of neural drive (Ross *et al.*, 2001), failure to fully activate the contracting musculature (through quadriceps EMG assessment) decreases force production and subsequently RSA performance (Racinais *et al.*, 2007; Mendez-Villanueva *et al.*, 2008). However, decline in the amplitude of EMG signals depends on the magnitude of the induced fatigue: on the one hand, when fatigue is mild (FI or S_{dec} <10%), a steady level of neural activation during RSA protocols is typically reported (Hautier *et al.*, 2000; Perrey *et al.*, 2010); on the other hand, when the fatigue level is more substantial (>10%), a concurrent decline in mechanical performance and the amplitude of EMG signals has consistently been reported across sprint repetitions (Mendez-Villanueva *et al.*, 2007; Racinais *et al.*, 2007; Mendez-Villanueva *et al.*, 2008; Billaut & Smith, 2010).

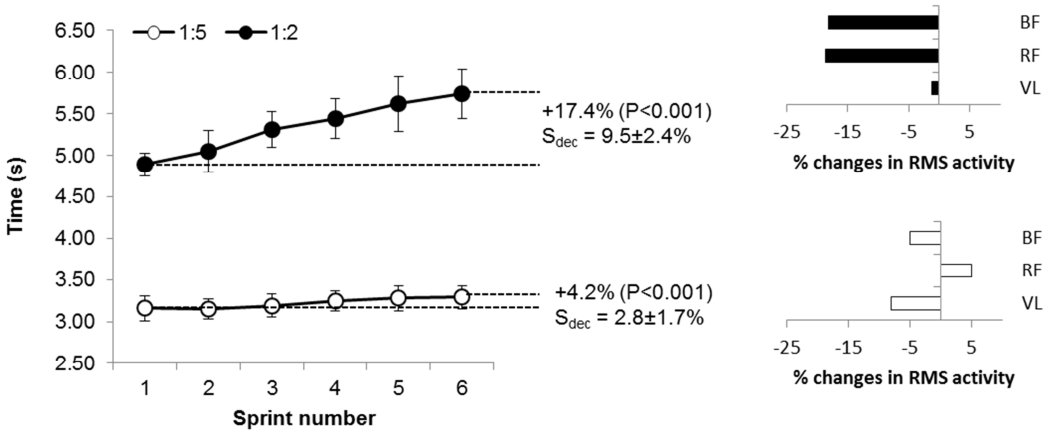

Figure 3. Effect of exercise-to-rest ratio (*i.e.*, 1:5 = 6 × 20 m – 20 s recovery *vs.* 1:2 = 6 × 35 m – 10 s recovery) on repeated running sprints performance and associated muscle activity. [adapted from (Girard *et al.*, 2011c; Brocherie *et al.*, 2015b)]. S_{dec}, sprint decrement score; RMS, root mean square; VL, *vastus lateralis* muscle; RF, *rectus femoris* muscle; BF, *biceps femoris* muscle.

This was indirectly verified in a team-sport setting: comparatively to RSA-induced mild fatigue (S_{dec} ~3% using a 1:6 exercise-to-rest ratio) where the amplitude of lower-limb EMG signals across running sprint repetitions was unaltered (Girard *et al.*, 2011c), the used of a more stressful exercise-to-rest ratio (*i.e.*, 1:2 corresponding to approximately twice longer sprints time and a two-fold decrease in recoveries duration) exacerbated the degree of fatigue, as confirmed by larger S_{dec} (~10%) (Fig. 3) and larger reductions in RMS activity in *rectus femoris* and *biceps femoris* muscles, while no changes occurred in *vastus lateralis* muscle (Brocherie *et al.*, 2015b).

1.2.3 Advancing the understanding of RSA determinants: neuro-mechanical variables

Maximal rate of force development. Following a contraction onset during an MVC, the time requested to produce the maximal force capacity generally exceeds 300 ms (Thorstensson *et al.*, 1976), which appears longer than most of muscular contraction (*i.e.*, <250 ms) characterising sprinting (Kuitunen *et al.*, 2002). The ability to rapidly generate force within the initial (<250 ms) phase of an MVC – *i.e.*, the rate of force development (RFD) – which in turn correlates with sprint performance (Tillin *et al.*, 2013), likely constitutes a surrogate for explosive strength and a more functional outcome measure (Girard & Millet, 2009) of the acute neuromuscular adjustments to repeated-sprint exercise. Although there is a growing interest in RSA-induced neuromuscular fatigue quantification [*i.e.*, pre-to-post sprint (Racinais *et al.*, 2007; Perrey *et al.*, 2010; Billaut *et al.*, 2013; Girard *et al.*, 2013b; Hureau *et al.*, 2014) or set (Goodall *et al.*, 2015; Pearcey *et al.*, 2015) force, voluntary activation, EMG, and twitch responses], very little is known on the RFD responses.

To date, only Girard *et al*. (2013b) have investigated the effect of repeated sprinting (*i.e.*, 10 × 6-s 'all-out' cycling sprints, followed, after 6 min of passive rest, by 5 × 6-s sprints; recoveries = 30 s) performance on post-exercise alterations in RFD of the knee extensors.

MVC (-12%) and RFD (-15 to -26% from the 0-30 to 0-200 ms epochs after contraction onset) decreased during brief (*i.e.*, 5 s) contractions after (*i.e.*, 3 min) the RSA test. Meanwhile, non-significant reductions in *vastus lateralis* RMS activities have been shown to accompany the deteriorated RFD values. However, because the delay between exercise termination and post-exercise neuromuscular testing was 3 min in the aforementioned study, any meaningful changes in the central nervous system (CNS) performance may have already recovered.

Running mechanics. During running, the mechanical behaviour of the musculoskeletal structures of the legs is often described as that of a spring loaded by the runner's mass, constituting the 'spring-mass model' (Blickhan, 1989; McMahon & Cheng, 1990) (Fig. 4). This model consists of a point mass supported by a single massless linear 'leg spring' and the main mechanical parameter studied when using the spring-mass model is the stiffness of the leg spring (K_{leg}), defined as the ratio of the maximal force in the spring to the maximum leg compression at the middle of the stance phase (Farley & Gonzalez, 1996).

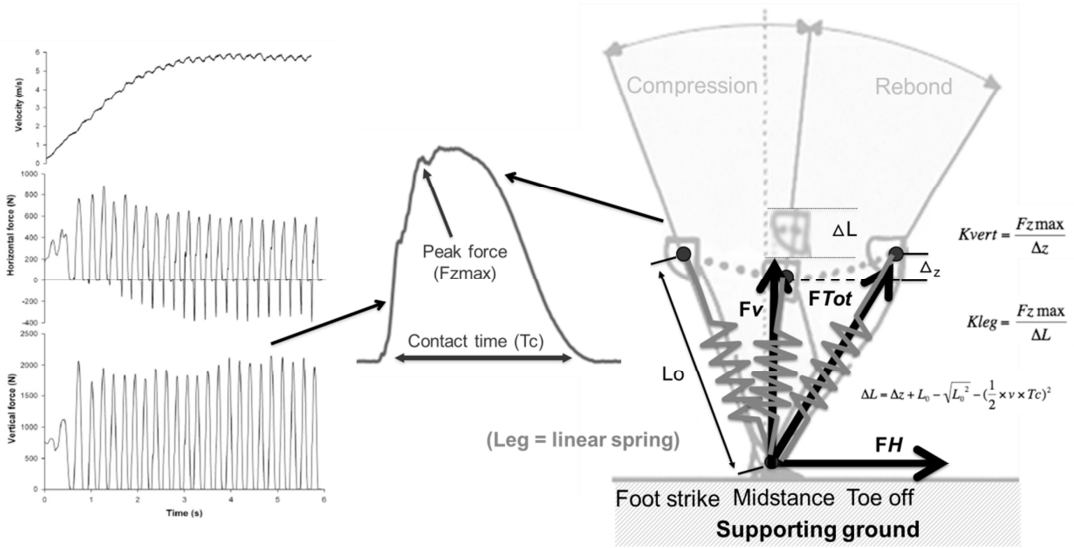

Figure 4. Schematic representation of GRFs recorded during a 6-s sprint and spring-mass model. [adapted from (McMahon & Cheng, 1990; Morin *et al.*, 2011a)]. Δ*L* Peak displacement of the leg spring (leg compression) during contact; Δ*z* Maximal downward displacement of the centre of mass during contact; F_H Horizontal force; F_{Tot} Total force; F_V Vertical force; Fz_{max} Maximal vertical force; K_{leg} Leg stiffness; K_{vert} Vertical stiffness; L_0 Lower-limb length; t_c Contact time

Moreover, although it does not correspond to any physical spring, the vertical stiffness (K_{vert}) is used to describe the vertical motion of the centre of mass during contact (McMahon & Cheng, 1990; Farley & Gonzalez, 1996) and is defined as the ratio of the maximal force to the vertical displacement of the centre of mass as it reaches its lowest point, that is, the middle of the stance phase.

Although not as extensively studied, changes in mechanical behaviour (stiffness regulation) may indirectly alter fatigue resistance during repeated running sprints (Ross *et al.*, 2001; Girard *et al.*, 2011a). It has been demonstrated that, through a stiffer system, greater elastic energy efficiency would improve the force production (*i.e.*, concentric phase) of bouncing gait (Farley *et al.*, 1991). Moreover, the correlation found between K_{leg} and sprinting performance (Chelly & Denis, 2001) or between spring-mass characteristics alterations and repeated 'all-out' runs (*i.e.*, 4 × 100 m – 2 min of rest) (Morin *et al.*, 2006) supports the initial premise that stiffness regulation is a vital component for setting stride frequency (S_F) (Farley *et al.*, 1991).

In the few available RSA-related biomechanical studies, specific impairments in sprinting mechanics [*i.e.*, increase in contact time (t_c) and swing time (t_{swing}), reductions in K_{vert} and S_F] have been connected with progressively slower sprint times/running velocities during over-ground repeated sprints [*i.e.*, 6 × 20 m – 20 s of passive recovery in under-19 footballers (Girard *et al.*, 2011c); 6 × 35 m – 10 s of passive recovery in elite footballers (Brocherie *et al.*, 2015b); or 12 × 40 m – 30 s of passive recovery in team- and racquet-sports athletes (Girard *et al.*, 2011b)]. Collectively, these findings show that the ability to tolerate ground impact/stretch loads decreases as fatigue develops with sprint repetitions. However, these observations emerged from studies using indirect measures of stride characteristics (*i.e.*, pressure insoles) (Girard *et al.*, 2011c; Brocherie *et al.*, 2015b) or direct sprint kinetics/kinematics assessments (*i.e.*, force platforms) on only few steps at various intervals of the sprint distance (Girard *et al.*, 2011b).

The recent validation of a motorised instrumented sprint treadmill (*i.e.*, ADAL3D-WR, Medical Development, HEF Tecmachine, Andrézieux-Bouthéon, France) (Morin *et al.*, 2010) which makes possible to perform reliable continuous (*i.e.* instantaneous and contact or step-averaged) measurements of instantaneous horizontal (F_H) and vertical (F_V) components of the resultant (total, F_{Tot}) ground reaction force during maximal sprints (including the typical acceleration phase) from a typical crouched sprint start (similar to team-sport scenarios) (Girard *et al.*, 2015c) will undoubtedly contribute to advance the understanding of fatigue-induced RSA neurophysiological adjustments (Fig. 5). A unique feature of this device is to allow the computation of the ratio of support-averaged net F_H to F_{Tot}[*], which gives an indication of the way athletes apply forces onto the ground (technical ability), independently from the amount of total force applied (physical capability) (Morin *et al.*, 2011a).

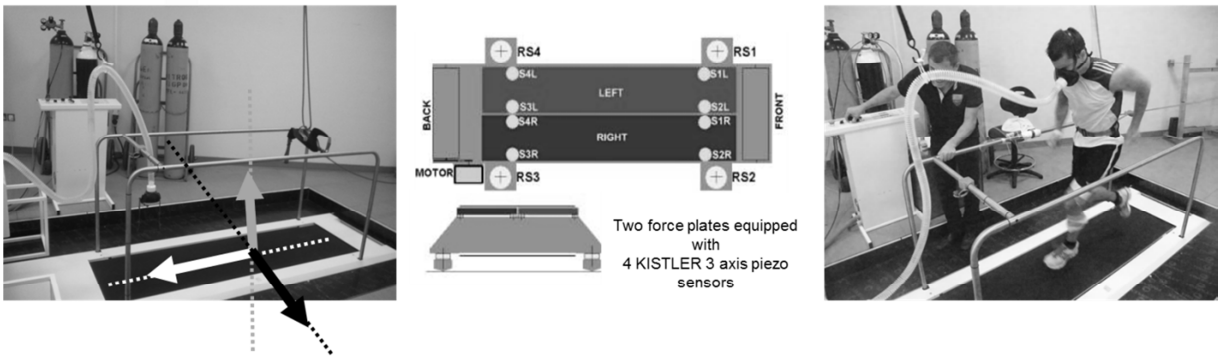

Figure 5. The ADAL3D-WR motorised instrumented sprint treadmill (Morin *et al.*, 2010). Treadmill with GRFs directions representation (left panel), design (central panel) and utilisation during hypoxic sprinting (right panel) views are pictured.

In the only available treadmill studies thoroughly describing changes in sprinting kinetics over repeated sprints, significant decrease in force production capacity, particularly in the ability to apply it horizontally [*i.e.*, approximately twofold larger reduction than F_V and F_{Tot}] has been observed (Morin *et al.*, 2011b; Girard *et al.*, 2015b). As sprint bouts and series were

[*] As this indicator of the overall technical ability (RF) of force application and orientation against the ground decrease linearly with the increase in speed over a sprint acceleration from null to top speed, an index of force application technique (D_{RF}) is computed as the slope of the linear RF-speed relationship. Thus for a given sprint, the higher D_{RF}, the more RF is maintained at high values despite increasing speed, and the more forward-oriented total force applied. (Morin *et al.*, 2011b).

repeated, the technical ability to apply force effectively against the ground was altered and led to shorter and less effective acceleration phase (Morin *et al.*, 2011b). In the aforementioned studies, however, these observations were restricted to thermo-neutral and/or normoxic conditions. It is possible that the addition of an environmental stress (*i.e.*, heat or hypoxia) leads to premature and excessive fatigue (*i.e.*, increased neurophysiological stress) and is associated with less efficient stride mechanics.

1.3 Influence of an environmental stress on team-sport related performance

Exercising in either warm or reduced O_2 content environments poses severe challenges to human regulatory systems. Compared to control test settings, an accelerated rate of locomotor muscle fatigue development is often observed when the same moderate- to high-intensity whole-body exercise is performed at a constant workload in conditions of high temperature (Gonzalez-Alonso *et al.*, 1999) or moderate to severe hypoxia (Amann & Kayser, 2009). Although the exact physiological mechanisms underpinning this environmental stress-related performance degradation are not fully understood, a variety of cardiorespiratory (*i.e.* lower cardiac output, decrease in systemic blood flow), metabolic (*i.e.* larger muscle/cerebral deoxygenation levels) and/or neuromuscular factors (*i.e.* reduced muscle activation, altered contractile function) could explain the earlier decision to stop exercise in such challenging conditions (Racinais & Oksa, 2010).

1.3.1 Match demands of team-sport competition in challenging environments

Performing team-sport competition in challenging environments is not an isolated phenomenon. For instance, most of the football FIFA World Cup tournaments are generally scheduled during the hottest months (*i.e.*, June-July; >30°C) with potentially high relative

humidity (RH; >70%RH). Moreover, of the last 19 football FIFA World Cup tournaments since 1930, eight hosting countries were located at altitude, with other age group tournament (*i.e.*, under-17 and under-20) being also organised in moderate altitude venues (Mexico City, Mexico and Manizales, Colombia).

Heat stress. When competition occurs in hot conditions, elite team-sport athletes consistently generate core temperatures >38.5°C (Mohr *et al.*, 2012; Nybo *et al.*, 2013; Aughey *et al.*, 2014). Reportedly, football total match distance (-7%) and high-intensity running (-26%) were reduced in environmental temperature of 43°C *vs*. 21°C (Mohr *et al.*, 2012). However, peak sprinting velocity was improved (+4%) in the hottest condition (Mohr *et al.*, 2012), suggesting that players may modulate their match activity patterns by reducing the volume of low-intensity running undertaken in order to preserve their ability to perform the hardest actions (*e.g.*, sprinting, accelerations) (Duffield *et al.*, 2009; Aughey *et al.*, 2014). Consequently, it is not surprising that similar declines (~2%) in repeated-sprint performance (3 × 30 m – 25 s of active recovery) in fatigued footballers were reported after completion of a match in hot (35-45 °C) *vs*. cool (21-22 °C) (Nybo *et al.*, 2013). That said, the generally lower distance covered (Mohr *et al.*, 2010; Mohr *et al.*, 2012) highlights the importance of acclimatisation before playing at higher temperature (Maughan & Shirreffs, 2010). However, the direct link with football performance at the international level has not been yet addressed.

Hypoxic stress. Owing to the large distance covered in match and the numerous brief explosive efforts (*e.g.*, accelerations, changes in pace and direction, sprints) to be performed, competing at altitude is likely to exacerbate fatigue in team-sport athletes. Recent data collected during official games of the 2010 FIFA World Cup (Nassis, 2012) and in preparation for the 2011 FIFA under-20 World Cup (Garvican *et al.*, 2014) reported that a reduction of 3-9% in total distance covered during matches played at 1200-1753 m compared

to sea level matches. Interestingly, while top-running velocity was not different at altitude compared to sea-level (Nassis, 2012), low- (*i.e.*, <14.9 km.h^{-1}), and high-velocity running (*i.e.*, 15.0-36.0 km.h^{-1}) were also reduced by 8% and 15% at altitude, respectively (Garvican *et al.*, 2014). Furthermore, using a 5 min rolling sample period for analysis gave an indication of transiently lower output, possibly fatigue, during matches. As a result, there were a 7%, 21% and 20% reduction in total distance covered, high-velocity running and acceleration frequency in the 5 min subsequent to the peak 5 min period at 1600 m compared to sea level, suggesting transient neuromuscular fatigue (Garvican *et al.*, 2014). Collectively, these observations suggest that non-acclimatised athletes (Gore *et al.*, 2008) may be at disadvantage when they have to perform at altitude.

1.3.2 Effect of environmental perturbation on laboratory-based repeated-sprint performance

Heat stress. Higher single sprint performance (*i.e.*, mean and/or peak running velocity or cycling power output) can be achieved following passive local muscle heating [*e.g.*, warm baths and/or heated blankets (Gray *et al.*, 2006)], active warm-up (Yaicharoen *et al.*, 2012a; Yaicharoen *et al.*, 2012b), passive heating [*e.g.*, elevation of core temperature by 1°C (Linnane *et al.*, 2004)], or hot ambient conditions [*e.g.*, heat exposure prior to exercise including an active warm-up (Falk *et al.*, 1998; Ball *et al.*, 1999; Yaicharoen *et al.*, 2012c; Girard *et al.*, 2013b)]. Among the potential mechanisms explaining why acute heat exposure may benefit single sprint performance (Ball *et al.*, 1999; Gray *et al.*, 2008), muscular biochemical and contractile adaptations, improving force production capacity, have been proposed (Karatzaferi *et al.*, 2004; Farina *et al.*, 2005; Gray *et al.*, 2006).

While these muscle-temperature-related gains may benefit for initial sprint performance during a repeated-sprint series, the elevation in core temperature in hotter environments may

exacerbate the cardiovascular and metabolic strain, resulting in larger performance decrement with successive efforts. For instance, after 40 min of intermittent cycling exercise in the heat and passive induced hyperthermia, core (~39.5°C) and muscle (~40.2°C at a depth of 3 cm) temperatures were elevated, thereby impairing the ability to produce power output during a subsequent 5 × 15 s cycling sprints – 15 s recovery (Drust *et al.*, 2005). Consequently, it seems that core temperature elevation (≥39°C) is the primary limiting factor of repeated-sprint performance in the heat, which by extension overwhelms the beneficial effect of a higher muscle temperature for initial sprint performance. This may potentially attributed to (i) an indirect inhibitory effect of the sensory afferent feedback (group III/IV muscle afferents) originating from heated muscles (Nybo *et al.*, 2014) and/or (ii) a greater reliance on anaerobic energy contribution (resulting from a lower blood flow and altered arterial O_2 delivery in the exercising muscles), thereby accelerating the decline in ATP and PCr levels and aggravating muscle lactate and H+ accumulation (Sawka *et al.*, 2011). This topic has been extensively developed in a recent comprehensive review (Girard *et al.*, 2015a).

Hypoxic stress. Performance data from track and field or team-sports competition in hypoxia (*i.e.*, altitude >1500 m) suggest that human maximal running velocity is not negatively affected (unchanged or even slightly improved). For example, average velocity for 100 m performed at Mexico City (2240 m) was 101.9% of the average velocity at sea level (Peronnet *et al.*, 1991). Additionally, average maximal running velocity collected from 21 matches played at 1401-1753 m corresponded to 102.4% of the average maximal running velocity from 23 matches at sea level during the FIFA World Cup 2010 (Nassis, 2012). Aside from the fact that reduced air density at altitude facilitates sprinting performance (Ward-Smith, 1984; Peronnet *et al.*, 1991; Levine *et al.*, 2008), the limited oxidative contribution (*i.e.*, rate of VO_2 >12% compared to normoxia) when sprints are performed in hypoxic environment is likely compensated by an increase in the rate of anaerobic energy release (Weyand *et al.*, 1999;

Morales-Alamo *et al.*, 2012). However, when maximal or near-maximal sprints are repeated in hypoxia, earlier and larger performance decrements are typically observed. This has been previously demonstrated in a pioneer study (Balsom *et al.*, 1994) which reported a reduction in O_2 availability during high-intensity intermittent exercise (*i.e.*, 10 × 6 s cycling sprints – 30 s recovery) induced higher post-exercise blood lactate (10.3 *vs*. 8.5 mmol.L^{-1}) and lower VO_2 (3.03 *vs*. 3.19 L.min^{-1}) in hypoxia [*i.e.*, hypobaric condition (barometric pressure 526 mmg) corresponding to a simulated altitude of 3000 m] *vs.* normoxia, thereby resulting in impaired ability to maintain power output (*i.e.*, -8.5% compared to normoxia).

More recent studies, mainly completed on a cycle-ergometer and involving athletes of various training backgrounds, have confirmed that normobaric hypoxia (FiO_2 ranging 0.14-0.16) induces earlier and larger decrement in RSA compared to normoxic conditions (Girard *et al.*, 2011a; Billaut & Buchheit, 2013; Billaut *et al.*, 2013).

It is generally accepted that sprints repetition in hypoxia *vs*. normoxia elevates heart rate (HR) (Bowtell *et al.*, 2014), minute ventilation (Bowtell *et al.*, 2014), O_2 debt (Balsom *et al.*, 1994; Bowtell *et al.*, 2014), muscle deoxygenation level (Billaut & Buchheit, 2013; Billaut *et al.*, 2013; Bowtell *et al.*, 2014), and lowers EMG signals of active musculature (Billaut *et al.*, 2013; Bowtell *et al.*, 2014). Further, Bowtell *et al*. (2014) investigated the effects of different hypoxia levels (FiO_2 0.12, 0.13, 0.14, 0.15, 0.21) on repeated-sprint performance (*i.e.*, 10 × 6 s 'all-out' running sprints – 30 s recovery) to construct a 'hypoxic dose' response. Although sprint performance was relatively resilient to moderate hypoxia (FiO_2 0.14-0.15), fatigue development was only significantly exacerbated at the severer hypoxic level (FI = -17.6% and S_{dec} = -9.9% at FiO_2 0.12) compared to normoxia (FI = -10.0% and S_{dec} = -4.9% at FiO_2 0.21) (Bowtell *et al.*, 2014). This indicates that RSA decrements with increased hypoxia severity may not follow a monotonic (*i.e.*, linear) pattern. In addition to limitations in energy supply (*i.e.*, PCr hydrolysis, anaerobic glycolysis, oxidative metabolism), metabolic by-product accumulation (*e.g.*, inorganic phosphate, H+), and failure to fully activate the contracting

muscle, the slower on-transient VO_2 response, as a result of reduced O_2 availability, would increase the magnitude of the O_2 deficit incurred during each sprint and thereby place more demand on anaerobic sources to maintain the required rate of ATP provision. The increased rate of fatigue under hypoxic conditions may have also been the result of a more rapid accumulation of inorganic phosphate during each sprint and a reduced rate of removal during recovery (Hogan *et al.*, 1999).

By modifying O_2 availability, hypoxia offers a relevant manipulation to examine how the neuro-mechanical factors and CNS interact with active skeletal muscles during repeated-sprint efforts. However, it is still unknown whether performing RSA in hypoxic environments exacerbate the degree of fatigue-induced changes in running mechanics, and how these modifications relate to larger peripheral and/or central fatigue.

1.4 Combined hypoxic training as a promising approach

The prevailing interest in hypoxic (or altitude) training originated with the 1968 Mexico City Olympics. Until now, individual endurance-sport athletes have mostly used hypoxic training with the primary goal to improve exercise performance at sea level and to acclimatize at altitude. To this end, several altitude training paradigms – 'live high-train high' (LHTH), 'live high-train low' (LHTL) or more recently 'live low-train high' (LLTH) approaches (Millet *et al.*, 2010; Millet *et al.*, 2013a) – have been introduced.

Over the last few years, media reports featured that several high profile team-sport clubs or national teams – mainly football but also rugby union and Australian football – undertake fitness programs in hypoxia during the early pre-season or in preparation of a major competition (*e.g.*, FIFA World Cup 2010 played at moderate altitude; Johannesburg, South Africa) in an attempt to gain a competitive edge. Most commonly, they use indifferently natural LHTH or LHTL to accumulate a sufficient 'hypoxic dose' (*i.e.*, 12 $h.day^{-1}$ for a

minimum of 2 weeks) to attain the associated physiological benefits [*i.e.*, increase in haemoglobin mass (Hb_{mass}), erythrocyte volume and ultimately VO_2max] (Wilber *et al.*, 2007; Billaut *et al.*, 2012). While LHTH method is widely used by teams to acclimatize before matches at altitude (Gore *et al.*, 2008; Billaut *et al.*, 2012), the actual benefit of such LHTH camps for physical performance improvement when returning near sea level can be questioned since the additional gains in physical performance (+1.5%, with large individual responses compared to sea-level training) (McLean *et al.*, 2013b) are low.

1.4.1 Usefulness of the 'gold-standard' LHTL method

To date, and despite on-going debate on (i) its efficiency in elite endurance athletes (Siebenmann *et al.*, 2012), (ii) its underlying mechanisms (Gore & Hopkins, 2005; Levine & Stray-Gundersen, 2005), and (iii) the importance of hypobaric *vs*. normobaric hypoxic stimuli (Millet *et al.*, 2012a; 2013b), LHTL is largely recognized as the 'Gold-Standard' method (Levine & Stray-Gundersen, 1997). The success of this altitude training intervention for elite athletes belong to an erythropoietic effect of chronic hypoxic exposure (Wehrlin *et al.*, 2006) initiated by residing at natural or simulated altitude, whilst maintaining a sea-level training intensity and high rates of O_2 flux (Wilber *et al.*, 2007).

An emerging concept is that positive gains associated with the LHTL method may rely on the magnitude of hypoxia-induced increase in Hb_{mass} (Levine & Stray-Gundersen, 2005). While an initial high Hb_{mass} will not allow substantial increase in Hb_{mass} following altitude training (Gore *et al.*, 1998) [although caution should be taken as some Hb_{mass} expansion were observed in highly-trained endurance athletes with high pre-intervention Hb_{mass} level (Saunders *et al.*, 2013)], an initially low Hb_{mass} will likely lead to meaningful enhancements in Hb_{mass} (Robach & Lundby, 2012). Bearing in mind that team-sports athletes are characterised by low to moderate Hb_{mass} (9-13 $g.kg^{-1}$) and/or VO_{2max} (55-65 $mL.min^{-1}.kg^{-1}$)

(Wachsmuth *et al.*, 2013; Brocherie *et al.*, 2015d) compared to endurance athletes (13-15 $g.kg^{-1}$ and 57-72 $mL.min^{-1}.kg^{-1}$, respectively) (Heinicke *et al.*, 2001), one may speculate that substantial gains in Hb_{mass} could occur in this athletic population after LHTL. Because aerobic metabolism dominates energy delivery in most team sports, it is likely that LHTL would benefit some team-sport athletes (Girard *et al.*, 2013a). The rationale for attempting to increase Hb_{mass} in team-sport athletes would be to increase their VO_{2max} and enhance blood buffer capacity, and thereby decrease relative exercise intensity during games and increase RSA tolerance, respectively (Bishop & Edge, 2006; Bishop *et al.*, 2011; Mendez-Villanueva *et al.*, 2013). Positive short-term benefits on Hb_{mass} have been reported after hypoxic exposure in football [+3% after 13 days of exposure at 3600 m (Wachsmuth *et al.*, 2013)], Australian Football League (AFL) [+3.5% after 18-19 days of pre-season moderate altitude (~2100 m) camp (McLean *et al.*, 2013a)], or water-polo [+3.7-4.5% after 10 days of simulated LHTL at 2500-3000 m (Garvican-Lewis *et al.*, 2013)] players. This is in line with the findings of a recent meta-analysis demonstrating that Hb_{mass} increases at approximately 1% per 100 h of altitude exposure regardless of the type of exposure [*i.e.*, LHTH (>2100 m) or LHTL (~3000 m)] (Gore *et al.*, 2013). Altogether, this suggests that a 2-week camp would probably be beneficial to aerobic power in a more realistic fashion compared to a 'classical' 4-week camp in a busy pre-season preparation.

1.4.2 From 'traditional' to innovative hypoxic methods

High-intensity intermittent exercise capacity not only depends on the blood O_2-carrying capacity, but also on molecular adaptations in the skeletal muscle [*i.e.*, hypoxia inducible factor-1α subunit (HIF-1α) pathway and its target genes] and the efficiency of the neuromuscular system. In this vein, LLTH approach, also called 'intermittent hypoxic training' (IHT), induces during a training session an additional stimulus [*i.e.*, due to larger

reduction in skeletal muscle O_2 pressure (Hoppeler & Vogt, 2001)] as compared to sea-level exercise. This could favourably impact team-sport specific patterns. It is unlikely that the LLTH's hypoxic dose is large enough to increase Hb_{mass} (Humberstone-Gough *et al.*, 2013). However, short and intense workouts characterising LLTH might induce specific adaptations at the molecular level in skeletal muscle tissue through the O_2 sensing pathway [capillary-to-fibre ratio, fibre cross-section area, myoglobin content (Mb) and oxidative enzyme activity such as citrate synthase (CS)] that either do not occur in normoxic conditions or, if they do so, do so to a lesser degree (Hoppeler & Vogt, 2001).

The technical development of new hypoxic devices that either decrease the pressure of the inspired air (hypobaric chamber) or reduce the concentration of O_2 in the inspired air by diluting it with extra nitrogen or filtering out O_2 (altitude tents, hypoxicator machines) had led to new promising training procedures [*i.e.*, intermittent hypoxic interval-training (IHIT), living high-training low and high (LHTLH)] (Millet *et al.*, 2010) to improve physical performance (Fig. 6), some being specifically tailored for team-sport use (*i.e.*, to better resist fatigue in the most intense periods of a game or towards match-end) (Billaut *et al.*, 2012; Girard *et al.*, 2013a; McLean *et al.*, 2014).

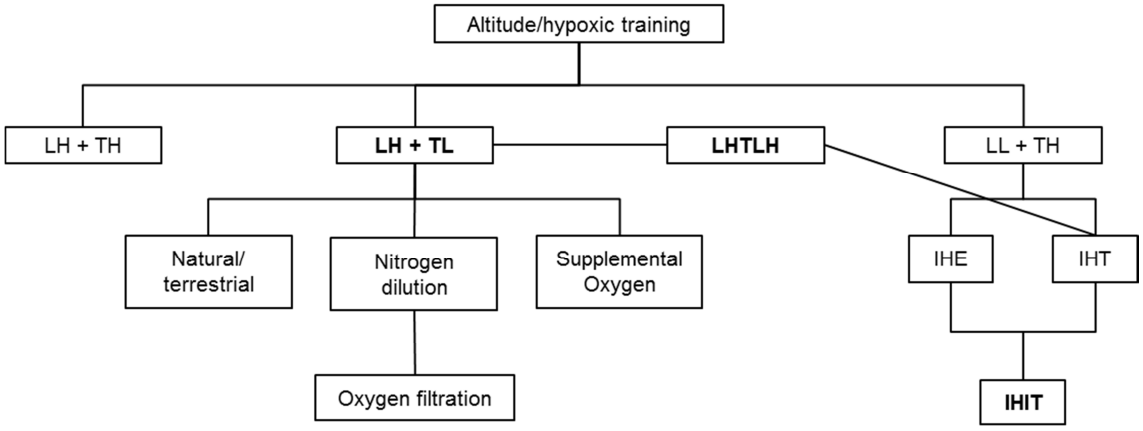

Figure 6. Different hypoxic methods [from (Millet *et al.*, 2010)]. LH, Living high; TH, training high; TL, training low; LHTLH, Living high-training low and high; LL, living low; IHE, intermittent hypoxic exposure; IHT, intermittent hypoxic training; IHIT, intermittent hypoxic interval-training.

In this view, the recent development of mobile inflatable simulated hypoxic facilities (Altitude Technology Solutions Pty Ltd, Brisbane, Queensland, Australia) (Fig. 7) would contribute to advance our understanding of hypoxia-induced physiological adaptations through ecological findings (Girard *et al.*, 2013c).

Figure 7. The mobile inflatable simulated hypoxic equipment (Girard *et al.*, 2013c). External 45 m running lane (1.8 m width and 2.5 m height) tunnel design (top panel), hypoxic system trailer (including a 55 kW screw compressor) (bottom left panel) and athlete sprinting inside the marquee (bottom right panel) views are pictured.

A recent literature review concludes that the additional benefits for sea-level performance of IHT compared to those of similar training under normoxic conditions are strikingly small (Faiss *et al.*, 2013a). For example, no significant difference in VO_2max was observed after 3 weeks of IHT training (*i.e.*, 5 × 60-90 min per week at simulated altitude of 3000 m) compared to similar sea-level training (Roels *et al.*, 2007a). The rationale for IHT relies on potentially greater muscle adaptations (*e.g.*, faster PCr resynthesis, larger CS activity) than for normoxic exercise (Terrados *et al.*, 1990; Holliss *et al.*, 2013). However, since the effects of

IHT on endurance performance measured in normoxia are '*minimal and inconclusive in trained athletes*' (Lundby *et al.*, 2012), one may question the functional significance of these physiological adaptations (Lundby *et al.*, 2009).

Repeated sprints in hypoxia. In 2013, a novel LLTH approach involving 'repeated-sprint training in hypoxia' (RSH) has been developed. RSH provides additional systematic benefits in comparison with the same training under normoxic condition (RSN, repeated sprints in normoxia) (Faiss *et al.*, 2013b; Galvin *et al.*, 2013; Puype *et al.*, 2013). For instance, 4 weeks of RSH (*i.e.*, 3 × 5 s 'all-out' cycling sprint – 20 s recovery at FiO_2 ~0.14; 2 sessions per week) was shown to delay fatigue during a RSA cycling test (*i.e.*, 10 s sprints – 20 s recoveries until exhaustion) by 40% compared to RSN (Faiss *et al.*, 2013b). An additional benefit of RSH *vs.* RSN was also found by Galvin *et al.* (2013) with well-trained academy rugby union and rugby league players' Yo-Yo intermittent recovery test level 1 (YYIR1) performance being improved by 33% after 4 weeks of training including 120 running sprints of 6 s performed at FiO_2 ~0.13. Because RSH is based on the repetition of 'all-out' efforts of short (*i.e.*, <30 s but ideally <10 s) duration separated by short periods of incomplete recovery, the efficiency of this intervention probably depends on the maximal intensity of the successive bouts of sprinting (separated by very short periods of recovery) to evoke potent adaptations at the molecular level and, possibly, an improved O_2 delivery to the mitochondria (Faiss *et al.*, 2013b). Indeed, the intensity of hypoxic training *per se* seems to modulate muscle performance at the molecular level with *'adaptations that compensate for the reduced availability of O_2 during exercise'* (Hoppeler & Vogt, 2001).

In the first RSH study (Faiss *et al.*, 2013b), the additional benefit of RSH was thought to involve improved O_2 extraction by the fast-twitch fibres (McDonough *et al.*, 2005) which are those predominantly recruited during sprints (Hautier *et al.*, 1996). Molecular adaptations in muscle tissue together with higher blood perfusion (presumably via nitric oxide-mediated

vasodilatation mechanisms) (Casey & Joyner, 2011; 2012) suggesting enhanced muscle blood flow supported this hypothesis of greater O_2 use by fast-twitch fibres after RSH. Since then, other LLTH studies have proved superior to training at sea level in enhancing peripheral adaptations [*i.e.*, oxidative capacity, capillary density (Vogt *et al.*, 2001; Dufour *et al.*, 2006; Roels *et al.*, 2007b; Hoppeler *et al.*, 2008) and muscle glycolytic (Puype *et al.*, 2013) as well as increased expression of HIF-1α and downstream genes to O_2 sensing and transport] (Vogt *et al.*, 2001; Zoll *et al.*, 2006; Lundby *et al.*, 2009; Schmutz *et al.*, 2010; Faiss *et al.*, 2013b; Holliss *et al.*, 2013).

More specifically to team sports, although the aforementioned RSH training intervention was performed on cycle ergometers (Faiss *et al.*, 2013b; Puype *et al.*, 2013) which represents an unusual activity for team-sport athletes or on a non-motorised treadmill (Galvin *et al.*, 2013), it could be speculated that a more ecological RSH training might influence team-sport specific physical performance (*e.g.*, RSA and YYIRs).

Live high-train low and high. As previously mentioned, the capacity of team-sport athletes to repeatedly perform high-intensity actions depends not only on their Hb_{mass} but also on their skeletal muscle tissue adaptations and neuromuscular system efficiency. Theoretically, for team-sport athletes and coaches looking to elicit concurrent 'aerobic' and 'anaerobic' adaptations to improve sea-level performance, 'live high-train low and high' [LHTLH; *i.e.*, 2-3 weeks of sleeping at 2500-3000 m with training at sea level, except for a few (two to three) hypoxic training sessions per week], suggested as early as 2010 (Millet *et al.*, 2010; Millet *et al.*, 2013a), is an attractive combination. However, it is currently unknown whether combining LHTL and RSH in a cohort of team-sport athletes would produce larger performance gains and associated physiological adaptations than would concurrent 'traditional' LHTL and RSN training.

1.5 Experimental considerations

In the present thesis, a multi-experimental approach has been employed to further study the neurophysiological mechanisms of fatigue resistance in the face of challenging environmental conditions.

1.5.1 Setting up the stage: Impact of heat stress on international football match outcomes

While the detrimental effect of altitude on football results has been previously investigated [*i.e.*, a lower likelihood to win for sea-level teams playing at altitude above 1200 m against altitude-acclimated teams (McSharry, 2007)], no study has yet determined the potential influence of heat stress (temperature, humidity) on the home advantage[*] phenomenon (Courneya & Carron, 1992; Pollard, 2006). Therefore, we aimed to add insight on environmental (*i.e.*, heat) effect on team-sport performance using match analysis data from the Gulf region (*i.e.*, specific context of the FIFA World Cup 2022). For this, we determined the effects of weather, rank and home advantage on international football match (n = 2008) results and scores in heat-acclimatised *vs*. non-acclimatised national teams (presented in Chapter 6, Article 1).

1.5.2 Shedding light on the neuro-mechanical manifestation of fatigue in hot and hypoxic environments

The purpose was two-folds: (i) to extensively describe the changes in mechanical performance and associated running kinematics/kinetics and spring-mass characteristics

[*] The home advantage is defined as *'the consistent finding that home teams in sports competitions win over 50% of the games played under a balanced home and away schedule'* (Courneya & Carron, 1992) and is dependent of different factors such as crowd support, travel effects, familiarity with local playing conditions, territoriality, altitude, referee bias, technique, tactic and management, as well as players' physiological and psychological condition (Pollard, 2006).

during repeated sprinting (*i.e.*, similar to team-sports) as performed in challenging environmental conditions (*i.e.*, hot and normobaric hypoxia compared to a control condition); and (ii) to better understand the nature of the underlying neuromuscular adjustments (*i.e.*, MVC and RFD).

Although there are compelling evidences to suggest earlier and larger RSA performance decrements with ambient heat (Drust *et al.*, 2005; Girard *et al.*, 2013b; Girard *et al.*, 2015a) or hypoxic stress (Billaut & Buchheit, 2013; Billaut *et al.*, 2013; Bowtell *et al.*, 2014), no direct (*i.e.*, same participants) comparison exists for the effects of heat and hypoxia on RSA and associated mechanical alterations. We therefore compared the performance changes and the accompanying alterations in running mechanics over a series of treadmill sprints (*i.e.*, 5 × 5 s sprints – 25 s recovery) performed by team-sport participants in severely hot (*i.e.*, 38°C/21% RH, FiO_2 0.21) and hypoxic (*i.e.*, 25°C/45% RH, FiO_2 ~0.13) environments *vs*. control (*i.e.*, 25°C/45% RH, FiO_2 0.21). This study is presented in Chapter 7, Article 2.

Further, based on previous studies (Bowtell *et al.*, 2014; Goods *et al.*, 2014) indicating a non-linear influence of the hypoxic stress, thereby suggesting a potential hypoxic 'threshold', we designed a randomized, double-blind study (presented in Chapter 8, Article 3) in order to comprehensively examine RSA performance (*i.e.*, 8 × 5 s – 25 s recovery) changes and accompanying running mechanical alterations under differing levels of acute normobaric hypoxia severity [*i.e.*, moderate (MH; FiO_2 0.17) or severe hypoxia (SH; FiO_2 0.13)] compared to normoxia [*i.e.*, near sea level (SL; FiO_2 0.21)]. Furthermore, given that there is *'extraordinarily little that changes with regard to maximal force-generating capacity with acute hypoxia'* (Perrey & Rupp, 2009), we hypothesised that the already-known decrease in RSA (*i.e.*, lower fatigue resistance) under SH would not be associated with more pronounced MVC and RFD alterations compared to SL or MH. In order to verify it, we assessed the pre- to post-RSA (*i.e.*, 1 min) knee extensor neuromuscular adjustments, with special reference to RFD (presented in chapter 9, article 4).

1.5.3 Implementing innovative hypoxic training methods to improve team-sport-related physical performance

First, in order to extend the putative benefit of the first RSH studies (Faiss *et al.*, 2013b; Galvin *et al.*, 2013; Puype *et al.*, 2013), we sought to demonstrate its effectiveness for improving football-specific physical performance in a cohort of under-18 footballers (chapter 10, article 5).

Further, based on the premise of promising combination of hypoxic methods, we investigated in a randomised, double-blind, controlled study design, the immediate (few days) and delayed (3 weeks) effects of the 'traditional' LHTL approach (*i.e.*, 14 days 'in-season' camp; >14h.day^{-1} at FiO_2 ~0.14), combined with either normobaric RSH (LHTL+RSH, namely LHTLH; FiO_2 0.14) or RSN (LHTL+RSN, namely LHTL; FiO_2 0.21) (both compared to controls, LLTL) on normoxic sports-specific performance (*i.e.*, RSA and YYIR2) in elite male field hockey players and on some potential underpinning mechanisms, namely Hb_{mass} gains and muscle molecular adaptations. Specific training consisted of 4 sets of 5 × 5 s maximal sprints – 25 s recovery with 5 min of rest between sets and were completed on a synthetic grass ground, inside a mobile inflatable simulated hypoxic equipment (Girard *et al.*, 2013c).

The rationale for this study was based on the assumption that, if the LHTL paradigm works to improve sea-level 'endurance' performance [relying on an increase in Hb_{mass} (Levine & Stray-Gundersen, 1997)] and if additional RSH works to improve sea-level RSA tolerance [via an improved neuromuscular system and/or molecular adaptations (Faiss *et al.*, 2013b; Millet *et al.*, 2013a; Brocherie *et al.*, 2015a)], then the physiological benefits of the LHTLH intervention must derive from the combination of these two hypoxic methods. This original research is presented in chapter 11, article 6 with a companion article addressing the associated skeletal muscle molecular (*i.e.*, HIF-1α pathway and its target genes) adaptations (chapter 12, article 7).

Chapter 2

Summary of experimental results

2. Summary of experimental results

2.1 Impact of heat stress on international football match outcomes

As hypothesised, our main results demonstrate that hot countries' national football teams [*i.e.*, national teams representative of the Gulf Cooperation Council (GCC) region], likely better heat-acclimatised, are at advantage when matches are played at higher temperature than usual and, to a lower extent, at higher humidity. Specifically, they have greater likelihood of favourable outcome (odds ratio = +4%) and higher goal difference (ß = 0.06) against equally ranked opponents with every 1°C increase in the usual temperature. However, the higher level of play in non-heat-acclimatised teams (*i.e.*, better FIFA-ranking) partly compensates for the heat-induced detrimental effects on the match outcomes when playing in hot conditions.

For more details, see chapter 6, article 1.

2.2 Shedding light on the neuro-mechanical manifestation of fatigue in hot and hypoxic environments

2.2.1 RSA-induced alterations in running mechanics did not differ in hot and hypoxia compared to control

Our results indicate that RSA (*i.e.*, S_{dec} = 7.9±3.0%, 2.4±3.4% and 3.1±3.9% for hypoxia, hot and control; $P<0.05$) is more impaired in hypoxia [end-exercise arterial O_2 saturation (SpO_2): ~84%] than in hot (end-exercise core temperature: ~38.6°C) environment when compared to a control condition. However, the nature and extent of fatigue-induced alterations in running kinetics, kinematics and spring-mass characteristics did not differ between the three environmental conditions. Irrespective of the environmental condition, significant changes occurred across sprint repetitions (all three conditions compounded) in selected running

kinetics (F_H, P<0.01) or kinematics (t_c and t_{swing}, both P<0.001; S_F, P<0.001) and spring-mass characteristics (K_{vert}, P<0.001; K_{leg}, P<0.01).

For more details, see chapter 7, article 2.

2.2.2 Hypoxia severity exacerbates sprinting mechanics

The main findings were that in SH, but not MH, impairments in RSA and the magnitude of accompanying alterations in kinetics/kinematics and spring-mass characteristics exceed those observed in SL (Fig. 8).

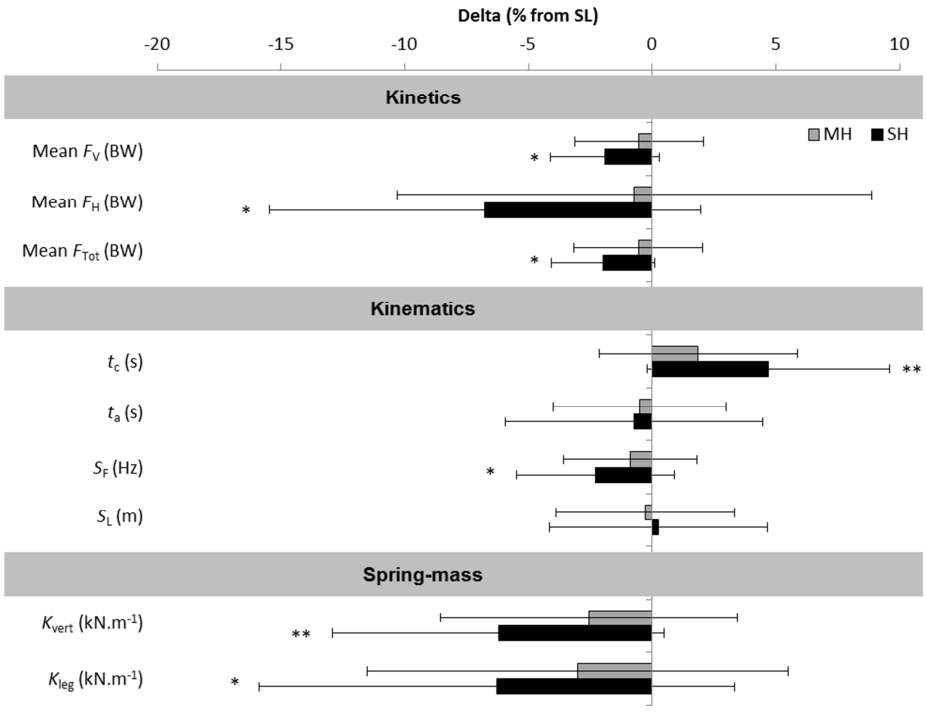

Figure 8. Comparison of averaged running mechanical data for the eight sprints in MH and SH in reference to SL. Values are mean ± SD, N = 13. * Significantly different from SL, P < 0.05 and ** P < 0.01.

Specifically, F_V, F_H and F_{Tot} decreased (P<0.001) from the first to the last repetition in all conditions (pooled values: -2.4±1.9%, -8.6±6.5% and -2.4±1.9%). Only F_H was significantly lower in SH compared to SL and MH (-4.9±5.9% and -5.2±5.4%, respectively; P<0.01). This

was further accompanied by larger kinematic (t_c: +4.0±2.9%, P<0.001 and +3.3±3.6%, P<0.05; respectively; and S_F: -2.3±2.0%, P<0.01 and -2.3±2.8%, P<0.05; respectively) and spring-mass characteristics (K_{vert}: -6.0±3.9% and -5.1±5.7%, P<0.01; respectively) fatigue-induced changes in SH compared to SL and MH. A notable finding is that most of the sprint performance alterations and accompanying running mechanics occurred within the first half (*i.e.*, sprints 1 to 4) of the RSA test with smaller changes during the second part (*i.e.*, sprints 5 to 8).

For more details, see chapter 8, article 3.

2.2.3 Larger hypoxia-severity-dependent alterations in knee extensors MVC, but not in RFD after repeated sprinting

The main results were that, compared to SL (-9±7%), a larger (P<0.05) reduction in MVC force occurred post-exercise in SH (-14±9%) but not in MH (-12±7%), with no difference between SL and MH (P>0.05). Irrespectively of condition (P>0.05), peak RFD (-6±11%; P<0.05), and normalised peak RMS activity for *vastus lateralis* (-8±11%; P=0.07) and *rectus femoris* (-14±11%; P<0.01) muscles were reduced post-exercise. Reductions (P<0.05) in absolute RFD occurred within the 0-100 (-8±9%) and 0-200 ms (-10±8%) epochs after contraction onset. After normalisation to MVC torque, there was no difference in RFD values. Additionally, the EMG rise for *vastus lateralis* muscle was similar (P>0.05), whereas it increased (P<0.05) for *rectus femoris* muscle during all epochs post-exercise, independently of the conditions (Fig. 9).

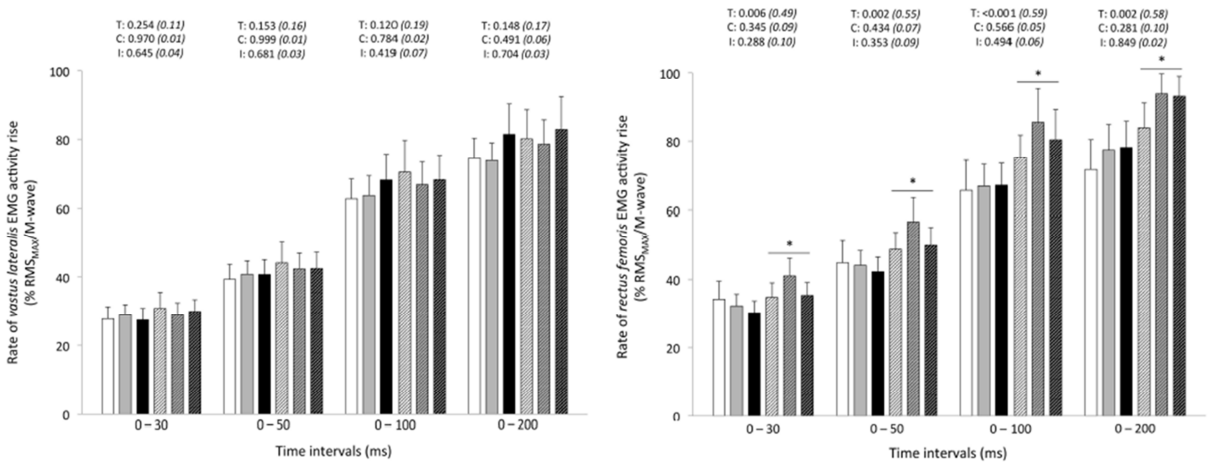

Figure 9. Rate of *vastus lateralis* (left panel) and *rectus femoris* (right panel) muscles EMG activity rise (%RMS_{MAX}/M-wave) during explosive knee extension obtained at 0-30, -50, -100 and -200 ms prior to (Pre-tests; full bars) and following (Post-tests, dashed bars) repeated-sprint exercise in normoxia (SL; FiO_2 0.21; white bars), moderate (MH; FiO_2 0.17; gray bars) and severe (SH; FiO_2 0.13; back bars) normobaric hypoxia. Values are mean ± SD (n = 13). T, C, and I respectively refer to ANOVA main effects of time, condition and interaction between these two factors with *P*-value and *partial eta-squared* (effect size) in parentheses. * Significantly different from Pre-tests, $P<0.05$.

For more details, see chapter 9, article 4.

2.3 Implementing innovative hypoxic training methods to improve team-sport-related physical performance

2.3.1 Verifying the efficacy of RSH in team sports

The major findings of this investigation were that (i) the addition of ten specific run-based training sessions (over a 5-wk period; ~60 min/training, 2 days/week) to the regular practice of highly-trained under-18 male footballers substantially improved several neuromuscular fitness components (counter movement jump: +6.5±1.9% *vs.* +5.0±7.6% for RSH and RSN, respectively; both $P<0.001$ with a *possibly beneficial* effect for RSH compared to RSN; sprinting times: -6.6±2.2% *vs.* -4.3±2.6% at 10 m to -1.7±1.7% *vs.* -1.3±2.3% at 40 m for RSH and RSN, respectively; *P* values ranging from <0.05 to <0.01 with a *possibly to likely beneficial* effect for RSH compared to RSN) related to on-field football physical performance, and (ii) high-intensity training in normobaric hypoxia in a team-sport applied setting (*i.e.*,

high-intensity intermittent and repeated-sprint runs, agility and conditioning exercises) appeared more efficient than the same training in normoxia at enhancing RSA (best time: -3.0±1.7% *vs.* -2.3±1.8%; both $P<0.05$ and mean time: -3.2±1.7%, $P<0.01$ *vs.* -1.9±2.6%, $P<0.05$ for RSH and RSN, respectively; with a *possibly beneficial* effect for RSH) and repeated-agility [*very likely* greater gains ($P<0.05$) for RSH than RSN (initial sprint: 4.4±1.9% *vs.* 2.0±1.7% and cumulated times: 4.3±0.6% *vs.* 2.4±1.7%] abilities, with the inclusion of direction changes. Meanwhile, maximal aerobic velocity remained unchanged throughout the protocol.

For more details, see chapter 10, article 5.

2.3.2 'Live High-Train Low and High': an attractive combination to elicit Hb_{mass} and concurrent aerobic and anaerobic adaptations

Our findings are clear and compelling (Fig. 10): first, similar increases in Hb_{mass} (LHTLH: +4.0%, $P<0.001$; LHTL: +3.0%, $P<0.001$) and specific aerobic fitness (YYIR2) (LHTLH: +21%, $P<0.01$; LHTL: +22%, $P<0.001$) were observed in the two intervention groups, despite a low altitude dose (≥200 h); while no change occurred in LLTL. Second, the YYIR2 performance was further enlarged at Post-2 in reference to Post-1 in the two intervention groups (LHTLH: +45%; LHTL: +19%, both $P<0.001$) while Hb_{mass} was maintained (LHTLH: +2.7%, $P<0.01$; LHTL: +3.0%, $P<0.001$). Third, RSA was improved in the two intervention groups (LHTLH: -3.6%, $P<0.001$; LHTL: -1.9%, $P<0.01$), yet with twice larger gains measured at Post-1 in LHTLH compared to LHTL. Three weeks after the intervention, RSA performance improvements were only maintained in the LHTLH group (-3.5%, $P<0.001$).

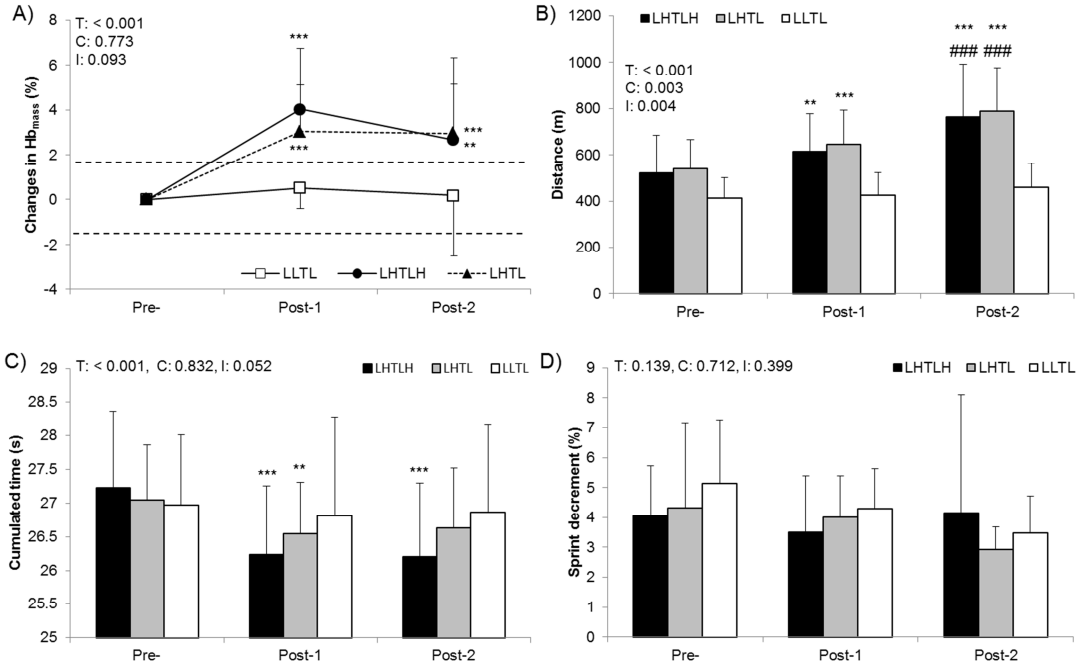

Figure 10. Mean changes in haemoglobin mass (Hb_{mass}; panel A) and performance changes in distance (m) covered during the Yo-Yo intermittent recovery level 2 test (YYIR2; panel B), cumulated sprint times (panel C) and sprint decrement score (panel D) during RSA test (8 × 20 m – 20-s passive recovery). Measurements were taken before (Pre-), immediately after (Post-1) and 3 wk after the intervention (Post-2) in the LHTLH, LHTL, and LLTL groups. Dashed lines represent the typical error of the CO rebreathing procedure in the present study (1.6%). C, condition effects; I, interaction effects; T, time effects. ***P*<0.01, significantly different from Pre. ****P*<0.001, significantly different from Pre. ### *P*<0.001, significantly different from Post-1.

For more details, see chapter 11, article 6.

2.3.3 HIF-1α and related genes transcription in human skeletal muscle are boosted by RSH superimposed to LHTL

From muscle biopsies taken from *the vastus lateralis* muscle before, immediately and 3 weeks after LHTLH intervention, HIF-1α subunit (+132%), vascular endothelial growth factor (VEGF) (+63%), Mb (+110%), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC1-α) (+120%) and mitochondrial transcription factor A (TFAM) (+63%) mRNA levels increased at Post-1 (all P≤0.05) in LHTLH, but not in LHTL or LLTL, and returned near baseline levels at Post-2 (Fig. 11). The protein expression of CS increased in LHTLH (+13%, *P*<0.001 at Post-1 and +20%, *P*<0.01 at Post-2) and LLTL (+38, *P*<0.01

at Post-1 and +58%, *P*<0.05 at Post-2), whereas it decreased in LHTL at Post-1 and Post-2 (both P<0.001).

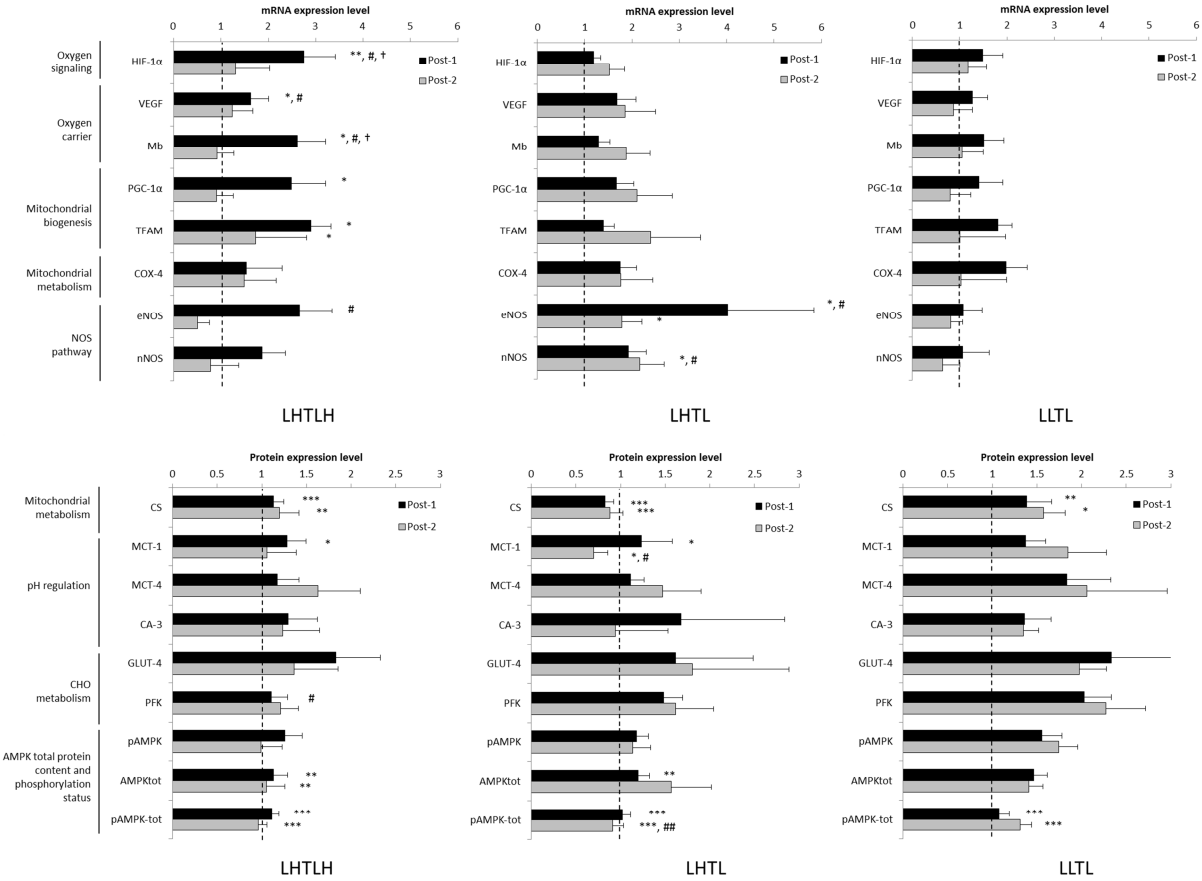

Figure 11. Relative changes in selected mRNA expression markers (upper panel) and relative protein expression of selected markers (lower panel) from baseline (Pre-) to the end of the intervention (Post-1) and after 3 weeks (Post-2). The intervention consisted in 14-days of passive normobaric hypoxic exposure combined with repeated maximal-intensity hypoxic exercise in hypoxia (LHTLH; n = 9) or normoxia (LHTL; n = 11). A control group followed a 'live low-train low' (LLTL; n =10) protocol. Black and grey bars represent Post-1 and Post-2 values of mRNA concentrations in *vastus lateralis* muscle, respectively. These values were normalized to baseline values (Pre-), which were set to 1 (dashed line). Values are means ± SD. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ *vs*. Pre-intervention; # $P < 0.05$ and ## $P < 0.01$ *vs*. LLTL and † $P < 0.05$ *vs*. LHTL.
HIF-1α, hypoxia inducible factor-1a; VEGF, vascular endothelial growth factor; Mb, myoglobin; COX-4, cytochrome oxidase 4; PGC1-α, proliferator-activated receptor gamma coactivator-1α; TFAM, mitochondrial transcription factor A; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase.
GLUT-4, glucose transporter 4; PFK, phosphofructokinase; CA-3, carbonic anhydrase III; CS, citrate synthase; MCT-1, monocarboxylate transporter 1; MCT-4, monocarboxylate transporter 4; AMPK, AMP-activated protein kinase.

For more details, see chapter 12, article 7.

Chapter 3

Discussion

3. Discussion

A better understanding of the neurophysiological and mechanical determinants of RSA is an important prerequisite to design training/ergogenic interventions that could delay the onset of fatigue and improve sport-specific physical performance in team-sport athletes. Further, investigating the effects of an additional environmental stress (*i.e.*, heat or hypoxia) when sprinting repeatedly would strengthen the knowledge of the RSA-related neurophysiological responses, which is potentially usefulness to develop innovative environmentally-based training.

An initial investigation was designed to determine the effect of heat stress on team-sport match outcome. We have demonstrated for the first time the potential influence of heat stress (*i.e.*, temperature, humidity) on the home advantage phenomenon (Pollard, 2006) in international football outcomes. While it confirms previous studies on the impact of weather variables on endurance-based performance (Ely *et al.*, 2007; Vihma, 2010), this also adds to the detrimental effect of altitude/hypoxia on team-sport competition results [*i.e.*, a lower likelihood to win for sea-level football teams playing at altitude >1200 m against altitude-acclimated teams (McSharry, 2007)]. Altogether, this highlights the importance of environmental acclimatisation or acclimation before training and/or competing in hot or hypoxic conditions.

Subsequently, the leading purpose of our work aimed to advance our understanding of the underlying regulatory mechanisms (*i.e.*, neuromuscular, metabolic and biomechanical) during repeated sprinting when performed under environmental stress. Although caution is required when comparing different environmental-mediated fatigue-induced responses [*i.e.*, hot (end-exercise core temperature: ~38.6°C) *vs*. hypoxia (end-exercise SpO_2: ~84%) *vs*. control], we

reported larger RSA performance deterioration in hypoxia and to a lower extent in hot *vs*. control (Girard *et al.*, 2016). While the lack of difference between hot and control (*i.e.*, cool ambient temperature and normoxia) condition may be explain by the fact that hyperthermia (*i.e.*, core temperature ≥38.5°C) occurred in both case, the greater hypoxic effect may relate to the decrease in convective factors of O_2 transport that occurred during the sprints, leading to a lower O_2 supply, as evidenced by the substantial hypoxemia level (*i.e.*, end-exercise SpO_2 <85%). Postulated mechanisms include a lower muscle reoxygenation capacity during recovery periods (Billaut & Buchheit, 2013; Brocherie *et al.*, 2015b) and/or a suboptimal muscle activation capacity stemming from lower oxygenation of the prefrontal cortex (Smith & Billaut, 2010). A unique feature of our study was also that hot and hypoxic stresses do not accentuate the extent of fatigue-induced changes in sprinting mechanics. However, it is worth mentioning that, in this specific environmental comparison, our analysis only included 5 sprints. Thus, in addition to a possible 'task-dependency' effect, the type and the severity of each environmental stressor may also determine the extent to which fatigue increases during the completion of RSA protocols. Reportedly, fatigue development during cycling RSA was exacerbated only under severe (FiO_2 0.12-0.14), but not moderate (FiO_2 0.14-0.16) hypoxic levels, when compared to normoxia (Bowtell *et al.*, 2014).

In this view, we further manipulated the O_2 availability [*i.e.*, FiO_2 0.21 (SL), 0.17 (MH) and 0.13 (SH)] to better describe the changes in propulsive power, sprint kinematics/kinetics and spring-mass characteristics during the repetition of 8 sprints. Accordingly with a previous treadmill sprinting study (Weyand *et al.*, 1999), initial sprint performance remained unaltered across conditions. As previously suggested (Calbet *et al.*, 2003), this may relate to an enhanced anaerobic energy release to compensate for the reduced aerobic ATP production. However, fatigue-induced decrement in propulsive power and in accompanying kinetics/kinematics (*i.e.*, t_c, S_F, F_H) and spring-mass characteristics (*i.e.*, K_{vert}) generally observed during normoxic RSA tests in team-sport athletes [*i.e.*, overground (Girard *et al.*,

2011b; Girard *et al.*, 2011c; Brocherie *et al.*, 2015b)] or treadmill (Morin *et al.*, 2011b; Delextrat *et al.*, 2013; Girard *et al.*, 2015b)] were significantly exacerbated in SH (FiO_2 0.13; ~3600 m) relative to normoxia (SL), while sprint performance was relatively resilient to MH (FiO_2 0.17; ~1800 m) exposure. This appears in line with previous findings (Bowtell *et al.*, 2014; Goods *et al.*, 2014), thereby confirming that hypoxia-induced RSA performance decrements did not follow a monotonic (*i.e.*, linear) pattern.

From a practical view stand, this clearly indicates that t_c, S_F, K_{vert} (*i.e.*, paramount to minimize the fatigue-related increase in t_c and concomitant decrease in S_F) (Girard *et al.*, 2011b; Girard *et al.*, 2011c; Brocherie *et al.*, 2015b) and applying forward-oriented total force against the ground (*i.e.*, due to the three-fold larger alteration for F_H than for F_V and F_{Tot}) (Girard *et al.*, 2011b; Morin *et al.*, 2011a; Morin *et al.*, 2011b) are the key mechanical determinants of fatigue resistance during RSA performance. Bearing in mind that the initial phase (*i.e.*, acceleration) of sprinting is essential in team sports, our observations do lend some support to the maximisation of the propulsive component of the ground reaction force (Nagahara *et al.*, 2014), via the combination of ground force production (preferably F_H) and S_F (Schache *et al.*, 2014) or *vice versa*. Given the importance to maintain maximal P_P levels across sprints repetition during a RSA protocol (Girard *et al.*, 2011a), and based on the fact that only SH (not MH) exacerbates the aforementioned mechanical alterations, one may postulate that SH is less suitable than MH for training purposes, even though larger physiological effects associated with SH *vs*. MH cannot be ruled out.

Another relevant finding is the biphasic profile of the alterations *(i.e.*, sprints 1 to 4 *vs*. sprints 5 to 8) observed during the RSA test, irrespectively of the condition. This appears in line with the gradual decrease in power output during the first sprints followed by a plateau-like phase previously observed during repeated cycling sprints [*i.e.*, 10 × 10 s 'all-out' sprints – 30 s passive recovery (Hureau *et al.*, 2014) or 10 × 10 s cycling sprints – 180 s recovery (Pearcey *et al.*, 2015)]. Interestingly, in this later study, the difference in the rate of decline in total

work observed during the first 5 sprints (-5.2%) *vs*. last 5 sprints (-3.3%) was associated with peripheral neuromuscular fatigue in the first 5 sprints, and both peripheral and central fatigue in the last 5 sprints, respectively. Thus, these earlier and larger performance and mechanical decrements in SH *vs*. MH and SL may relate to larger neuromuscular fatigue levels. Recent evidence using manipulations of pre-existing fatigue levels (Hureau *et al.*, 2014) and hypoxia severity (Billaut *et al.*, 2013) would support the view that power output and EMG are adjusted during repeated sprinting for the purpose of limiting the development of peripheral fatigue beyond a constant threshold. While SpO_2 values remained >90% in MH, the fact that initial SpO_2 values were <90% in SH and decreased to ~82% (ranging 76-89%) across sprint repetitions may support the notion of a critical 'threshold' (i.e., SpO_2 values of 70-75% derived from self-paced exercises) where CNS hypoxia would primarily (*e.g.*, over peripheral fatigue) influence exercise performance (Amann *et al.*, 2007). However, because using step changes in FiO_2 as hypoxic stimulus is associated with larger inter-individual variability in the degree of arterial hypoxemia (and related stimulus at the muscle level) as opposed to pre-determined values of SpO_2 (Chapman, 2013), clamping SpO_2 may be useful to deepen the understanding of hypoxia-induced neuro-mechanical alterations.

Along with mechanical performance decrements, we have reported larger post-exercise reduction in MVC of knee extensors in SH (-14%) *vs*. SL (-9%), which appears in line with previous study (Billaut *et al.*, 2013). Interestingly, we also observed a graded effect of hypoxia severity on post-RSA MVC losses, thereby extending previous findings (Christian *et al.*, 2014). In a companion article (Girard *et al.*, 2015d), we have further demonstrated that RMS activity values of *vastus lateralis, rectus femoris* and *biceps femoris* muscles (among others) decreased significantly across our RSA protocol, confirming that neural factors may have played a role in fatigue-related decrement in sprint performance (Bowtell *et al.*, 2014; Brocherie *et al.*, 2015b). Our post-RSA alterations of maximal normalised EMG activity (RMS_{MAX}/M-wave ratio) measured in *vastus lateralis* (-8%) and *rectus femoris* (-14%)

muscles supported this observation. Our results also feature an earlier and larger central down-regulation of skeletal muscle recruitment in the severer condition, even though this observation is restricted to the *rectus femoris* muscle only. Exacerbated performance decrements under SH are likely to be explained by a reduced neural drive to the active musculature, arising secondary to a stronger reflex inhibition due to brain hypoxia (*i.e.*, decreased brain oxygenation independently of afferent feedback and peripheral fatigue (Millet *et al.*, 2012b) or a hypoxia-induced increased level of intramuscular metabolites known to stimulate group III-IV muscle afferents (Hogan *et al.*, 1999).

A further result was that hypoxia severity did not modify the post-exercise RFD responses [*i.e.*, unchanged early phase RFD (30 and 50 ms) and similar decline in peak and absolute RFD values in the late phase (100 and 200 ms) of muscle contraction]. Similarly, heat stress has been reported to not exacerbate knee extensors' RFD decreases after cycling RSA (Girard *et al.*, 2013b). Our results also highlight the influence of maximal strength in maintaining normalised RFD values at all time intervals after repeated sprinting in differing hypoxic conditions. Practically, based on the prevalence of neuromuscular qualities to determine RSA performance (Brocherie *et al.*, 2014), this pinpoints the relevance of power-related exercise/training [*e.g.*, heavy-resistance (4 sets of 5 repetitions with 90% of the 1-repetition maximum) or plyometric training (drop jump)] to improve RSA-induced fatigue resistance.

The question of whether the same is true when completing similar (repeated-) sprint exercise in hypobaric hypoxia (*i.e.*, at natural altitude), known to induce severer physiological responses (Millet *et al.*, 2012a) and eventually larger neuromuscular alterations, would need to be specifically addressed. While reduced air resistance at terrestrial altitude is likely decreasing the energy cost of running at high velocities, and thereby improve single sprint performance (Ward-Smith, 1984; Peronnet *et al.*, 1991; Levine *et al.*, 2008), however, when sprints are repeated, hypobaric hypoxia would induce higher work of breathing responses and more detrimental neuromuscular consequences than exposure to normobaric hypoxia.

The last goal of our work was to design an innovative hypoxic training intervention to elicit concurrent 'aerobic' and 'anaerobic' adaptations to improve team-sport-related physical performance. Given the prevalence of neuromuscular qualities determining RSA (Brocherie *et al.*, 2014; Brocherie *et al.*, 2015b), we first verified the usefulness of RSH on team-sport physical performance. We demonstrated that a 5-week in-season period including the addition of two weekly high-intensity intermittent running-based training sessions to the usual football training routine of highly-trained under-18 male footballers improved several neuromuscular fitness components related to on-field football physical performance. Of interest, high-intensity training in normobaric hypoxia was more efficient than the same training in normoxia at enhancing repeated-agility ability, with the inclusion of direction changes. The larger improvement in the repeated-agility test (~4% for RSH *vs.* ~2% for RSN), which arguably requests higher muscular solicitation (*e.g.*, eccentric load during the braking phase of change of direction) is likely due to the greater hypoxic-induced power-related gains (*i.e.*, explosive strength and sprinting performance). We suggested that this improved repeated-agility ability (*i.e.*, as a result of a better production/force application technique) was due to neuromuscular adaptations, as evidenced by greater EMG signal amplitudes during post-hypoxic intervention MVCs (Manimmanakorn *et al.*, 2013). Based on these findings, we have recommended adding hypoxic explosive exercises [*i.e.*, heavy resistance or plyometric / agility / sprint drills] to improve power-related factors and fatigue resistance. However, the fact that aerobic quality was not improved raises the question of its development when using RSH in isolation.

Therefore, if the LHTL paradigm works to improve sea-level endurance performance and if additional RSH works to improve sea level-specific performance (*i.e.*, higher RSA tolerance) (Faiss *et al.*, 2013b; Millet *et al.*, 2013a; Brocherie *et al.*, 2015a), then the physiological benefits of the LHTLH method must derive from the combination of these two hypoxic methods.

For the first time, we have demonstrated the usefulness of combining hypoxic training methods when attempting to improve sea-level performance in elite team-sport athletes. After a short-term 'in-season' simulated altitude camp (*i.e.*, 14 days of LHTL exposure + 6 RSH sessions; FiO_2 0.14) Hb_{mass} values increased to a similar extent than previous studies involving team-sports athletes (Garvican-Lewis *et al.*, 2013; McLean *et al.*, 2013a; Wachsmuth *et al.*, 2013) and appears consistent with the model estimated (*i.e.*, 1% for every 100 h) by Gore *et al*. (2013). Of interest, with similar room confinement time for our two intervention groups, the addition of six RSH sessions (~5 h FiO_2 0.14) for LHTLH had no measurable impact on Hb_{mass} increase, thereby indicating that 'hypoxic dose' is the main factor for Hb_{mass} increase and that RSH *per se* has no 'erythropoietic' effect. This also suggests that the proposed RSH-related mechanisms [*i.e.*, metabolic (PCr resynthesis enhancement; muscle and/or cerebral de/reoxygenation kinetics), molecular (HIF-1α and downstream genes), neural (muscle contractility/activation and preferable fast-twitch fibres selection) and biomechanical (running economy)] (Faiss *et al.*, 2013a) were not blunted by the LHTL exposure.

Regarding physical performance, an immediate improved specific aerobic fitness and RSA occurred in the two intervention groups, with a twofold superior benefit seen in LHTLH compared to LHTL for the latter. With comparable Post-1 Hb_{mass} gain between our two intervention groups, the twice-larger improvement in RSA in LHTLH is likely linked to the aforementioned RSH-specific adaptations. Therefore, by using sport-specific ecological training and testing setting, we strengthen the validity of previous studies using RSH training (Faiss *et al.*, 2013b; Galvin *et al.*, 2013; Puype *et al.*, 2013; Brocherie *et al.*, 2015a) and provide evidence that both 'aerobic' and 'anaerobic' adaptations acutely improve sea-level team-sport physical performance. Consequently, this allows us to confirm the premise on the potential benefits of combining hypoxic methods (Millet *et al.*, 2010; Millet *et al.*, 2013a) and to update the panorama of the different hypoxic methods currently available (Fig. 12).

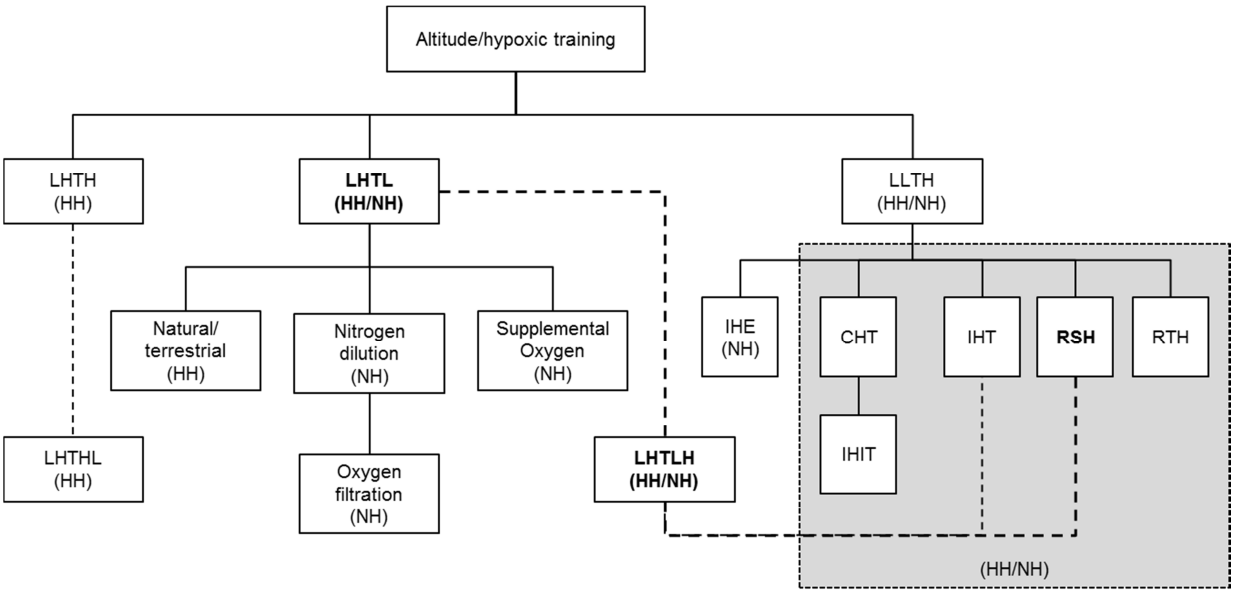

Figure 12. Updated panorama of the different hypoxic methods currently available for a range of athletes engaged in endurance and team–sport disciplines. [adapted from Millet et al. (2013)] with investigated methods highlighted in bold. LHTH, 'live high-train high'; LHTHL, 'live high-train high and low'; LHTL, 'live high-train low'; LHTLH, 'live high-train low and high'; LLTH, 'live low-train high'; IHE, intermittent hypoxic exposure; CHT, continuous hypoxic training; IHT, interval hypoxic training; RSH, repeated sprint training in hypoxia; RTH, resistance training in hypoxia; IHIT, IHE during interval-training; NH, normobaric hypoxia; HH, hypobaric hypoxia.

Finally, our results indicate that the main mechanisms for improved sea-level performance after LHTLH exposure rely at least in part on molecular adaptations of factors implicated in the regulation of O_2 signalling and carrying, mitochondrial biogenesis, as well as of enzymes implicated in mitochondrial metabolism. The more largely enhanced mRNA level of HIF-1α after LHTLH suggests that the addition of RSH (but not RSN) plays an important role for up-regulating the activation of the HIF-1α pathway and its downstream genes (Vogt *et al.*, 2001; Zoll *et al.*, 2006; Faiss *et al.*, 2013b). Activation of HIF-1α is known to lead to cellular adaptations [*i.e.*, O_2 carrying-capacity (Wenger & Gassmann, 1997), neovascularization (Forsythe *et al.*, 1996), glucose oxidation (Wenger & Gassmann, 1997)], which in turn would positively influence exercise capacity in humans (Vogt *et al.*, 2001; Zoll *et al.*, 2006; Faiss *et al.*, 2013b). Accordingly, the mRNA levels for the capillary growth factor VEGF, *i.e.*, an HIF-1-regulated gene (Wenger & Gassmann, 1997; Semenza, 1999; Semenza *et al.*, 1999) and Mb mRNA, significantly increased after LHTLH, whereas no changes were observed in both LHTL and LLTL. This corroborates previous works, which demonstrated Mb mRNA or

protein levels enhancement after hypoxic endurance exercise (Terrados *et al.*, 1990; Vogt *et al.*, 2001). In addition to an exercise intensity effect (Vogt *et al.*, 2001), the shift from type I (oxidative) to type II (glycolytic) fibres in hypoxia (Ishihara *et al.*, 1994; Itoh *et al.*, 1995) could at least in part explain differences in VEGF gene expression between LHTLH and LHTL. Meanwhile, as previously suggested (Millet & Faiss, 2012), RSH (compared to RSN) would enhance the behaviour of fast twitch fibres via larger blood perfusion levels (*i.e.*, microvascular O_2 delivery) (McDonough *et al.*, 2005) and/or compensatory vasodilation (Casey & Joyner, 2012). In addition to the heterogeneity of the ratio between local muscle blood flow, metabolic rate and O_2 utilization (Koga *et al.*, 2014), it is therefore conceivable that the molecular effects – *i.e.*, up-regulating HIF-1α pathway and its downstream genes – of RSH are more manifest in type II muscles, especially given their greater reliance on fractional O_2 extraction compared to type I muscles.

When RSH was used in isolation (Faiss *et al.*, 2013b), a down-regulation in mitochondrial biogenesis (PGC-1α and TFAM) and unchanged oxidative capacity (CS), was observed. Contrastingly, LHTLH induced larger mitochondrial adaptations and improved skeletal respiratory capacity and muscle function (*i.e.*, CS enzymatic adaptation), thereby suggesting a preponderant role of the combination of LHTL with RSH for muscle phenotypic adaptations.

A further important observation was that, when players were retested 3 weeks after completion of the hypoxic intervention, the majority of the positive molecular responses already disappeared. In the meantime, both LHTLH and LHTL displayed maintenance of Hb_{mass} and enhancement of YYIR2 performance at Post-2 in reference to Post-1, whereas preservation of hypoxia-induced RSA gains was only observable in LHTLH. The fact that no hypoxic exposure occurred during the 3 weeks post-intervention (before Post-2) – be it during LHTL or RSH – indicates that the lack of external hypoxic 'stimulus' could be responsible for the rapid reversal of beneficial muscle function adjustments, while it apparently did not affect Hb_{mass} (Brocherie *et al.*, 2015c). Meanwhile, as this post-intervention period did not include

any RSH session (as performed six times during the 14-days intervention), the influence that of such exercise modality could have on normalisation of molecular responses should not be overlooked.

Other factors likely to influence the maintenance of performance (*i.e.*, training responsiveness and exercise capacity) after an hypoxic training stimulus include the time decay in Hb_{mass}, ventilatory acclimatisation, and biomechanical and neuromuscular factors associated with force production (Chapman *et al.*, 2014). Further, in agreement with the absence of negative effect recently observed on running mechanics after LHTL (Stickford *et al.*, 2016), hypoxia-induced improvement in the neural drive of active musculature (Bowtell *et al.*, 2014) may have up-regulated musculoskeletal stiffness, leading to faster S_F and thereby better sea-level RSA (Bishop & Girard, 2013).

Of practical relevance, periodisation of hypoxic training is very challenging and appears critical in order to peak fitness at the most appropriate time during team-sport competition (*i.e.*, for a single match, league championship and/or tournament play). Before solid recommendations can be formulated, understanding the time course for the onset and decay of any ergogenic effects, and the frequency of stimulus required for maintenance, is vital for developing strategies that can be used by team-sport athletes. This specific area of research warrants further attention.

Chapter 4

Conclusion and perspectives

4. Conclusion and perspectives

In conclusion, the general aim of this work was to gain a better understanding of neurophysiological determinants of RSA, while using acute and chronic manipulations of environmental stress. In the first part, we have demonstrated the effect of heat stress on football match outcome. In the second part, we have highlighted that (i) hot and hypoxic stresses do not accentuate the extent of fatigue-induced changes in sprinting mechanics, (ii) hypoxia-induced RSA performance, biomechanical (*i.e.*, mainly t_c, S_F, F_H and K_{vert}) and neurophysiological (*i.e.*, MVC, EMG) alterations are exacerbated with severe but not moderate hypoxia in reference to normoxia and do not follow a monotonic (*i.e.*, linear) pattern, whereas (iii) RFD modifications are not modified by hypoxia severity. These different observations bring practical insights in the setting of environmental stress in training routine. Lastly, in the third part, we have first verified the putative benefit of the 'repeated sprint training in hypoxia' in a team-sport context. Additionally, we have established the potential advantage of the 'live high-train low and high' intervention to induce concomitant 'aerobic' and 'anaerobic' adaptive mechanisms via blood O_2 carrying-capacity improvement (*i.e.*, Hb_{mass}) and muscle molecular changes (*i.e.*, HIF-1α and related genes). Overall, by using a sport-specific ecological training and test setting, our findings open new frontiers to optimise future environmental training applications (*e.g.*, appropriate periodisation to implement isolated and/or combined methods).

In perspectives, the concept of cross-tolerance – *i.e.*, acclimation to one environmental stressor (*i.e.*, heat) which could enhance adaptation to various other stressors (*i.e.*, hypoxia or vice versa) (Hale & Mefferd, 1958; Hale, 1969) – deserves further attention. Hence, this concept has emerged as a time-efficient solution in athletes training, as it is believed to activate common protective pathways (*i.e.*, HIF-1α signalling, expansion of plasma volume or improved cardiac efficiency) (White *et al.*, 2014). There is also a growing interest in the

concept of combining heat and hypoxia stressors (Takeno *et al.*, 2001; Tipton, 2012), and evidence is emerging to support such an ergogenic 'cocktail' (Takeno *et al.*, 2001; Buchheit *et al.*, 2013). However, given the variety of physiological adaptations with single environmental stress, clarifying and isolating precise mechanisms of any performance effects with combined environmental stressors will likely be challenging. While both approaches may potentially increase and/or speed-up competition preparation of team-sport athletes, evidence of the efficacy of the cross-tolerance concept or combined stressors is still needed.

Finally, considering the role of hypoxic pathways during normoxic exercise, the potential additive effects of accentuating muscle hypoxia by manipulating O_2 availability during exercise might be also a valuable 'therapeutic strategy' in hypertension-, obesity- or ageing-related clinical areas. Future studies are needed to evaluate the extent of this bourgeoning research topic.

« En atteignant un sommet,
on voit ce qui se trouve derrière la montagne,
et tout est encore plus vaste que ce qu'il y avait devant. »
Ueli Steck.

Chapter 5

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Chapter 6

Article 1 – Influence of weather, rank, and home advantage on football outcomes in the gulf region

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Influence of Weather, Rank, and Home Advantage on Football Outcomes in the Gulf Region

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ABSTRACT

BROCHERIE, F., O. GIRARD, A. FAROOQ, and G. P. MILLET. Influence of Weather, Rank, and Home Advantage on Football Outcomes in the Gulf Region. *Med. Sci. Sports Exerc.*, Vol. 47, No. 2, pp. 401–410, 2015. **Purpose**: The objective of this study was to investigate the effects of weather, rank, and home advantage on international football match results and scores in the Gulf Cooperation Council (GCC) region. **Methods**: Football matches (n = 2008) in six GCC countries were analyzed. To determine the weather influence on the likelihood of favorable outcome and goal difference, generalized linear model with a logit link function and multiple regression analysis were performed. **Results**: In the GCC region, home teams tend to have greater likelihood of a favorable outcome ($P < 0.001$) and higher goal difference ($P < 0.001$). Temperature difference was identified as a significant explanatory variable when used independently ($P < 0.001$) or after adjustment for home advantage and team ranking ($P < 0.001$). The likelihood of favorable outcome for GCC teams increases by 3% for every 1-unit increase in temperature difference. After inclusion of interaction with opposition, this advantage remains significant only when playing against non-GCC opponents. While home advantage increased the odds of favorable outcome ($P < 0.001$) and goal difference ($P < 0.001$) after inclusion of interaction term, the likelihood of favorable outcome for a GCC team decreased ($P < 0.001$) when playing against a stronger opponent. Finally, the temperature and wet bulb globe temperature approximation were found as better indicators of the effect of environmental conditions than absolute and relative humidity or heat index on match outcomes. **Conclusions**: In GCC region, higher temperature increased the likelihood of a favorable outcome when playing against non-GCC teams. However, international ranking should be considered because an opponent with a higher rank reduced, but did not eliminate, the likelihood of a favorable outcome. **Key Words:** ATHLETIC PERFORMANCE/PHYSIOLOGY, ENVIRONMENTAL EXPOSURE, HEAT STRESS, TEMPERATURE, SOCCER

In December 2010, football's governing body—i.e., the Federation of International Football Associations (FIFA)—officially appointed Qatar as host for the FIFA World Cup 2022. Working against the Qatar bid was the challenging environmental conditions that players are likely to face during the hot months of June/July (e.g., average in July is 37°C, ranging 34°C–41°C). Hot environments are known to be detrimental for athletic performance (e.g., repeated sprint ability, capacity to sustain high-intensity intermittent work) (35) and can potentially be harmful to players' health (3). Exposure to a severe heat stress can lead to hyperthermia and premature fatigue, which may be reflected by a marked dehydration (e.g., >2% loss of body mass in football players) (39) and concomitant reductions in stroke volume, brain and active muscle blood flow (secondary to hypotension), brain metabolism, substrate depletion, and large increases in body core and brain temperatures (3). Playing football in hostile conditions can exacerbate those symptoms (15), leading to heat exhaustion and life-threatening exertional heatstroke (45).

Domestic and international football matches are often played in challenging environmental conditions where the temperature can exceed 35°C, with or without a high relative humidity. For instance, the final match of the 2008 Beijing Olympic Games was played at an air temperature approaching 42°C (25). While the literature on match-related performance changes in such hostile conditions is not extensive, players competing in hot conditions generally cover less distance (6,28–30). This pronounced reduction in high-intensity running (29) could be related to a likely increase of injury incidence toward the end of a match (16) played in the heat. This field-based applied research highlights the importance of heat acclimatization, particularly for teams playing away matches at higher temperature and/or humidity (25). These two variables are well known for limiting the thermoregulatory control (7,43).

Although it is recognized that teams that are acclimatized to heat may be at advantage, the direct link with football performance at the international level has not been reported

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or quantified yet. Meanwhile, home advantage has been recognized as a worldwide phenomenon in football (33) that influences the outcome of matches but varies considerably between countries and over time. Although several explanations including crowd support, travel effects, familiarity with local playing conditions, territoriality, altitude, referee bias, technique, tactic and management, as well as players' physiological and psychological condition have been proposed to account for this diversity (27,33), no dominant factor influencing home advantage has been isolated. It is likely that the individual factors interact with each other in a manner yet to be established and that home advantage is the result of their combined effect (33). To our knowledge, no study has yet focused on the potential influence of heat stress (temperature, humidity) on the home advantage phenomenon. Therefore, we investigated the effects of heat and humidity on international football match results and scores in the Gulf region, i.e., specific context of the FIFA World Cup 2022. To do so, we applied statistical analyses aiming to control other factors as the home advantage or the difference in international ranking between the playing teams. We hypothesized that temperature and humidity would positively affect international football match results in/toward national football squads already heat-acclimatized when playing in hot conditions (i.e., Gulf Cooperation Council (GCC) countries playing home in hot ambient conditions).

METHODS

The International Gulf Cooperation Council Arab states' football results and scores offer a direct measure of the performance of different teams at multiple temperatures and humidities, which can be linked to their ability to acclimatize. For this, a data set containing FIFA-recognized Olympic and A level football scores for six national teams representing GCC (i.e., Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and United Arab Emirates) was assembled. A total of 2008 matches of these teams over 55 yr between 1957 and 2012 played either at home or not (i.e. away or neutral ground) was used to assess the influence of temperature and humidity on football outcome. It should be noted that matches played in neutral venue accounted for 12.4% only and that, in most of the case, these matches were organized in neighbor countries, which have very similar weather condition (e.g., Qatar playing against Kuwait in the United Arab Emirates).

Two Web sites were selected, from which relevant information on football and weather data were extracted for meaningful worldwide comparisons. First, match results and scores were collected on the official internet Web site of FIFA and corresponding teams FIFA world ranking at the exact month and year of match was extracted (12). A written and informed consent was therefore not required from individual players. Second, average dry bulb temperature (°C) and relative humidity (%) during the month preceding the matches were collected both in home countries and in all opponents' sides from the "Weather Underground" Web site (44), which centralized climatic data from weather stations owned by government agencies referenced by the World Meteorological Organization (http://www.wmo.int/pages/index_fr.html). A monthly average seemed appropriate to reflect the environmental condition experienced by the different teams because a yearly average temperature would not reflect the weather fluctuation and, in opposite, a too short window (e.g. one week) would not reflect the medium-term acclimatization process. Temperature and humidity of the day for all matches for the three different venues (home, played in GCC; away, played in the opponent' country; or neutral, played neither at home nor away) were also collected.

Estimates of home advantage were calculated as the number of matches won or lost expressed as percentage of the total number of matches for all the FIFA international matches played by the six GCC teams. The average percentage of home wins for the six GCC teams was 63% ± 9%, ranging from 48% (Bahrain) to 74% (Saudi Arabia). The two best percentages are from the two higher FIFA-ranked countries in our data set, Kuwait and Saudi Arabia. The total number of Asian Cup victory and participation in the final phase of FIFA World Cups was one and three for Kuwait and Saudi Arabia, respectively. This observation points out the importance of the FIFA ranking system in our analysis. More importantly, such home advantage suggests a possible influence of temperature on football performance in the Gulf region, which is categorized as BWh (i.e., B, arid; W, desert; and h, hot arid) in the Köppen–Geiger classification (32). Then, we defined two groups as GCC and other worldwide opponents (non-GCC (nGCC)) to control the differing performances of the individual teams. The distribution of the usual temperature during matches played by both groups (Fig. 1A and B) serves to highlight the rational for performing this study.

To investigate the influence of temperature and humidity on match results and scores, we first defined two dependent variables: (i) the likelihood of a favorable outcome and (ii) the difference between the number of goals scored and the number of goals conceded (Δgoals). Second, five independent variables were used: (i) the difference in team FIFA-ranking (Δrank), (ii) the home advantage, (iii) the temperature difference (ΔT) between the match venue temperature and usual monthly temperature of a specific team, (iv) the relative humidity difference (ΔH) between the match venue humidity and monthly humidity of a specific team, and (v) the saturated vapor pressure of water (e^*_w) difference (Δe^*_w) between the match venue and monthly saturated vapor pressure of water of a specific team. Finally, two additional independent variables were used to determine the best heat stress variable predicting the likelihood of favorable outcome and goal difference: (vi) the heat index (HI) difference (ΔHI) between the match venue and monthly HI of a specific team and (vii) the wet bulb globe temperature (WBGT) difference (ΔWBGT) between the match venue WBGT and monthly WBGT of a specific team.

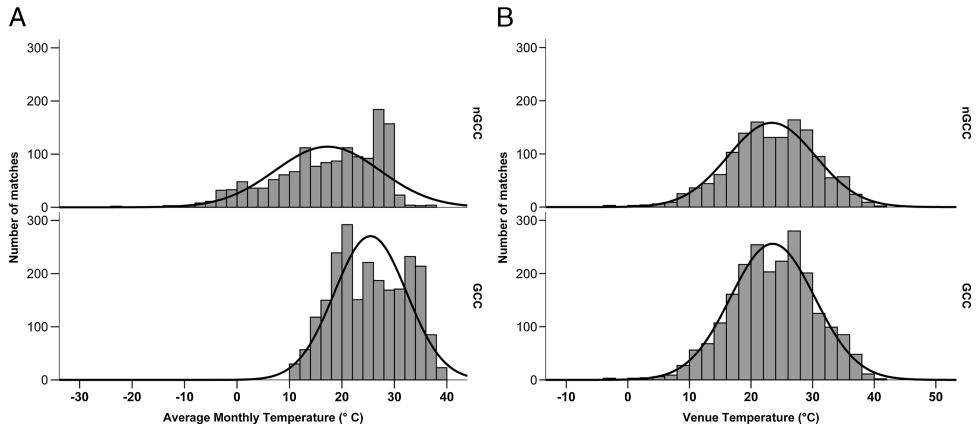

FIGURE 1—Distribution of the monthly temperature (A) and match day temperature (B) for GCC countries and nGCC opponents.

These variables are defined and calculated as follows:

(i) The likelihood of a favorable outcome was defined to categorize a positive result (i.e., win or draw vs loss), as we claim that a draw could be considered as a positive result. This may be the case during qualification phases, which are mainly used in international football context. Moreover, this distinction is of importance because GCC teams generally have poorer ranking than their opponents.

(ii) Although two distinct approaches on how to model the match outcomes (i.e., modeling win–draw–loss match results or modeling the goals scored and conceded by each team) (13) coexist, we considered Δgoals as the final explanatory variable. When the differences between the goals- and results-based models' performances seem to be relatively small, some advantage is gained by using goals-based model because of a richer data set, permitting covariate performance. The Δgoals variable is zero when both the home country and opponent's game results in a draw and is positive when the home country wins. Likewise, a negative number indicates a loss.

(iii) Since its introduction, the FIFA ranking was used to create a reliable measure for comparing national football teams' progression and ability. For example, the average ranking over the data set period is 77 ± 5 (range, 46–96) for the six GCC teams, whereas Japan (the First Asian Confederation team) and Brazil (considered as the best ever team in the world) are, respectively, 31 ± 11 and 2 ± 2. To consider the relative strength of the opposition, Δrank for home team and all opponents was expressed as a positive (i.e., home side playing against a lower FIFA-ranked; i.e., a stronger country) or negative difference.

(iv) The home advantage specifically refers to the ground effect (playing at home or not). In the specific case of matches played in neutral venue, the home team was selected as defined by FIFA.

(v) Because the key variables, temperature and humidity, change over matches for the same host country and visiting opponents, the difference between each usual variable at home and venue was considered. Hence, the variable "temperature difference" (ΔT, expressed in degrees Celsius) is zero when both home and venue have the same temperature, is positive when the GCC team is playing at temperature higher than the usual temperature, and is negative when the GCC team is playing at a cooler temperature.

(vi) Readings for the relative humidity (ΔH, expressed in percent) and temperature differences are similar and correspond to the difference between the home and venue's ratios of the partial pressure of water vapor in the air to the saturated vapor pressure of water at a given temperature.

(vii) The saturated vapor pressure of water (e^*_w, expressed in hectopascals) reflects the absolute humidity gradient and was computed using an updated formula proposed by Buck (5):

$$e^*_w = 6.1121 \exp\{[18.678 - (T/234.5)][T/(257.14 + T)]\} \quad [1]$$

where T is the ambient dry bulb temperature (°C).

(viii) The HI (expressed in degrees Celsius) and the WBGT (expressed in degrees Celsius) are two empirical indexes of environmental heat stress. The first is derived from a collection of equations (40). To simplify the model, the parameters involved in HI calculation are given as assumed magnitudes in the following equation:

$$\begin{aligned} \mathrm{HI} = {} & -42.379 + 2.04901523\,T + 10.14333127\,H - 0.22475541\,TH \\ & - \left(6.83783 \times 10^{-3}\right) T^2 - \left(5.481717 \times 10^{-2}\right) H^2 \\ & + \left(1.22874 \times 10^{-3}\right) T^2 H + \left(8.5282 \times 10^{-4}\right) T H^2 \\ & - \left(1.99 \times 10^{-6}\right) T^2 H^2 \end{aligned} \quad [2]$$

where T is the ambient dry bulb temperature (°F = °C(9/5) + 32) and H is the relative humidity (%).

(ix) The WBGT has been validated and given ISO (ISO/DIS 7933:1984) certification and, therefore, represents the preferred measure available (14) when properly measured. However, given that not all facilities have the equipment required to measure WBGT, the following

approximate formula that relies on temperature and humidity (1) is generally used:

$$\mathrm{WGBT} = 0.567T + 0.393[H/100 \times 6.105 \times \exp(17.27T/237.7 + T)] + 3.94 \quad [3]$$

where T is the dry bulb temperature (°C) and H is the relative humidity (%).

For e^*_w, HI, and WBGT, we calculated difference as previously described for ΔT and ΔH. Readings are identical for Δe^*_w, ΔHI, and ΔWBGT. Favorable outcome (i.e., win or draw vs loss) was dichotomous, whereas the other dependent outcome (i.e., goal difference) was continuous. All other independent variables (temperature, relative and absolute humidity, HI, WBGT, and ranking) were continuous.

Statistics

To determine the influence of temperature and humidity on the likelihood of favorable outcome, generalized linear model with a logit link function was performed. Three models resulting from different combinations of the important predictor variables were considered: no predictors (model 1), addition of Δrank and home advantage (model 2), and subsequent addition of interaction term $\Delta T \times$ opponent GCC/nGCC (model 3). This last model was repeated for all other weather variables (ΔH, Δe^*_w, ΔHI, and ΔWBGT). The parameter estimates were reported as odds ratios (OR) with 95% of confidence interval (95% CI). The Akaike Information Criterion (AIC), as a measure of the relative quality of a statistical model for a given set of data (i.e. the "smallest" AIC is the "best"), provided a means for model selection.

A similar set of multiple regression analysis was executed to test the influence of weather variables on Δgoals as a dependent variable. This variable served as a better representation measure for the overall performance. Although this variable was discrete, it closely resembled a normal distribution. Therefore, normality assumptions were reasonably held. Similar to favorable outcome, three models were constructed to predict goal difference as match outcome. The parameter estimates were reported as beta coefficient (β) with 95% CI. For all procedures, a P value <0.05 was considered as cutoff for significance. To help choose between two sets of model terms, the model with smaller AIC was considered "better". All data were coded and analyzed in the Statistical Package of Social Sciences (SPSS 21.0).

TABLE 1. Parameter estimates following generalized linear model (logit link) for each model on the favorable outcome of GCC countries in international football matches.

Variable	Model 1[a]	Model 2[b]	Variable	Model 3[c]
Intercept	1.99 (1.79–2.21)**	1.51 (1.24–1.84)**	Intercept	1.51 (1.24–1.83)**
ΔT	1.04 (1.03–1.06)**	1.03 (1.01–1.06)**	$\Delta T \times$ vs GCC	0.99 (0.93–1.05), P = 0.674
			$\Delta T \times$ vs nGCC	1.04 (1.02–1.06)**
Δrank		0.99 (0.99–0.99)**	Δrank	0.99 (0.99–0.99)**
Home advantage		1.65 (1.25–2.18)**	Home advantage	1.66 (1.26–2.20)**
AIC	1233	1423		1424
Intercept	1.77 (1.61–1.96)**	1.25 (1.05 –1.49)*, P = 0.011	Intercept	1.14 (0.95–1.37), P = 0.146
ΔH	1.00 (1.00–1.01), P = 0.401	1.00 (1.00–1.01), P = 0.288	$\Delta H \times$ vs GCC	0.98 (0.97–1.00)*, P = 0.016
			$\Delta H \times$ vs nGCC	1.01 (1.00–1.01)*, P = 0.011
Δrank		0.99 (0.99–0.99)**	Δrank	0.99 (0.99–0.99)**
Home advantage		2.00 (1.54–2.59)**	Home advantage	2.18 (1.67–2.85)**
AIC	1672	1430		1422
Intercept	1.98 (1.78–2.20)**	1.52 (1.25–1.84)**	Intercept	1.51 (1.24–1.83)**
Δe^*_w	1.02 (1.01–1.03)**	1.02 (1.01–1.03)**	Query$\Delta e^*_w \times$ vs GCC	0.99 (0.96–1.02), P = 0.603
			$\Delta e^*_w \times$ vs nGCC	1.02 (1.01–1.03)**
Δrank		0.99 (0.99–0.99)**	Δrank	0.99 (0.98–0.99)**
Home advantage		1.65 (1.25–2.16)**	Home advantage	1.66 (1.27–2.18)**
AIC	2389	1490		1489
Intercept	1.84 (1.66–2.03)**	1.34 (1.13 –1.59)**	Intercept	1.34 (1.13 –1.59)**
ΔHI	1.01 (1.01–1.02)**	1.01 (1.00–1.02), P = 0.013	ΔHI × vs GCC	1.00 (0.97–1.03), P = 0.758
			ΔHI × vs nGCC	1.02 (1.00–1.03)**
Δrank		0.99 (0.99–0.99)**	Δrank	0.99 (0.99–0.99)**
Home advantage		1.86 (1.43–2.40)**	Home advantage	1.86 (1.44–2.41)**
AIC	2296	1425		1426
Intercept	1.94 (1.75–2.14)**	1.50 (1.25–1.80)**	Intercept	1.45 (1.20–1.75)**
ΔWBGT	1.06 (1.04–1.08)**	1.05 (1.03–1.08)**	ΔWBGT × vs GCC	0.95 (0.89–1.01), P = 0.078
			ΔWBGT × vs nGCC	1.07 (1.04–1.10)**
Δrank		0.99 (0.99–0.99)**	Δrank	0.99 (0.99–0.99)**
Home advantage		1.65 (1.26–2.16)**	Home advantage	1.71 (1.30–2.24)**
AIC	2270	1415		1402

Parameter estimates are presented as OR with 95% CI.
Generalized linear models:
[a]Model 1, intercept-only model.
[b]Model 2, intercept, ranking difference (Δrank), and home advantage.
[c]Model 3, intercept, ranking difference (Δrank), home advantage, and interaction term variable (ΔT, ΔH, Δe^*_w, ΔHI, or ΔWBGT) × opponent (GCC or nGCC).
*$P < 0.05$.
**$P < 0.001$.

RESULTS

Probability of Favorable Outcome

Table 1 presents the parameter estimates for the likelihood of a favorable outcome (win or draw vs loss) of GCC countries obtained after applying generalized estimate equations. Model 1 clearly demonstrated that ΔT was a significant explanatory variable to predict the favorable outcome when used independently. In model 2, the addition of Δrank and home advantage was significant ($P < 0.001$) but without influencing the effect of ΔT. The OR = 1.03 (95% CI, 1.01–1.06) indicating that with every one unit increase in ΔT (i.e., playing at a 1°C higher temperature than usual temperature) the likelihood of favorable outcome increases by 3% for the GCC side. However, when interaction term was added (model 3), results suggest that this advantage was only significant when playing against nGCC opponents (OR, 1.04 (1.02–1.06); $P < 0.001$). Conversely, ΔH did not appear of relevance when used independently (OR, 1.00 (1.00–1.01); $P = 0.401$) (Table 1, model 1) or when adjusted for Δrank and home advantage (OR, 1.00 (1.00–1.01); $P = 0.288$) (Table 1, model 2). However, from model 3, ΔH increased the likelihood of favorable outcome slightly when playing against an nGCC team (OR, 1.01 (1.00–1.01); $P = 0.011$) but not against a GCC opponent (OR, 0.98 (0.97–1.00); $P = 0.016$). Besides, Δe^*_w gave a similar trend for models 1, 2, and 3, with a lower OR and a lower statistical fit for the likelihood of a favorable outcome than those for ΔT and ΔWBGT (Table 1, models 1, 2, and 3). Alongside, after adjustment for ΔT and Δrank (Table 1, model 2) home advantage increased the odds of favorable outcome for GCC teams by 65% (OR, 1.65 (1.25–2.18); $P < 0.001$) and remained similar after inclusion of interaction with opposition (66%, $P < 0.001$) (Table 1, model 3). Meanwhile, the likelihood of a favorable outcome for a GCC team decreased by 1% (OR, 0.99 (0.99–0.99); $P < 0.001$) when playing against a 1-unit stronger opponent (i.e., with a lower FIFA-ranked team).

As an example, based on model 3 (Table 1), by playing in hot (e.g., 30°C) versus cool (e.g., 20°C) conditions (ΔT = 10°C) against an nGCC opponent deemed to be of similar level (Δrank = 0), a GCC team would increase its likelihood of a favorable outcome by 48% (OR, 1.48 computed as $\exp[\Delta T \times \ln(\text{OR})]$, where OR, 1.04). However, with a stronger opposition (i.e., when playing against a squad ranked 25 places ahead in the FIFA ranking, Δrank = 25), the likelihood of favorable outcome would be attenuated (OR, 0.78 or −22% computed as $\exp[\Delta\text{rank} \times \ln(\text{OR})]$ where OR, 0.99).

Table 2. Parameter estimates following multiple linear regression for each model on the goal difference of GCC countries in international football matches.

Variable	Model 1[a]	Model 2[b]	Variable	Model 3[c]
Intercept	0.35 (0.24 to 0.46)**	−0.13 (−0.31 to 0.05), $P = 0.0163$	Intercept	−0.13 (−0.31 to 0.05), $P = 0.144$
ΔT	0.06 (0.04 to 0.07)**	0.04 (0.02 to 0.06)**	$\Delta T \times$ vs GCC	−0.01 (−0.07 to 0.05), $P = 0.709$
			$\Delta T \times$ vs nGCC	0.04 (0.02 to 0.06)**
Δrank		−0.02 (−0.02 to −0.01)**	Δrank	−0.02 (−0.02 to −0.01)**
Home advantage		0.55 (0.31 to 0.80)**	Home advantage	0.56 (0.32 to 0.80)**
AIC	7913	4992		4990
Intercept	0.20 (0.09 to 0.31)**	−0.36 (−0.52 to −0.20)**	Intercept	−0.44 (−0.61 to −0.27)**
ΔH	0.00 (0.00 to 0.01), P=0.025	0.01 (0.00 to 0.01)*, $P = 0.040$	$\Delta H \times$ vs GCC	−0.02 (−0.03 to 0.00)*, $P = 0.023$
			$\Delta H \times$ vs nGCC	0.01 (0.00 to 0.01)**
Δrank		−0.02 (−0.02 to −0.01)**	Δrank	−0.02 (−0.02 to −0.01)**
Home advantage		0.79 (0.56 to 1.02)**	Home advantage	0.86 (0.63 to 1.09)**
AIC	7927	4994		4985
Intercept	0.40 (0.26 to 0.53)**	−0.17 (−0.35 to 0.01), $P = 0.061$	Intercept	−0.18 (−0.36 to 0.00), $P = 0.053$
Δe^*_w	0.03 (0.02 to 0.04)**	0.01 (0.00 to 0.02)**	$\Delta e^*_w \times$ vs GCC	0.00 (−0.03 to 0.03), $P = 0.970$
			$\Delta e^*_w \times$ vs nGCC	0.02 (0.01 to 0.03)**
Δrank		−0.02 (−0.02 to 0.02)**	Δrank	−0.02 (−0.02 to −0.01)**
Home advantage		0.60 (0.36 to 0.84)**	Home advantage	0.55 (0.30 to 0.79)**
AIC	5522	5251		5252
Intercept	0.25 (0.15 to 0.36)**	−0.27 (−0.42 to −0.11)**	Intercept	−0.27 (−0.43 to −0.11)**
ΔHI	0.02 (0.01 to 0.03)**	0.01 (0.00 to 0.02)**	ΔHI × vs GCC	−0.01 (−0.04 to 0.02), $P = 0.385$
			ΔHI × vs nGCC	0.02 (0.01 to 0.03)**
Δrank		−0.02 (−0.02 to −0.01)**	Δrank	−0.02 (−0.02 to −0.01)**
Home advantage		0.69 (0.46 to 0.91)**	Home advantage	0.69 (0.47 to 0.92)**
AIC	7912	4986		4984
Intercept	0.32 (0.21 to 0.42)**	−0.14 (−0.30 to 0.02), $P = 0.096$	Intercept	−0.18 (−0.34 to −0.02)*
ΔWBGT	0.07 (0.06 to 0.09)**	0.06 (0.04 to 0.08)**	ΔWBGT × vs GCC	−0.05 (−0.10 to 0.01), $P = 0.107$
			ΔWBGT × vs nGCC	0.07 (0.05 to 0.09)**
Δrank		−0.02 (−0.02 to −0.01)**	Δrank	−0.02 (−0.02 to −0.01)**
Home advantage		0.55 (0.32 to 0.78)**	Home advantage	0.59 (0.36 to 0.82)**
AIC	7865	4966		4952

Parameter estimates are presented as beta coefficient (β) with 95% CI.
Multiple regression analysis:
[a]Model 1, intercept-only model.
[b]Model 2, intercept, ranking difference (Δrank), and home advantage.
[c]Model 3, intercept, ranking difference (Δrank), home advantage and interaction term variable (ΔT, ΔH, Δe^*_w, ΔHI, or ΔWBGT) × opponent (GCC or nGCC).
*$P < 0.05$.
**$P < 0.001$.

Goal Difference

Table 2 shows parameter estimates on Δgoals in international football matches obtained after applying multiple regressions. In this case, a similar set of changes as for predicting likelihood of favorable outcome was observed. When adjusted for Δrank and home advantage, the effect of ΔT was significant; with every 1°C increase in temperature, Δgoals increased (β = 0.04; 95% CI, 0.02–0.06; $P < 0.001$) (Table 2, model 2). However, addition of interaction term revealed significant effect of ΔT on Δgoals but only against an nGCC opponent (β = 0.04 (0.02–0.06), $P < 0.001$) (Table 2, model 3). Furthermore, as Δrank increased between two teams, the estimate to score more goals decreased ($\beta = -0.02$ (-0.02 to -0.01), $P < 0.001$) (Table 2, models 2 and 3) after adjustment for home advantage and ΔT.

Based on model 3, Figure 2A and B illustrates the relationship between adjusted Δgoals and temperature variables. When GCC teams played against nGCC opponents, playing at higher temperature than usual corresponded with the largest goals differences (Fig. 2A and Table 2, model 3) ($P < 0.001$). But gradually with ΔT above +10°C, the adjusted Δgoals remained stable. Interestingly, there was no effect of ΔT when playing against GCC opponents (Fig. 2B and Table 2, model 3) (P = 0.709). As relative temperature did not reflect absolute temperature (e.g., ΔT = 10°C could result from venue temperature of 10°C–20°C or 25°C–35°C), we also plotted adjusted Δgoals against venue (match) temperature (Fig. 3A and B). Of importance, matches played against nGCC teams (Fig. 3A) displayed an increasing trend in Δgoals with ΔT, whereas it remains stable when matches were played against GCC opponents (Fig. 3B).

Environmental Indicators

To determine the best heat stress predictor on the likelihood of favorable outcome (Table 1) or on goal difference (Table 2), we compared the different environmental variables collected or calculated: ΔT, ΔH, Δe^*_w, ΔHI, and ΔWBGT based on AIC values for model fit. As independent variables (model 1), ΔT and ΔWBGT provided the best values to predict favorable outcome (Table 1), whereas Δe^*_w delivered the best AIC when estimating Δgoals (Table 2). After adjustment for Δrank and home advantage (model 2), both ΔT and ΔWBGT were still the best relevant factors for favorable outcome and Δgoals, whereas Δe^*_w showed the lowest statistical fit. Meanwhile, ΔH seemed to be of lower importance in affecting the favorable outcome and Δgoals (Tables 1 and 2, models 1 and 2). ΔHI was not sensitive enough to predict favorable outcome or Δgoals (Tables 1 and 2) despite being significant but seemed to be a better indicator than ΔH and Δe^*_w in predicting Δgoals (Tables 1 and 2, model 2).

As a summary, ΔT and ΔWBGT seemed the most appropriate weather variables to predict match results and Δgoals. However, ΔH was the only variable found to significantly

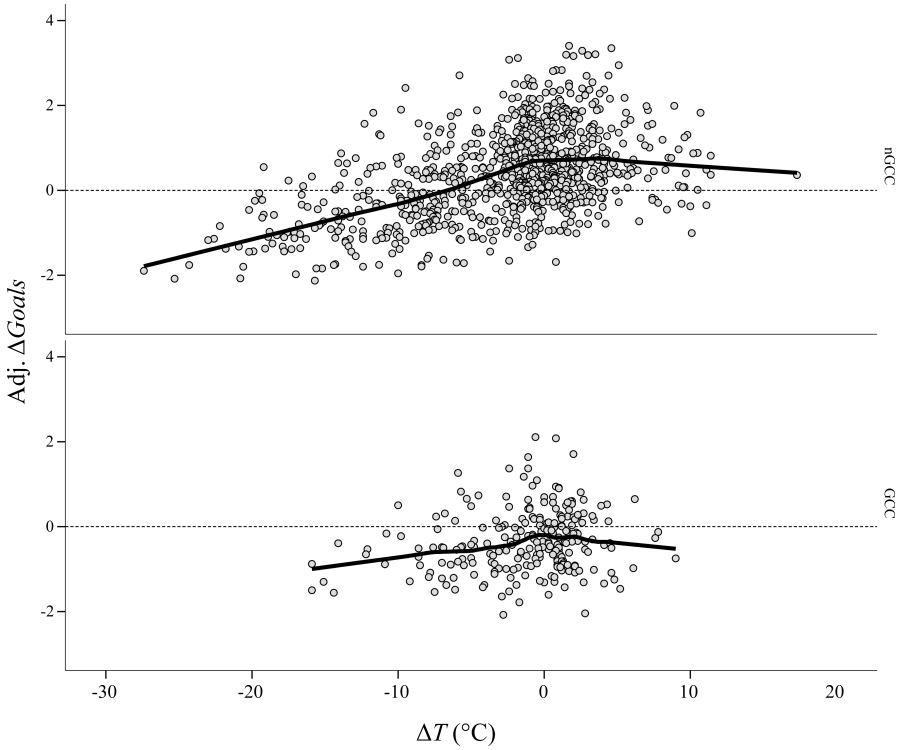

FIGURE 2—Effect of temperature difference (Δ*T*) on the distribution of adjusted goal difference (adj. Δgoals) for matches played vs GCC countries (low panel) and nGCC opponents (top panel).

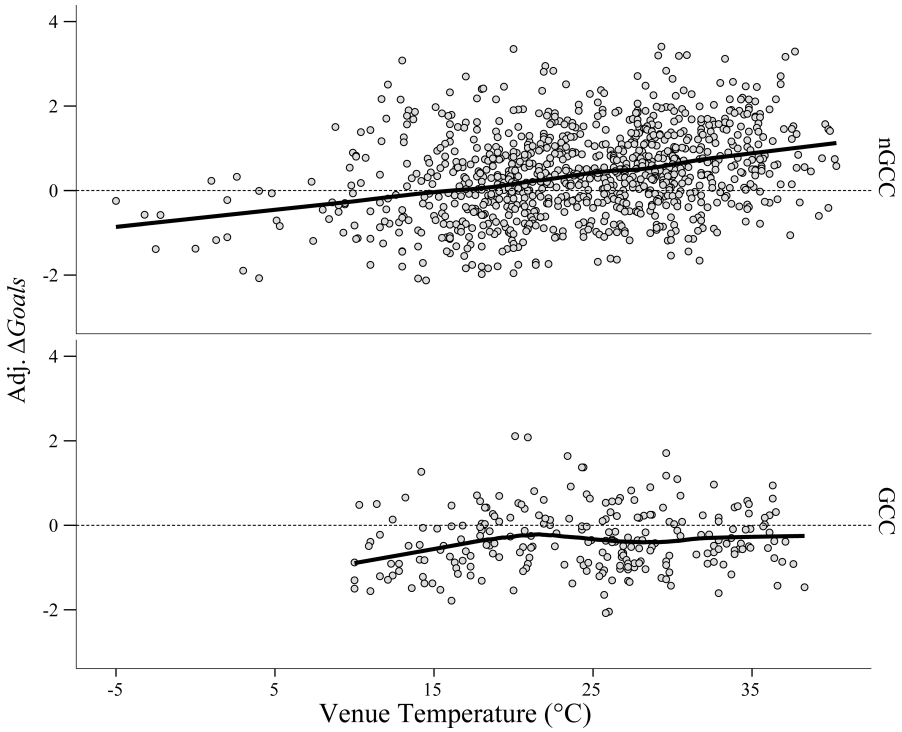

FIGURE 3—Effect of venue temperature on the distribution of adjusted goal difference (adj. Δgoals) for matches played vs GCC countries (low panel) and nGCC teams (top panel).

($P < 0.05$) influence favorable outcome and Δgoals against GCC opponents.

DISCUSSION

The purpose of this study was to examine the effects of heat and humidity on international football outcomes (i.e., results and scores). Our results confirm the existence of home advantage for the Gulf region countries. In addition, as hypothesized, we showed that the differences in heat stress conditions (temperature and humidity) between home and away teams significantly affected the outcome of international football matches in this region and are therefore an integral component of the home advantage. Hot countries' national football squads (i.e., GCC), likely better heat-acclimatized, tend to have greater likelihood of favorable outcome (+4%) (Table 1) and higher goal difference (β = 0.06) (Table 2) against equally ranked nGCC countries with every 1°C increase in the usual temperature. To our knowledge, this is the first time that weather conditions are shown as being influential of football results, whereas a home advantage has already been highlighted for altitude-acclimatized teams in the mountaineers region of South America (27). One interesting observation is that the parameter estimates on Δgoals increased when playing at a temperature of 20°C and higher. It was previously demonstrated that living and exercising in natural outdoor hot ambient conditions—e.g., desert region, characterized by an annual average dry air temperature of 20°C or more during the daylight—increased work capacity (9). Apart from the physiological heat adaptation (i.e., body temperature regulation), the main difference with nonacclimatized population includes other factors such as learning pacing strategies and psychological attributes (3).

When playing at cooler temperature, the advantage of GCC teams was considerably reduced, specifically against nGCC opposition. This would therefore suggest that the "heat advantage" in GCC countries is a specific phenomenon that is not necessarily transferable to cooler conditions. However, this assumption should be taken with caution because historical match data of GCC teams suggest that they had played in cooler condition mostly against better-ranked opponents. Conversely, the advantages of heat acclimatization are well established (2,18,21,34,38,41,46). An efficient heat acclimatization procedure usually leads to decreased core temperature, lower temperature threshold for sweating (21), plasma volume expansion, enhanced myocardial efficiency and improved cardiovascular adjustments (46), reduced oxygen uptake at a given power output, and muscle glycogen sparing (47). It is therefore reasonable to postulate that such exercise-heat acclimatization would have an ergogenic effect in football players' training and competing in hot environments (34,41). Nonetheless, it is still unclear if those benefits are also extendable to temperate conditions (11,41). Interestingly, Buchheit et al. (4) found that an in-season training camp in the heat (35°C) significantly expanded plasma volume and enhanced football-specific physical performance (approximately 7% in Yo-Yo intermittent recovery test level 1) in temperate conditions (22°C). However, because no control group was employed,

the observed improvements could possibly represent a "training effect".

During exercise in a hot dry environment, heat exchange is typically limited by high ambient and skin temperatures and a reduced thermal gradient for heat transfer, and the effectiveness of evaporation is primarily determined by relative humidity (19). Our results indicate that the relative humidity difference was of lower importance than temperature difference in affecting the matches' outcomes. Although differences in humidity have been factored into studies investigating the relationship between environmental conditions and competitive race performance in athletes (10,26), the specific influence of relative humidity on performance and on the physiological response to exercise remains largely unexplored. However, the available data would indicate that it is modest effect relative to effect of ambient temperature (10,17,24). High relative humidity increases sweat drippage, decreases sweat vaporization, and reduces heat loss through evaporation, which creates a physiological disadvantage (38) and results in severe dehydration. By clamping the relative humidity and increasing the dry bulb temperature, a recent study reported that intermittent sprint performance was impaired to the same extent in hot dry (40.2°C ± 0.2°C and 33.1% ± 4.9% of relative humidity) and hot humid (33.7°C ± 0.5°C and 78.2% ± 2.3%) conditions when compared with that in temperate (21.1°C ± 1.3°C and 48.6% ± 8.4%) condition (17). These findings corroborate our results, indicating the predominance of temperature rather than humidity in affecting the match outcome, except for teams already acclimatized where relative humidity appeared more sensitive.

However, it is well known that the amount of heat lost by the human body through evaporation is not dictated by relative humidity but by the difference in the partial pressure of water vapor between the skin and the ambient air (absolute humidity gradient). Hence, the physiological strain, and by extension performance, can be different for the same percentage of relative humidity at different ambient temperatures because of a different evaporative power. The results of this study confirm this point, as the estimates differed between relative and absolute humidity. Therefore, caution is required for the relative humidity interpretation in the present study because this variable was not independently associated with physiological strain. Recent studies demonstrating that skin temperature and not core temperature is the "primary factor" for performance decay (36,37) highlight the importance of considering other weather variables such as absolute humidity (saturated vapor pressure of water at a prescribed temperature and/or dew point temperature) or wind speed. This is especially crucial in warm/hot conditions because hypohydration (>2% of body mass loss) exacerbates the heat stress effect (20).

In an attempt to determine heat stress predictors, our results suggest that the best-fitted variables are the temperature and WBGT approximation, whereas humidity and HI have less importance. The WBGT is the commonly recommended measure to be used to reflect environmental heat stress, particularly during hot and humid days. However, the use of dry bulb temperature is easier than the approximation formula for WBGT. Moreover, there are other effects of weather such as wind speed and solar radiation, which were not evaluated here but could have affected match outcomes to some extent. For example, solar shortwave radiation has been significantly correlated (R ranging from 0.41 to 0.71) with the finishing time in marathon (43). This may lead to incorrect estimates of thermal stress, particularly in cloudy and windy conditions as well as during nighttime and early morning conditions when the sun is low or is below the horizon (14). However, intermittent activities such as football may reflect different responses to such weather factors.

Strengths and Limitations

The strength of this study is the novel approach of using a large football database containing results of 2008 matches played at multiple temperatures over a 55-yr period. Previous studies offering insight on the effects of heat on football have been restricted to small sample sizes (4,28). In addition, by analyzing football results for the entire GCC region, we reduced the influence of any specific country in the measurement of the relationship between match outcomes and environmental variables.

One weakness of the present study lies in the difficulty of controlling external factors—such as the tactics, crowd support, or travel effects—that can influence the outcome of football matches. Although the large data sample used in this study probably attenuated the influence of these factors, one cannot rule out that over a so long period (55 yr), other factors such as football FIFA "laws of the game" changes, (e.g., introduction of the yellow and red card system in the 1970s, revision of the offside law in the 1990s), climate (e.g., air pollution, global warming), or even geopolitics and socioeconomic changes (31) in the GCC region might also have influenced the outcome variables investigated in this study. However, this is beyond the scope of the present study.

Another point to be mentioned concerns the FIFA world ranking system. Since its introduction, the FIFA world ranking system has been continuously criticized and resulted in different revision of the calculation procedure. However, despite controversies and debates (22,42) on the efficiency of the FIFA ranking system and the proposition to use alternative systems (e.g., Elo rating system), the FIFA world ranking system is still considered the main benchmark. Our results suggest that the team ranking difference (Δrank) play an important role, which tends to disguise the environmental effect. Possible explanations could reside in the fact that (i) the away teams with better ranking (most likely nGCC teams) likely have better fitness and/or technical and tactical level than GCC teams, which could be useful to repeatedly execute accurate football skills without being acclimatized, and (ii) these away higher-ranked teams usually respect and follow recommendations and safety guidelines based on scientific evidence when traveling for a match to be played in hot

environments (3,14), which could be helpful when coping with heat (4,29,34). A detailed analysis of well-trained footballers' activity patterns during a football match in the heat underlined higher peak sprint velocity and technical quality (i.e., successful passes and crosses and ball possession) despite a lower total distance (−7%) and marked decline (−26%) in the amount of high-intensity runs (29).

Many other factors (e.g., crowd support, travel effects) were also recognized to influence the home advantage phenomenon in football (33) and might probably interact between them. However, the integration of a too high number of independent variables may lead to a too complex model with difficulty in interpretation. Inversely, excluding some important variables may result in misleading estimates of the effects on the dependent variables. Because the main purpose of this study was to investigate the potential influence of weather variables (mainly temperature and humidity) on the football results and scores, using the home advantage phenomenon, we therefore selected the main variables of interest. Moreover, to date and to the best of our knowledge, neither any independent factor was demonstrated to be more important than the other when investigating home advantage and neither any interaction between independent variables was highlighted.

It is also important to recognize that virtually all GCC players play in the GCC leagues during their entire career and most of these players have African roots (e.g., Sudan, Nigeria), which may confer an advantage over Caucasian players. Hence, Caucasian players have been described as having higher body dimension and composition, which may negatively influence their ability to play in hot conditions because of increased heat production (8,23). However, this assumption awaits scientific evidence.

CONCLUSIONS

Although it is known that heat acclimatization reduces the likelihood of heat illness and partly restores performance when exercising in hot environments, the direct link of heat stress (temperature and humidity) on football match outcomes has not been previously quantified. Our findings demonstrate that GCC football teams are at increased advantage when matches are played at higher temperature than usual and, to a lower extent, at higher humidity. However, the higher level of play in non–heat-acclimatized teams partly compensates for the heat-induced detrimental effects on the match outcomes when playing in hot conditions. Despite a ranking impact, the results of this study strengthen the effect of previous field-based studies highlighting the effect of weather variables (7,10,43) and the importance of heat acclimatization (3,14,34).

Nevertheless, some challenging questions need to be addressed with comprehensive new sport-specific research. First, more investigations are requested regarding the influence of the different weather factors, which may differ regarding the sport-specific performance (e.g., football vs marathon). Second, conductive heat exchange could be modulated by different running speed and anthropometrical determinants of exercise thermoregulation (i.e., body mass, surface area-to-body mass ratio). Future statistical analyses might include such data to better quantify their effects on heat stress. Finally, the development of a specific model using weather and biometeorological variables could be used for further predictions to evaluate expected performance variations with changing weather conditions. This may have a significant effect on forecasting performance, which may be of particular importance when scheduling training and competition in a hostile environment.

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Chapter 7

Article 2 – Running mechanical alterations during repeated treadmill sprints in hot versus hypoxic environments. A pilot study

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Running mechanical alterations during repeated treadmill sprints in hot *versus* hypoxic environments. A pilot study

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ABSTRACT

We determined if performance and mechanical running alterations during repeated treadmill sprinting differ between severely hot and hypoxic environments. Six male recreational sportsmen (team- and racket-sport background) performed five 5-s sprints with 25-s recovery on an instrumented treadmill, allowing the continuous (step-by-step) measurement of running kinetics/kinematics and spring-mass characteristics. These were randomly conducted in control (CON; 25°C/45% RH, inspired fraction of oxygen = 20.9%), hot (HOT; 38°C/21% RH, inspired fraction of oxygen = 20.9%; end-exercise core temperature: ~38.6°C) and normobaric hypoxic (HYP, 25°C/45% RH, inspired fraction of oxygen = 13.3%/simulated altitude of ~3600 m; end-exercise pulse oxygen saturation: ~84%) environments. Running distance was lower ($P < 0.05$) in HOT compared to CON and HYP for the first sprint but larger ($P < 0.05$) sprint decrement score occurred in HYP *versus* HOT and CON. Compared to CON, the cumulated distance covered over the five sprints was lower ($P < 0.01$) in HYP but not in HOT. Irrespective of the environmental condition, significant changes occurred from the first to the fifth sprint repetitions (all three conditions compounded) in selected running kinetics (mean horizontal forces, $P < 0.01$) or kinematics (contact and swing times, both $P < 0.001$; step frequency, $P < 0.001$) and spring-mass characteristics (vertical stiffness, $P < 0.001$; leg stiffness, $P < 0.01$). No significant interaction between sprint number and condition was found for any mechanical data. Preliminary evidence indicates that repeated-sprint ability is more impaired in hypoxia than in a hot environment, when compared to a control condition. However, as sprints are repeated, mechanical alterations appear not to be exacerbated in severe (heat, hypoxia) environmental conditions.

ARTICLE HISTORY
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KEYWORDS
Heat; hypoxia; repeated-sprint ability; sprinting mechanics

Introduction

Many sporting events are organized in hot environments (e.g., 2016 Olympic Games in Brazil) or at altitude (e.g., 2010 FIFA World Cup in South Africa), so the understanding of the impact of heat stress and hypoxia on in-competition physical performance is paramount. In team sports, both hot ambient conditions (i.e., environmental temperature >30°C) (Mohr & Krustrup, 2013; Mohr, Nybo, Grantham, & Racinais, 2012) and hypoxia (i.e., altitude >1500 m) (Garvican, Hammond, Varley, & Gore, 2014; Nassis, 2013) negatively alter match activity patterns. Reportedly, exertional heat stress elicits substantial decrements upon the completion of football-specific activities (i.e., jumps) (Mohr & Krustrup, 2013) and leads to pacing strategies (i.e., reduced amount of low-intensity running to preserve high-intensity actions) (Aughey, Goodman, & McKenna, 2014) due to increasing body temperatures, which would be pivotal to the outcome of a game. Likewise, with altitude ascent above sea level, lowered oxygen delivery to active tissues compromises aerobic capacity and inhibits recovery from high-intensity intermittent activity, thereby reducing the ability to sprint to the ball within a game (Aughey et al., 2013; Garvican et al., 2014).

Although there are compelling evidences to suggest that earlier and larger performance decrements occur when consecutive sprints (i.e., repeated-sprint ability) are undertaken with elevated heat stress (Drust, Rasmussen, Mohr, Nielsen, & Nybo, 2005; Girard, Brocherie, & Bishop, 2015) or limited oxygen availability (Billaut & Buchheit, 2013; Smith & Billaut, 2010), no direct (i.e., same participants) comparison exists for the effects of heat and hypoxia on repeated-sprint ability. Furthermore, while previous studies have mainly used cycle ergometry, the recent demonstration of exacerbated alterations in sprint capacity and resulting neuromuscular fatigue levels following cycling *versus* running repeated-sprint ability call into question the relevance of these findings in team sports (Rampinini et al., in press). The recent validation of an instrumented sprint treadmill (Morin, Samozino, Bonnefoy, Edouard, & Belli, 2010) made it possible to assess the instantaneous changes in both running velocity and ground reaction forces during maximal sprints similar to game play. For instance, Morin, Samozino, Edouard, and Tomazin (2011b) reported significant decrease

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in force production capacity and even larger deteriorations in the ability to apply forces horizontally during acceleration during repeated 6-s sprints. However, these observations have been made in cool/normoxic conditions.

The aim of the present study was to compare the performance changes and the accompanying alterations in running mechanics over a series of treadmill sprints performed in severely hot and hypoxic environments. While it was anticipated that, similar to control (e.g., cool, normoxic) condition (Morin et al., 2011b), the ability to apply/orient force would be more deteriorated than the total force production, we further hypothesised that heat stress and hypoxia would progressively exacerbate the magnitude of these alterations.

Methods

Participants

Six male recreational team- (football, rugby, basketball) or racket- (tennis, squash) sport players (34.0 ± 4.0 years; 178.1 ± 7.4 cm; 75.9 ± 9.7 kg; 2–4 h physical activity per week) participated in the study. Although residing in Qatar, the participants (all foreigners) were not accustomed to sprinting in the heat, as the study was conducted in the winter. They were all born and raised at <1500 m and had not travelled to elevations >1000 m in the 3 months prior to investigation. They gave their informed, written consent prior to the commencement of the experiment. Experimental protocol was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of *Shafallah Medical Genetics Center*.

Experimental procedure

About 1 week prior to the first experimental session, participants undertook a familiarisation session consisting of short (<5 s) treadmill sprints at increasing intensities, with full recovery between each sprint. Sprints were repeated until participants felt comfortable with their running technique (i.e., 7–10 trials were generally needed). Afterwards, they performed three maximal 5-s single sprints separated by 2 min of passive rest, and the complete repeated-sprint ability test after 10 min of passive rest.

On three occasions, participants performed (in a counterbalanced randomised crossover design), at the same time of day (±1 h) and 4–5 days apart, five 5-s treadmill sprints with 25-s recovery. The trials were conducted in control (CON; 25°C/45% RH, inspired fraction of oxygen = 20.9%), hot (HOT; 38°C/21% RH, inspired fraction of oxygen = 20.9%) and normobaric hypoxic (HYP, 25°C/45% RH, inspired fraction of oxygen = 13.3%/simulated altitude of ~3600 m; Altitrainer, SMTEC SA, Nyon, Switzerland) environments with participants wearing similar sports gear (running shoes, short and T-shirt). These environmental conditions were chosen to reflect the extremes of heat (i.e., ambient temperature exceeding 35°C; Middle Eastern and Equatorial regions) and hypoxia (i.e., simulated altitude above 3000 m; mountainous regions of Pacific Latin America) that players may encounter during their practice. Our experimental conditions match field observations of football activity [i.e., decreases in total distance covered and at high-intensity (>14–15 km · h^{-1}) of ~7–8% and ~23–26%, respectively, compared to control] in either hot (43°C/12% RH) (Mohr et al., 2012) or hypoxic (natural altitude of 3600 m) (Aughey et al., 2013) conditions. Strong verbal encouragement was given during all maximal efforts. Participants were asked to avoid vigorous exercise and alcohol for 24 h, caffeine for 12 h and food for 2 h before all trials. They also kept a 24 h food diary prior to testing and replicated this diet before the three trials. During the period of testing, they were instructed to maintain their normal sleeping habits (>7 h/night) and normal diet (avoiding nutritional supplements). Participants were instructed to drink 4–6 mL of water per kilogram of body mass every 2.5 h on the day before each experimental session to ensure euhydration at the start of exercise; this has resulted in urine specific gravity (Pal-10-S, Vitech Scientific, West Sussex, UK) values of <1.015 g/mL before all trials. They were permitted to drink *ad libitum* during the warm-up procedure.

The repeated-sprint ability test was preceded by a warm-up consisting of 10 min of running at 10 km · h^{-1}, followed by 15 min of sprint-specific muscular warm-up exercises [i.e., 3 × (high knee, high heels, butt-kick, skipping for ~10 s with 30-s walking in between), followed by 3 × (three steps accelerations at a subjective "sense of effort" of 7, 8 and 9 on a modified Borg CR10 scale) (Christian, Bishop, Billaut, & Girard, 2014), then by 2 × (3-s sprints at a subjective "sense of effort" of 8 and 9 on the modified Borg CR10 scale], and finally three maximal 5-s sprints separated by 2 min of passive rest. In order to prevent any pacing strategy, the best sprint was used as the criterion score. The participants had to achieve at least 95% of the best trial during the first sprint of the repeated-sprint ability test, which was fulfilled in all of the three testing sessions. Participants were then allowed 5 min of free cool down prior to the repeated-sprint ability test. Duration of each trial (i.e., from the beginning of the warm-up until the end of the repeated-sprint ability test) was ~45 min.

Instrumented sprint treadmill

The sprints were performed on an instrumented motorised treadmill (ADAL3D-WR, Medical Development – HEF Tecmachine, Andrézieux-Bouthéon, France). For a detailed description of this device, see Morin et al. (2010); Morin, Edouard, and Samozino (2011a). Briefly, it is mounted on a highly rigid metal frame fixed to the ground through four piezoelectric force transducers (KI 9077b; Kistler, Winterthur, Switzerland) and installed on a specially engineered concrete slab to ensure maximal rigidity of the supporting ground. This motorised treadmill allows participants to sprint and produce realistic acceleration and high running velocities (Morin et al., 2011a). A single-pass waist and a stiff rope (1 cm in diameter, ~2 m length) were used to tether participants to the 0.4-m vertical rail anchored to the wall behind them. When correctly attached, they were required to lean forward in a typical and standardised crouched sprint-start position with their left foot forward. Repeated-

sprint ability was assessed from covered distance data using three scores: the largest (i.e., initial in all cases) distance ran, the cumulated distance covered over the five sprints (i.e., sum of the five sprints) and the sprint decrement score (Glaister, Howatson, Pattison, & McInnes, 2008).

Mechanical variables

Data were continuously sampled at 1000 Hz over the sprints, and after appropriate filtering (Butterworth-type 30 Hz low-pass filter; Adirun, Tecmachine, Andrézieux-Bouthéon, France), instantaneous data of vertical, net horizontal and total (i.e., resultant) ground reaction forces were averaged for each support phase (vertical force above 30 N) over the 5-s sprints, and expressed in body weight (BW). The index of force application technique representing the decrement in ratio of forces (ratio of forces = horizontal forces/total forces) with the increasing belt velocity ($m \cdot s^{-1}$) was computed as the slope of the linear ratio of forces-running velocity relationship calculated from the step-averaged values between the second step and the step at top running velocity (Morin et al., 2011a). These data were completed by measurements of the main step kinematic variables: contact time (s), aerial time (s), swing time (s), step frequency (Hz) and step length (m). Lastly, for each 5-s sprint, horizontal forces were used with the corresponding average belt velocity to compute net power output in the horizontal direction (propulsive power = horizontal forces × running velocity, $W \cdot kg^{-1}$). Each sprint trial included 15–18 ground contacts. After excluding the last two ground contacts, the remaining last three consecutive steps were used for the final analysis of sprint kinetics/kinematics (Brocherie, Millet, & Girard, 2015).

A linear spring-mass model of running (Butler, Crowell, & Davis, 2003; Coleman, Cannavan, Horne, & Blazevich, 2012; Morin, Dalleau, Kyrölainen, Jeannin, & Belli, 2005), applied in previous interventional [variations in running velocity (Arampatzis, Brüggemann, & Metzler, 1999) or repeated-sprint ability-fatigue (6 × 35 m with 10 s of active recovery: Brocherie et al., 2015; 12 × 40 m with 30 s of passive rest: Girard, Micallef, & Millet, 2011)] and reliability (Pappas, Paradisis, Tsolakis, Smirniotou, & Morin, 2014) studies, was used to investigate the main mechanical integrative parameters characterising the lower limb behaviour during running. Vertical stiffness ($kN \cdot m^{-1}$) was calculated as the ratio of peak vertical forces (N) to the maximal vertical downward displacement of centre of mass (m), which was determined by double integration of vertical acceleration of centre of mass over time during ground contact. Leg stiffness ($kN \cdot m^{-1}$) was calculated as the ratio of peak vertical forces to the maximum leg spring compression [maximal vertical downward displacement + $L_0 - \sqrt{L_0^2 - (0.5 \times \text{running velocity} \times \text{contact time})^2}$, m], both occurring at mid-stance. Initial leg length (L_0, great trochanter to ground distance in a standing position) was determined from participant's stature as $L_0 = 0.53 \times \text{stature}$ (Morin et al., 2005).

Responses to exercise

Heart rate (all conditions), ratings of perceived exertion (all conditions) and pulse oxygen saturation (CON and HYP trials only) were monitored exactly 10 s following each sprint, respectively, via a wireless Polar monitoring system (Polar Electro Oy, Kempele, Finland), the Borg 6–20 scale and fingertip oximeter (Palmsat 2500, NONIN Medical Inc., Plymouth, MI, USA). Upon arrival on testing days (CON and HOT trials only), the telemetric temperature pill for monitoring core temperature (VitalSense®, Mini Mitter, Respironics, Herrsching, Germany) was inserted at the length distance of a gloved index finger beyond the anal sphincter. Skin temperatures of the chest, upper arm, thigh and lower leg were monitored via temperature data loggers (iButtons, Maxim Integrated, USA) and were used to calculate the mean skin temperature, using the equation of Ramanathan (1964).

Statistical analysis

Values are expressed as mean ± sd. Two-way repeated-measures analysis of variance (ANOVAs) [Time (Sprint number 1, 2, 3, 4 and 5) × Condition (CON, HOT and HYP)] was used to compare physiological/perceptual, running performance and mechanical responses. To assess assumptions of variance, Mauchly's test of sphericity was performed using all ANOVA results. A Greenhouse–Geisser correction was performed to adjust the degree of freedom if an assumption was violated, while a Bonferroni post hoc multiple comparison was performed if a significant main effect was observed. For each ANOVA, partial eta-squared was calculated as measures of effect size. Values of 0.01, 0.06 and values above 0.14 were considered as small, medium and large, respectively. All statistical calculations were performed using SPSS statistical software V.21.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $P < 0.05$.

Results

Repeated-sprint ability

Distance ran during the first 5-s sprint was lower ($P < 0.05$) in HOT compared to CON and HYP (Table 1) but a larger ($P < 0.05$) sprint decrement score (7.9 ± 3.0% *vs*. 2.4 ± 3.4% and 3.1 ± 3.9%) occurred in HYP *versus* HOT and CON. Compared to CON (116.5 ± 6.3 m), the cumulated distance covered over the five sprints was shorter ($P < 0.01$) in HYP (110.5 ± 6.6 m) but not in HOT (112.1 ± 10.1 m), with no difference between HOT and HYP.

Responses to exercise

Heart rate (CON: 146 ± 14 bpm *vs*. 168 ± 10 bpm; HOT: 150 ± 11 bpm *vs*. 171 ± 8 bpm; HYP: 148 ± 23 bpm *vs*. 169 ± 15 bpm) and ratings of perceived exertion (CON: 12.3 ± 0.8 *vs*. 16.0 ± 1.6; HOT: 12.9 ± 0.2 *vs*. 17.1 ± 1.6; HYP: 12.0 ± 0.9 *vs*. 16.3 ± 1.3) increased from the first to the fifth repetitions ($P < 0.001$), irrespective of the environmental conditions, yet with higher ($P < 0.05$) ratings of

Table 1. Changes in performance, force production and application technique variables during the repeated-sprint ability test in control (CON), hot (HOT) and hypoxic (HYP) conditions.

		Sprint number							
	Condition	1	2	3	4	5	1–5 sprints average	1–5 sprints changes (%)	ANOVA main effects (Effect Sizes)
Distance covered (m)	**CON**	24.10 ± 1.97	23.58 ± 1.15	23.32 ± 1.05	22.93 ± 1.31*	22.53 ± 1.22*	**23.29 ± 1.26**	**−6.2 ± 4.9**	T < 0.001 (0.66)
	HOT	22.97 ± 1.97	22.45 ± 1.91	22.59 ± 2.36	22.42 ± 2.30*	21.62 ± 2.12*	**22.41 ± 2.02**	**−5.7 ± 7.2**	C = 0.088 (0.38)
	HYP	24.05 ± 2.15	22.03 ± 1.13	21.84 ± 0.83	21.35 ± 1.70*	21.27 ± 1.26*	**22.11 ± 1.33**	**−11.3 ± 4.6**	I = 0.004 (0.41)
Mean velocity ($m \cdot s^{-1}$)	**CON**	6.42 ± 0.34	6.20 ± 0.19	6.11 ± 0.23	6.02 ± 0.19*	5.91 ± 0.21*	**6.13 ± 0.19**	**−7.7 ± 5.2**	T < 0.001 (0.72)
	HOT	6.25 ± 0.51	6.08 ± 0.40	5.95 ± 0.40	5.84 ± 0.49*	5.68 ± 0.50*	**5.96 ± 0.42**	**−8.8 ± 8.0**	C = 0.176 (0.29)
	HYP	6.28 ± 0.34	5.93 ± 0.24	5.83 ± 0.23	5.71 ± 0.31*	5.63 ± 0.23*	**5.87 ± 0.24**	**−10.1 ± 4.6**	I = 0.339 (0.19)
Average vertical forces (Body weight)	**CON**	1.68 ± 0.34	1.67 ± 0.19	1.64 ± 0.23	1.67 ± 0.19	1.66 ± 0.21	**1.66 ± 0.06**	**−1.4 ± 5.2**	T < 0.252 (0.23)
	HOT	1.62 ± 0.34	1.63 ± 0.19	1.60 ± 0.23	1.58 ± 0.19	1.60 ± 0.21	**1.61 ± 0.06**	**−1.7 ± 8.0**	C = 0.242 (0.26)
	HYP	1.69 ± 0.34	1.65 ± 0.19	1.68 ± 0.23	1.67 ± 0.19	1.66 ± 0.21	**1.67 ± 0.10**	**−1.6 ± 4.6**	I = 0.416 (0.17)
Average horizontal forces (Body weight)	**CON**	0.22 ± 0.05	0.22 ± 0.05	0.20 ± 0.04	0.20 ± 0.04	0.20 ± 0.04*	**0.21 ± 0.04**	**−6.0 ± 12.2**	T < 0.006 (0.50)
	HOT	0.23 ± 0.03	0.23 ± 0.03	0.22 ± 0.03	0.20 ± 0.03	0.21 ± 0.04*	**0.22 ± 0.03**	**−9.7 ± 12.7**	C = 0.064 (0.42)
	HYP	0.20 ± 0.05	0.20 ± 0.06	0.19 ± 0.05	0.19 ± 0.05	0.18 ± 0.05*	**0.19 ± 0.05**	**−9.3 ± 17.0**	I = 0.867 (0.08)
Average total forces (Body weight)	**CON**	1.70 ± 0.07	1.68 ± 0.07	1.65 ± 0.05	1.68 ± 0.05	1.67 ± 0.07	**1.68 ± 0.06**	**−1.6 ± 3.7**	T < 0.171 (0.26)
	HOT	1.64 ± 0.06	1.64 ± 0.07	1.62 ± 0.09	1.59 ± 0.07	1.61 ± 0.07	**1.62 ± 0.06**	**−1.9 ± 3.5**	C = 0.247 (0.25)
	HYP	1.71 ± 0.12	1.67 ± 0.09	1.69 ± 0.10	1.68 ± 0.09	1.67 ± 0.10	**1.68 ± 0.10**	**−1.7 ± 3.8**	I = 0.352 (0.19)
Propulsive power ($W \cdot kg^{-1}$)	**CON**	13.77 ± 2.95	13.02 ± 2.41	11.71 ± 2.26	11.96 ± 2.43*	11.77 ± 2.24*	**12.45 ± 2.32**	**−13.2 ± 12.5**	T < 0.001 (0.68)
	HOT	14.05 ± 1.40	13.42 ± 0.99	12.51 ± 1.26	11.51 ± 1.57*	11.64 ± 2.72*	**12.63 ± 1.33**	**−17.0 ± 17.9**	C = 0.051 (0.45)
	HYP	11.22 ± 2.28	11.40 ± 3.05	10.75 ± 2.65	10.42 ± 2.76*	9.96 ± 2.26*	**10.95 ± 2.52**	**−18.4 ± 15.7**	I = 0.687 (0.68)
Index of force application technique	**CON**	−0.065 ± 0.008	−0.069 ± 0.011	−0.075 ± 0.009	−0.076 ± 0.013	−0.076 ± 0.008*	**−0.072 ± 0.009**	**17.1 ± 10.7**	T < 0.012 (0.60)
	HOT	−0.066 ± 0.010	−0.064 ± 0.011	−0.069 ± 0.012	−0.076 ± 0.011	−0.084 ± 0.025*	**−0.072 ± 0.013**	**25.6 ± 26.8**	C = 0.984 (0.03)
	HYP	−0.071 ± 0.011	−0.067 ± 0.020	−0.075 ± 0.019	−0.070 ± 0.013	−0.076 ± 0.023*	**−0.072 ± 0.016**	**6.6 ± 20.7**	I = 0.319 (0.19)

Notes: Values are mean ± SD. T, C, I – time, condition and interaction effects, respectively. *significantly different from sprint number 1 ($P < 0.05$).

perceived exertion values for the average of five sprints in the HOT trial (15.1 ± 1.2) compared to HYP (14.1 ± 1.2) and CON (14.1 ± 1.3). No statistically significant differences were found for the average of five sprints for core temperature between HOT and CON (38.57 ± 0.30°C *vs*. 38.50 ± 0.31°C), whereas skin temperature was elevated in the heat (37.33 ± 1.05°C *vs*. 33.26 ± 1.01°C; $P < 0.001$). Arterial oxygen saturation values decreased ($P < 0.001$) from the first to the last sprint in the HYP trial (88.8 ± 1.8 *vs*. 83.8 ± 4.8%), whereas it did not change in CON (97.3 ± 0.8 *vs*. 97.2 ± 1.7%).

Mechanical variables

Irrespective of the environmental condition, significant changes occurred from the first to the fifth repetitions in selected running kinetics (horizontal forces: −8.3 ± 10.3%, $P < 0.01$; Table 1) or kinematics (contact time and swing time: +12.2 ± 5.4% and +4.7 ± 4.4%, both $P < 0.001$; step frequency: −8.1 ± 3.2%, $P < 0.001$; Table 2) and spring-mass characteristics (peak vertical forces: −2.6 ± 2.4%, $P < 0.05$; maximal vertical downward displacement: +19.5 ± 8.3%, $P < 0.001$; leg compression: +7.3 ± 7.5%, $P < 0.05$; vertical stiffness: −17.8 ± 8.3%, $P < 0.001$; leg stiffness: −8.3 ± 5.8%, $P < 0.01$; Table 3). No significant interaction between time and conditions was found for any mechanical parameter.

Discussion

Repeated-sprint ability performance

Although exacerbated impairments in sprinting capacity in hot (Drust et al., 2005; Girard, Brocherie, et al., 2015) or hypoxic (Billaut & Buchheit, 2013; Smith & Billaut, 2010) *versus* cool or normoxic conditions have already been documented, sprint duration, type of recovery, number of sprint repetitions and training status of the participants all varied, which complicates comparisons of the extent of environmental-mediated fatigue-induced decrements in repeated-sprint ability between conditions. To our knowledge, the present study is

Table 2. Changes in running kinematics during the repeated-sprint ability test in control (CON), hot (HOT) and hypoxic (HYP) conditions.

		Sprint number							
	Condition	1	2	3	4	5	1–5 sprints average	1–5 sprints changes (%)	ANOVA main effects (Effect Sizes)
Contact time (s)	**CON**	0.141 ± 0.007	0.149 ± 0.007	0.156 ± 0.006*	0.154 ± 0.007*	0.157 ± 0.006*	**0.151 ± 0.005**	**11.6 ± 5.5**	T < 0.001 (0.83)
	HOT	0.147 ± 0.002	0.150 ± 0.005	0.156 ± 0.007*	0.161 ± 0.008*	0.167 ± 0.009*	**0.156 ± 0.005**	**13.2 ± 7.3**	C = 0.459 (0.12)
	HYP	0.145 ± 0.014	0.151 ± 0.016	0.157 ± 0.012*	0.160 ± 0.013*	0.161 ± 0.008*	**0.155 ± 0.013**	**11.7 ± 5.5**	I = 0.066 (0.29)
Aerial time (s)	**CON**	0.094 ± 0.016	0.095 ± 0.012	0.094 ± 0.008	0.096 ± 0.011	0.095 ± 0.010	**0.095 ± 0.011**	**2.2 ± 9.5**	T = 0.106 (0.30)
	HOT	0.087 ± 0.008	0.092 ± 0.012	0.091 ± 0.011	0.092 ± 0.009	0.093 ± 0.011	**0.091 ± 0.010**	**7.5 ± 6.5**	C = 0.477 (0.14)
	HYP	0.093 ± 0.010	0.095 ± 0.011	0.097 ± 0.009	0.097 ± 0.007	0.097 ± 0.010	**0.096 ± 0.009**	**4.3 ± 7.4**	I = 0.685 (0.12)
Swing time (s)	**CON**	0.324 ± 0.026	0.337 ± 0.026	0.345 ± 0.017*	0.343 ± 0.024*	0.347 ± 0.023*	**0.339 ± 0.022**	**7.2 ± 3.1**	T < 0.001 (0.79)
	HOT	0.319 ± 0.016	0.326 ± 0.030	0.337 ± 0.023*	0.340 ± 0.016*	0.350 ± 0.022*	**0.334 ± 0.022**	**9.9 ± 5.8**	C = 0.145 (0.32)
	HYP	0.329 ± 0.026	0.342 ± 0.030	0.350 ± 0.024*	0.354 ± 0.025*	0.354 ± 0.022*	**0.346 ± 0.025**	**7.8 ± 4.1**	I = 0.546 (0.15)
Step frequency (Hz)	**CON**	4.28 ± 0.29	4.12 ± 0.27*	4.02 ± 0.21*	4.03 ± 0.27*	3.98 ± 0.23*	**4.09 ± 0.25**	**−6.7 ± 3.9**	T < 0.001 (0.82)
	HOT	4.29 ± 0.17	4.15 ± 0.23*	4.05 ± 0.14*	3.97 ± 0.14*	3.86 ± 0.14*	**4.06 ± 0.15**	**−9.9 ± 3.3**	C = 0.457 (0.15)
	HYP	4.24 ± 0.32	4.10 ± 0.40*	3.96 ± 0.26*	3.92 ± 0.24*	3.90 ± 0.17*	**4.02 ± 0.27**	**−7.7 ± 4.5**	I = 0.206 (0.22)
Step length (m)	**CON**	1.54 ± 0.18	1.53 ± 0.13	1.53 ± 0.10	1.51 ± 0.13	1.50 ± 0.10	**1.53 ± 0.13**	**−2.2 ± 6.7**	T < 0.623 (0.12)
	HOT	1.48 ± 0.15	1.50 ± 0.17	1.50 ± 0.15	1.48 ± 0.21	1.48 ± 0.16	**1.49 ± 0.16**	**−0.3 ± 6.3**	C = 0.257 (0.25)
	HYP	1.53 ± 0.17	1.47 ± 0.16	1.48 ± 0.11	1.47 ± 0.12	1.46 ± 0.10	**1.48 ± 0.13**	**−4.0 ± 8.8**	I = 0.370 (0.18)

Notes: Values are mean ± SD. T, C, I – time, condition and interaction effects, respectively. *significantly different from sprint number 1 ($P < 0.05$).

Table 3. Changes in spring-mass characteristics during the repeated-sprint ability test in control (CON), hot (HOT) and hypoxic (HYP) conditions.

		Sprint number							
	Condition	1	2	3	4	5	1–5 sprints average	1–5 sprints changes (%)	ANOVA main effects (Effect Sizes)
Peak vertical forces (N)	**CON**	1973 ± 388	1922 ± 297	1879 ± 244	1904 ± 280	1889 ± 267*	**1914 ± 295**	**−3.5 ± 4.9**	T = 0.023 (0.42)
	HOT	1885 ± 281	1915 ± 306	1881 ± 297	1864 ± 257	1856 ± 307*	**1880 ± 288**	**−1.6 ± 3.6**	C = 0.798 (0.05)
	HYP	1919 ± 272	1903 ± 249	1887 ± 225	1870 ± 222	1865 ± 233*	**1889 ± 239**	**−2.6 ± 2.5**	I = 0.336 (0.19)
Centre of mass vertical displacement (m)	**CON**	0.027 ± 0.004	0.030 ± 0.004	0.031 ± 0.003*	0.031 ± 0.004*	0.032 ± 0.004*	**0.030 ± 0.003**	**+16.2 ± 9.1**	T < 0.001 (0.85)
	HOT	0.027 ± 0.002	0.029 ± 0.003	0.030 ± 0.002*	0.032 ± 0.002*	0.033 ± 0.003*	**0.030 ± 0.002**	**+23.5 ± 9.4**	C = 0.255 (0.24)
	HYP	0.028 ± 0.004	0.030 ± 0.005	0.032 ± 0.004*	0.033 ± 0.004*	0.033 ± 0.003*	**0.031 ± 0.004**	**+18.8 ± 11.6**	I = 0.253 (0.21)
Leg compression (m)	**CON**	0.144 ± 0.019	0.152 ± 0.020	0.162 ± 0.018	0.154 ± 0.017	0.154 ± 0.014*	**0.153 ± 0.016**	**+8.1 ± 10.2**	T = 0.034 (0.39)
	HOT	0.148 ± 0.024	0.149 ± 0.022	0.154 ± 0.019	0.158 ± 0.028	0.160 ± 0.018*	**0.154 ± 0.021**	**+9.2 ± 8.4**	C = 0.511 (0.13)
	HYP	0.146 ± 0.025	0.145 ± 0.029	0.152 ± 0.022	0.152 ± 0.022	0.150 ± 0.014*	**0.149 ± 0.022**	**+4.4 ± 11.4**	I = 0.439 (0.17)
Vertical stiffness ($kN \cdot m^{-1}$)	**CON**	72.4 ± 9.5	65.5 ± 7.8	60.7 ± 5.7*	62.1 ± 7.1*	60.1 ± 5.8*	**64.2 ± 6.9**	**−16.4 ± 6.4**	T < 0.001 (0.83)
	HOT	69.4 ± 7.3	65.9 ± 6.4	61.9 ± 6.4*	58.8 ± 5.2*	55.4 ± 5.8*	**62.3 ± 5.7**	**−19.9 ± 7.1**	C = 0.477 (0.14)
	HYP	69.4 ± 11.4	64.5 ± 11.9	59.3 ± 6.9*	57.8 ± 7.4*	56.8 ± 5.0*	**61.6 ± 8.2**	**−17.1 ± 8.1**	I = 0.160 (0.24)
Leg stiffness ($kN \cdot m^{-1}$)	**CON**	13.9 ± 2.7	12.8 ± 1.7	11.7 ± 0.6	12.5 ± 1.1*	12.3 ± 1.0*	**12.7 ± 1.3**	**−9.9 ± 10.1**	T = 0.007 (0.49)
	HOT	12.8 ± 1.2	13.1 ± 1.6	12.3 ± 1.5	11.9 ± 1.3*	11.6 ± 1.4*	**12.3 ± 1.1**	**−9.4 ± 8.2**	C = 0.655 (0.08)
	HYP	13.4 ± 2.5	13.5 ± 2.7	12.6 ± 1.5	12.5 ± 1.5*	12.5 ± 1.3*	**12.9 ± 1.8**	**−5.7 ± 9.4**	I = 0.408 (0.18)

Notes: Values are mean ± SD. T, C, I – time, condition and interaction effects, respectively. *significantly different from sprint number 1 ($P < 0.05$).

the first one where the same participants performed the same repeated-sprint ability test in control, hot and hypoxic conditions. Our data support that repeated-sprint ability is further compromised in HYP and to a lower extent in HOT (not statistically different), when directly compared to CON. Nevertheless, the "task-dependency" of the responses implies that heat stress and hypoxia could not be considered as "generic phenomena". Hence, the type and the severity of each environmental stressor may well determine to which extent fatigue increases during each repeated-sprint ability test. For instance, large performance decrements solely occur in hotter conditions when consecutive sprints induce marked hyperthermia (core temperature >38.5°C) (Girard, Brocherie, et al., 2015). Compared to hot-dry environments, the ability of the body to extract heat through sweating is impaired in hot-humid conditions because sweat cannot readily evaporate off the body (Sawka, Leon, Montain, & Sonna, 2011), which will lead to greater hyperthermia and physiological strain and eventually larger impairment in repeated-sprint ability outcomes. Along the same lines, fatigue development during cycling repeated-sprint ability was exacerbated only under severe (inspired fraction of oxygen = 12–14%), but not moderate (inspired fraction of oxygen = 14–16%) hypoxic levels, when compared to normoxia (Bowtell, Cooke, Turner, Mileva, & Sumners, 2014; Goods, Dawson, Landers, Gore, & Peeling, 2014). As such, caution is needed when extrapolating our findings.

Physiological and perceptual responses

Larger repeated-sprint ability deterioration in HYP may relate to the decrease in convective factors of oxygen transport that occurred during the sprints, leading to a lower oxygen supply, as evidenced by the substantial hypoxemia level, with oxygenation saturation values below 85% at the end of exercise. Postulated mechanisms include a lower muscle reoxygenation capacity during recovery periods (Billaut & Buchheit, 2013) and/or a suboptimal muscle activation capacity stemming from lower oxygenation of the prefrontal cortex (Smith & Billaut, 2010). Furthermore, unchanged or enhanced short-term power output resulting from transient heat exposure, presumably attributable to improved muscle contractility, is a well-established finding (Girard, Brocherie, et al., 2015). In this study, the lack of difference between HOT and CON trials might arise from the narrow difference in core temperature (±0.10°C) and heart rate values. The fact that the participants also became "hyperthermic" in the CON trial (i.e., core temperature ≥38.5°C) indicates that the effect of the warm-up dominates over external heat in determining thermal strain. Interestingly though, our data indicate that for repeated-sprint efforts performed under severe heat stress, participants were able to overcome the thermal sensation linked to the environment (higher skin temperature and ratings of perceived exertion values) to maintain repeated-sprint ability close to CON conditions.

The development of hyperthermia and the concomitant rise in cardiovascular strain led to an increase in relative exercise intensity. Based on the rate of heat storage, it has also been proposed that muscle recruitment is adjusted or "down-regulated" in order to prevent thermal injury (Tucker, Rauch, Harley, & Noakes, 2004). During short repeated-sprint efforts pacing may occur, as it is influenced by manipulation of prior knowledge of sprint number (Billaut, Bishop, Schaerz, & Noakes, 2011). In the absence of surface EMG recording, it is difficult to accept or reject the hypothesis that the slowed sprints were due to a reduced neural drive. During hotter games, however, players apparently reduce the amount of low-intensity running to preserve high-intensity actions, while pacing strategies (i.e., influenced by tactics, opposition) during actual match play are difficult to predict (Aughey, Goodman, & McKenna, 2014). Furthermore, the direct effect of hypoxia in reducing the motor drive to the working muscles was shown to be only moderate at much higher altitude (inspired fraction of oxygen = 0.11) (Millet, Aubert, Favier, Busso, & Benoit, 2009), and it is therefore unlikely that this central regulation is paramount in the present study, where participants knew the number of sprint repetitions to be completed.

Sprint kinetics

In line with previous studies conducted in cool/normoxic conditions, our data showed that reductions in horizontal force production exceed those in the vertical direction in CON trial (Delextrat, Baliqi, & Clarke, 2013; Girard et al., 2011; Morin et al., 2011b). For example, the patterns and ranges of the present sprinting kinetics alterations mirror those previously reported (−1.8%, −8.4% and −2.4% for averaged vertical, horizontal and total forces, respectively) for the completion of five 6-s sprints with 24 s of rest (Morin et al., 2011b). A unique aspect of our study was that hot ambient and hypoxic conditions do not accentuate the extent of fatigue-induced changes in sprint kinetics. Disregarding the environmental conditions, our data confirm that producing large amounts of horizontal rather than large amounts of total forces to the ground is paramount to better preserve sprint capacity as fatigue develops (Morin et al., 2011b). Applying ground reaction impulse in a more horizontal direction is crucial for the ability to accelerate from a standing start, as it explains 44% and 61% of the variance of running speed at 8 m (Kawamori, Nosaka, & Newton, 2013) and 16 m (Hunter, Marshall, & McNair, 2005) from the start, respectively. Furthermore, the lower index of force application technique values (i.e., steeper slope of the ratio of forces-running velocity relationship) observed over the series indicates progressively shorter and less effective acceleration phases. However, with identical index of force application technique values (−0.072) for the five sprints across all three conditions, heat stress or hypoxia did not further deteriorate force application technique as participants became fatigued.

Sprint kinematics

The ability to tolerate impact/stretch loads progressively deteriorated across the five successive sprints. Specifically, substantial increases in contact and swing times occurred as fatigue developed, leading to a monotonic large decrease in step frequency, while flight time and step length were well

preserved. Our results are in line with previous repeated-sprint ability studies, either on a treadmill (Delextrat et al., 2013; Morin et al., 2011a) or over the ground (Brocherie et al., 2015; Girard et al., 2011), with work-to-rest ratios ranging from 1:2 to 1:6. Moreover, the differences in performance between HYP and other trials seem too narrow for inducing large kinematic differences in stride efficiency. During a team-sport game, however, maximal efforts are often clustered with players performing multiple bouts of sprinting actions (Waldron & Highton, 2014). Conceivably, a single set repeated-sprint ability model may only poorly reflect the complex match activity patterns in team sports (Serpiello, McKenna, Stepto, Bishop, & Aughey, 2011). This implies that the above results would need to be confirmed under "real world settings" (Carling, 2013).

Spring-mass characteristics

Estimates of mechanical stiffness of the lower limbs are closely related to jumping and sprinting abilities, running economy as well as injury incidence (Butler et al., 2003). Across repetitions, without any influence of the environmental conditions, peak vertical forces decreased while both maximal vertical downward displacement and leg compression increased to a lower extent; this resulted in monotonic reductions in vertical stiffness, which are closely related to running velocity changes. While this corroborates previous field-based repeated-sprint ability conclusions (Brocherie et al., 2015; Girard et al., 2011), an interesting finding, however, is that leg stiffness followed a similar behaviour. Although Arampatzis et al. (1999) found that leg stiffness increases concomitantly with running velocity, fluctuations in leg stiffness values during field-based repeated-sprint ability tests have hitherto been reported as not significant despite profound slowing of running velocity in the most demanding protocols (Brocherie et al., 2015; Girard et al., 2011). In line with present results, however, a ~10% reduction in leg stiffness has been observed from the first to the fifth sprint repetitions when a larger cohort of athletes (n = 13) executed the same repeated-sprint ability test (Girard, Brocherie, Morin, Degache, & Millet, 2015). Further studies are needed to clarify this contention.

Limitations

The major weakness of this pilot study probably relates to the small number of participants involved. It is therefore likely that the statistical power of the current study may have been insufficient to identify meaningful differences linked to the environmental conditions *per se* in some of the mechanical variables, potentially due to large variability within and across days or a lower reliability in fatigued conditions. Of note, measurements of vertical stiffness and leg stiffness as well as related kinematic parameters (e.g., contact time, aerial time, step frequency and step length) when running for 30 s at a constant running velocity of 4.4 m · s^{-1} were found to be highly reliable (intraclass correlation coefficients ranging between 0.86 and 0.99) for both intra-day and inter-day designs (Pappas et al., 2014). More specifically, we have indicated that reliable running mechanical data can be derived from single 5-s sprints (three sprints separated by 2 min of passive recovery) on this instrumented treadmill on the same day and between days (5–7 days apart) (Girard, Brocherie, Morin, & Millet, in press). Reportedly, intra-session reliability was high (intraclass correlation coefficients >0.94 and coefficients of variation <8%) for performance outcomes (distance covered, mean velocity and propulsive power) and associated running mechanics. Furthermore, inter-session reliability was good for performance indices (0.83 < intraclass correlation coefficients < 0.89 and coefficients of variation < 10%) and high for kinetics (Intraclass correlation coefficients > 0.94 and coefficients of variation < 5%) and ranged between good and high for all kinematic (0.88 < intraclass correlation coefficients < 0.95 and coefficients of variation ≤ 3.5%) variables.

Although data were continuously collected, our analysis was based on averaging the representative steps near maximal running velocity for each 5-s sprint, which implies that interpretation of our results must remain specific to this phase of the sprint. Interestingly though, averaging data over "all steps" or only a few steps during early, middle or late phases of 5-s sprints provides similar mechanical outcomes during repeated treadmill sprinting (Girard, Brocherie, Morin, Degache, et al., 2015).

Finally, participants wore a facemask that was connected to the Altitrainer apparatus by a ~1.8-m long pipe during CON and HYP. An effect on performance induced by mask breathing *per se* during testing is unlikely, since its resistance and increase in dead space is negligible (i.e., no additional specific work of the respiratory muscles) compared to "normal breathing" (Sheel, 2002). In these conditions, we assumed that the influence of mask breathing on our observed repeated-sprint ability outcomes is likely to be negligible and therefore did not modify the main findings of this study.

Conclusion

This study was designed to directly compare the magnitude of the running performance and mechanical alterations during repeated treadmill sprinting in severe heat and hypoxic conditions. Preliminary evidence indicates that repeated-sprint ability is more impaired in hypoxia than in hot environment when compared to a control condition. However, the nature and extent of fatigue-induced alterations in running kinetics, kinematics and spring-mass characteristics did not differ between the three environmental conditions. Despite this, there is a possibility (yet unknown) that other specific phases of the sprint (e.g., early acceleration, deceleration phases) would be more sensitive to heat stress or hypoxia, but this needs to be further investigated. Specific strategies, including heat acclimation protocols, mixed methods of cooling and/or maintenance of hydration status, have proven to be efficient at mitigating heat-related decrements in repeated-sprint performance (Girard, Brocherie, et al., 2015), while tolerance for exercise of this nature is also improved with altitude training/acclimatisation (Girard et al., 2013). How this potential environmentally mediated improvement

in repeated-sprint ability would also lead to biomechanical adaptations still needs to be thoroughly documented.

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Disclosure statement

The authors do not have any conflict of interest or personal relationships with other people or organisations that could inappropriately influence this work.

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Chapter 8

Article 3 – Mechanical alterations to repeated treadmill sprints in normobaric hypoxia

8. Article 3 - Mechanical alterations to repeated treadmill sprints in normobaric hypoxia

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Abstract

Purpose. Compelling evidences suggest larger performance decrements during hypoxic *vs*. normoxic repeated sprinting, yet the underlying mechanical alterations have not been thoroughly investigated. Therefore, we examined the effects of different levels of normobaric hypoxia on running mechanical performance during repeated treadmill sprinting.

Methods. Thirteen team-sport athletes performed eight, 5-s sprints with 25-s of passive recovery on an instrumented treadmill in either normoxia near sea level (SL; FiO_2 = 20.9%), moderate (MH; FiO_2 = 16.8%; corresponding to ~1800 m altitude) or severe normobaric hypoxia (SH; FiO_2 = 13.3%; ~3600 m).

Results. Net power output in the horizontal direction did not differ (P>0.05) between conditions for the first sprint (pooled values: 13.09±1.97 $W.kg^{-1}$) but was lower for the eight sprints in SH, compared to SL (-7.3±5.5%, P<0.001) and MH (-7.1±5.9%, P<0.01), with no difference between SL and MH (+0.1±8.0%, P=1.00). Sprint decrement score was similar between conditions (pooled values: -11.4±7.9%, P=0.49). Mean vertical, horizontal and resultant ground reaction forces decreased (P<0.001) from the first to the last repetition in all conditions (pooled values: -2.4±1.9%, -8.6±6.5% and -2.4±1.9%). This was further accompanied by larger kinematic (mainly contact time: +4.0±2.9%, P<0.001 and +3.3±3.6%, P<0.05; respectively; and stride frequency: -2.3±2.0%, P<0.01 and -2.3±2.8%, P<0.05; respectively) and spring-mass characteristics (mainly vertical stiffness: -6.0±3.9% and -5.1±5.7%, P<0.01; respectively) fatigue-induced changes in SH compared with SL and MH.

Conclusion. In severe normobaric hypoxia, impairments in repeated-sprint ability and in associated kinetics/kinematics and spring-mass characteristics exceed those observed near sea level and in moderate hypoxia (*i.e.*, no or minimal difference). Specifically, severe hypoxia accentuates the RSA fatigue-related inability to effectively apply forward-oriented ground reaction force and to maintain vertical stiffness and stride frequency.

Introduction

Despite distinct skills, tactics and movement patterns, team sports are characterized by alternating high-intensity actions (*i.e.*, accelerated runs with frequent directional changes, jumps and kicks) with incomplete recoveries. During the most intense periods of the game or toward match-end, team-sport athletes experience fatigue as manifested by temporary reductions in high-velocity running and especially peak/mean sprint distances (19), which are both likely to indirectly influence the game outcome (13). The reduction in partial pressure of oxygen (PO_2) (as seen from moderate-to-high altitudes, hypoxia) is known to lead to premature/larger fatigue during games. For instance, playing soccer at altitude (> 1500 m; inspired fraction of O_2, FiO_2 < 17%) detrimentally affects the total distance covered (40), the completion of high-intensity efforts and the ensuing recovery (14). However, sprint-related measures are highly variable due to match-specific factors (tactics, opposition) and environmental conditions (reduction in air density) (15). Caution is therefore needed when inferring the potential mechanisms underpinning the aforementioned altitude-mediated decrements on soccer performance.

Repeated-sprint ability (RSA) tests conducted in the laboratory offer more controlled conditions to shed light on the potential factors compromising fatigue resistance. Previous studies, mainly completed on a cycle-ergometer and involving athletes of various training backgrounds, have consistently indicated that normobaric hypoxia (FiO_2 ranging 14-16%) does not influence single sprint performance, but induces earlier and larger decrement in repeated-sprint performance compared with sea-level conditions (2-4, 19). It is generally accepted that sprints repetition in hypoxia *vs*. normoxia elevates heart rate (HR) (5), minute ventilation (5), O_2 debt (2, 5), muscle deoxygenation level (3-5) and growth hormone response (28) and lowers surface electromyogram of active musculature (4, 5). The more recent studies addressing the effects of hypoxia on RSA have specifically tested team-sport

athletes on non-motorized treadmills, which favorably increase the validity of their findings. In one of these studies, highly-trained team-sport athletes were able to match sea-level performance in hypoxic conditions (FiO_2 = 14%) during ten, 6-s treadmill running sprints separated by 24 s of passive rest (38). Other RSA treadmill studies comparing graded levels of FiO_2 reported that the decrements in RSA with increased hypoxia severity may not follow a monotonic (*i.e.*, linear) pattern (5, 23). This was notably demonstrated by Goods et al. (23) who showed using a multiple sets paradigm (*i.e.*, 3 sets of 9 × 4-s treadmill sprints) that peak power output was only further reduced (third set) at FiO_2 ~12% *vs*. ~21% but not at FiO_2 14-16%.

In the few available biomechanical studies that have been conducted near sea level, specific impairments in sprinting mechanics [*i.e.*, increase in contact time (t_c) and swing time (tswing), reductions in vertical stiffness (K_{vert}) and stride frequency (S_F)] have been connected with progressively slower sprint times/running velocities during over-ground repeated sprints [*i.e.*, 6 x 20 m – 20 s of passive recovery in U19 footballers (21); 6 x 35 m – 10 s of passive recovery in elite footballers (7); or 12 x 40 m – 30 s of passive recovery in team- and racquet-sports athletes (20)]. Collectively, these findings show that the ability to tolerate ground impact/stretch loads decreases as fatigue develops with sprint repetitions. Recently, we used a "recovery of performance approach" where hypoxia severity of an initial set of repeated sprints was manipulated and observed that hypoxia had no residual effect during a subsequent set performed in normoxia (18). Under this later study frame, only three out of eight repetitions (*i.e.*, sprints 1, 4 and 8) were investigated during the initial set of sprints. Consequently, the time course of changes in sprint mechanical performance could not be accurately described. Hence, the decay in performance during RSA protocols is not linear since fatigability is generally larger during the first *vs*. the last sprint repetitions, as peripheral disturbances are almost immediate and neural factors also contribute to impaired performance during the early stages of such exercise (19, 22).

Motorized instrumented treadmills now allow valid (35) and reliable (17) continuous (step-by-step) measurement of instantaneous horizontal and vertical components of the resultant (total) ground reaction force (GRF) during maximal sprints (including the typical acceleration phase) similar to team-sport scenarios. A unique feature of this device is to allow the computation of the ratio of support-averaged net horizontal to total force (RF), which gives an indication of the way athletes apply forces onto the ground (technical ability), independently from the amount of total force applied (physical capability) (34). In the only available treadmill studies thoroughly describing changes in sprinting kinetics over repeated sprints, significant decrease in force production capacity, particularly in the ability to apply it horizontally (*i.e.*, approximately two-fold larger reduction than vertical and total forces) has been observed (16, 36). As sprint bouts and series were repeated, the technical ability to apply force effectively against the ground was altered and led to shorter and less effective acceleration phase (36). In the above studies, however, these observations were restricted to normoxic conditions. It is likely (yet unknown) that increasing hypoxia severity above moderate levels (severe hypoxia) leads to premature and excessive fatigue and is associated with less efficient stride mechanics. Preventing excessive fatigue-induced changes resulting from the additional load imposed by the hypoxic stress during training routines [repeated sprints in hypoxia; (6)] or competition would help to improve athletic performance.

The aim of this study was therefore to comprehensively examine performance changes and accompanying running mechanical alterations over a series of treadmill sprints under differing levels of acute normobaric hypoxia severity compared with sea level. We hypothesized that deterioration of RSA and associated alterations in kinetics/kinematics and spring-mass characteristics would be more impaired under severe hypoxia (SH) compared with sea-level (SL) and moderate hypoxia (MH) conditions (*i.e.*, no or minimal difference).

Methods

Subjects. Thirteen male volunteers (mean ± SD age, 31.2 ± 4.8 years; stature, 178.4 ± 6.6 cm; body mass, 74.3 ± 8.2 kg) who were recreationally active (4.5 ± 2.5 $h.wk^{-1}$) in intermittent sports (*i.e.*, football, futsal, tennis, squash) took part in the study. The sample size was estimated from alterations in ground reaction forces as measured during a previous RSA treadmill test (36), requiring ≥ 12 subjects for a two-way repeated-measures analysis of variance (ANOVAs) with 3 conditions (ß = 0.85, α = 0.05). All subjects were lowlanders, with no recent exposition/acclimatization to altitude and free of musculoskeletal pain or injuries. In the 6 months preceding the study, their training included activity-specific (*i.e.*, technical and tactical skills), aerobic (*i.e.*, continuous and intermittent) and anaerobic (*i.e.*, strength, sprints, change of direction) exercises. Although subjects' training content largely focused on accelerated runs, their sprinting skills are deemed to be "moderate" compared to "elite" (*i.e.*, national to international level) sprinters (41) and/or footballers (7). They were requested to report to the laboratory in a rested and hydrated state, at least 3 h postprandial and having avoided strenuous exercise in the preceding 48 h. They were also asked to refrain from caffeine for 12 h and alcohol for 24 h before each visit. Written informed consent was obtained from participants, and the study was approved by the Shafallah Medical Genetics Center Ethics committee, and conducted according to the Declaration of Helsinki.

Experimental design. About 1 week prior to testing, subjects undertook a complete preliminary session where they performed short (<5 s) 'familiarization' treadmill sprints at increasing intensities while wearing a facemask for habituation (*i.e.*, with the hypoxic system turned off), with full recovery and until being comfortable with the running technique required (which generally required 7-10 trials). Then they performed three maximal 5-s single sprints, separated by 2 min of passive recovery, and after 5 min of rest, the RSA test (see "Repeated sprint ability" section) in full. All of them satisfied the criteria of having a

coefficient of variation < 2.2% for distance covered across three successive trials (17). Strong verbal encouragement was given during all maximal efforts, with no feedback provided, except for the time remaining during recoveries (*i.e.*, 10 s, 5 s and "3-2-1" s countdown before each sprint). Additionally, subjects were reminded during each recovery period that the subjective awareness of effort expended during the next sprint effort must be maximal.

Subjects reported to the laboratory on three different occasions at least 3-4 days apart to complete an experimental session (~45 min from the beginning of the warm-up until the end of the RSA test). The study was designed as a randomized, double-blind fashion protocol in which subjects and investigators (except one) were blinded toward the environmental conditions. The efficacy of blinding procedure was evaluated after each experimental session by questionnaires in which subjects were asked whether they believed they were exercising in SL, MH or SH. Upon arrival at the laboratory (well-ventilated at a constant temperature of ~25°C and ~40% relative humidity), subjects were instrumented for physiological measurements (see "Physiological responses" section). A normoxic standardized warm-up lasting ~15 min [5 min of running at 10 $km.h^{-1}$, 10 min of sprint-specific athletic drills (*i.e.*, 3 x (skipping, high knee, butt-kick, high heels for ~10 s with 30-s walking in between); 3 x short bursts of accelerations at a subjective "sense of effort" of 7, 8 and 9 on a modified Borg CR10 scale); and 2 x 3-s sprints at a subjective "sense of effort" of 8 and 9 on the modified Borg CR10 scale] was completed. Afterwards, three maximal 5-s sprints, separated by 2 min of passive recovery, were completed. The best of these three trials was used as the criterion score for the subsequent series, to ascertain that no pacing occurred. Finally, after a facemask connected to a portable hypoxic generator (see "Altitude simulation" section) had been attached on subjects, they were allowed 5 min of free rest (*i.e.*, passive standing) prior to the repeated-sprints protocol. Total hypoxic exposure corresponded to exactly 9 min, with facemask remaining attached during the entire RSA test, including the eight sprints as well as between-sprint recoveries.

Altitude simulation. Normobaric hypoxia was obtained by mixing nitrogen into ambient air under control of FiO_2 (Altitrainer, SMTec SA, Nyon, Switzerland). This gas-mixing system enriches the inspired air by adding a fixed quantity of nitrogen via a 30 L mixing chamber, with the dilution being constantly controlled by a PO_2 probe (with a precision of ± 0.82 Torr and safety set at FiO_2 = 9.7%). This device allows the production of large quantities of a hypoxic gas mixture (up to 200 $L.min^{-1}$), with an easily adjustable O_2 fraction over a large range, and a short response time (between 15 and 45 s), expressed either by the equivalent altitude or by the O_2 partial pressure, taking into account the barometric pressure. Subjects, always breathing through the same set-up (also in normoxia), inhaled the mixture contained in the buffer tank through a Hans Rudolph two-way respiratory valve. During testing, they were exposed to near sea-level (SL; FiO_2 = 20.9%), moderate (MH; FiO_2 ~16.8% corresponding to a simulated altitude of 1800 m) and severe (SH; FiO_2 ~13.3%; 3600 m) hypoxia. While it cannot be completely ruled out, the influence of mask breathing on our RSA outcomes is likely minimal (small resistance and negligible increase in dead space).

Instrumented sprint treadmill. The sprints were performed on an instrumented motorized treadmill (ADAL3D-WR, Medical Development – HEF Tecmachine, Andrézieux-Bouthéon, France). Briefly, it is mounted on a highly rigid metal frame fixed to the ground through four piezoelectric force transducers (KI 9077b; Kistler, Winterthur, Switzerland) and installed on a specially engineered concrete slab to ensure maximal rigidity of the supporting ground. This motorized treadmill allows subjects to sprint and produce acceleration and high running velocities due to the use of a constant motor torque (17, 35, 36). This corresponded to the motor torque necessary to overcome the friction on the belt due to subject's body weight, which was set to 160% of the default torque after preliminary testing. This default torque value was selected for allowing subjects to sprint in a comfortable manner and produce their maximal effort without risking loss of balance. It was measured by requiring the subject to

stand unmoving at the center of the treadmill's belt and by increasing the driving torque until observing a movement of the belt greater than 2 cm over 5 s.

A single-pass waist and a stiff rope (*i.e.*, 1 cm in diameter, ~2 m length) were used to tether subjects to the 0.4-m vertical rail anchored to the wall behind them. An additional safety harness attached to an overhead suspension (*i.e.*, with sufficient slack not to impede natural running mechanics) was fastened to the subjects to support them above the treadmill belt in the event of a fall. When correctly attached, they were required to lean forward in a typical and standardized crouched sprint-start position with their left foot forward. This starting position was used and standardized all along the sprint series. After a 5-s countdown ("5 s, 3-2-1-Go" given by both visual and audio instructions by the same investigator), the treadmill was released, and the belt began to accelerate as subjects applied a positive horizontal force.

Repeated sprint ability. The RSA test consisted of performing eight 5-s treadmill sprints interspersed by 25-s of passive recovery. The construct of the 5–25 s repeated-sprint design was used to match one of the most common effort-rest ratios seen in the literature [*e.g.*, (4)]. RSA was assessed from net power output in the horizontal direction (P_P) data using three scores: the largest (*i.e.*, during the first sprint in all cases) P_P, the cumulated P_P over the eight sprints (*i.e.*, sum of the eight sprints) and the sprint decrement score [*i.e.*, ([cumulated P_P / (largest P_P × 8)] – 1) × 100] (19).

Mechanical variables. Data were continuously sampled at 1000 Hz over the sprints allowing determination of the beginning of the sprints (defined as the moment the belt velocity exceeded 0.2 $m.s^{-1}$). After appropriate filtering (Butterworth-type 30 Hz low-pass filter), instantaneous data of vertical, net horizontal and resultant (*i.e.*, total) GRF were averaged for each support phase (vertical force above 30 N) over the 5-s sprints (F_V, F_H and F_{Tot}, respectively), and expressed in body weight (BW). The index of force application technique

(D_{RF}) representing the decrement in ratio of forces (RF = $F_H.F_T^{-1}$) with the increasing belt velocity (V, m.s^{-1}) was computed as the slope of the linear RF-V relationship calculated from the step-averaged values between the second step and the step at top V (34). These data were completed by measurements of the main step kinematic variables: t_c (s), aerial time (t_a, s), t_{swing}, (s), S_F (Hz) and step length (S_L, m). Last, for each 5-s sprint, F_H was used with the corresponding average V to compute P_P ($P_P = F_H.V$, W.kg^{-1}).

A linear spring-mass model of running (33), was used to investigate the main mechanical parameters characterizing the lower limbs behavior during running. K_{vert} (kN.m^{-1}) was calculated as the ratio of peak vertical force (F_{Zmax}, N) to the vertical maximal downward displacement of CM (Δ_Z, m), which was determined by double integration of vertical acceleration of center of mass over time during ground contact. Leg stiffness, the stiffness of the leg spring (K_{leg}, kN.m^{-1}), was calculated as the ratio of F_{Zmax} to the maximum leg spring compression [$\Delta L = \Delta z + L_0 - \sqrt{L_0^2 - (0.5.V.t_c)^2}$, m]. Initial leg length (L_0, great trochanter to ground distance in a standing position) was determined from subject's stature as L_0 = 0.53.*stature* (33).

Physiological responses. HR was monitored telemetrically with a Polar transmitter– receiver (Wearlink T-31; Polar Electro Oy, Kempele, Finland) and recorded every 5 s. Pulse O_2 saturation (SpO_2) was monitored via fingertip pulse oximetry (Palmsat 2500, Nonin Medical Inc, Plymouth, USA). HR and SpO_2 were obtained before (*i.e.*, after a 5-min seated period) and at the end of the warm-up procedure (*i.e.*, after 5 min of rest while breathing the hypoxic mixture, just before starting the RSA test). Both HR watch (RS400, Polar Electro Oy, Kempele, Finland) and oximeter receiver were attached on the handrails on the sides of the treadmill in a manner to not allow subjects to view any data, according to the blinding procedure. Together with HR and SpO_2, ratings of perceived exertion (RPE) were recorded using the Borg 6-20 scale (*i.e.*, 6 = no exertion at all, 20 = maximal exertion) exactly 10 s

following each sprint. A capillary blood sample was taken from the fingertip and analyzed for blood lactate concentration ([La]) with the Lactate Pro (LT-1710, Arkray, Japan) portable analyzer before the warm-up and 2 min after the RSA test.

Statistical Analyses. Each sprint trial included 15-18 ground contacts. After excluding the last two ground contacts, the remaining three last consecutive steps were used for final analysis of sprint kinetics/kinematics and spring-mass characteristics (7). Importantly, the effects of treadmill sprints repetition (fatigue) on performance and mechanical outcomes do not differ between early, middle or late phases of 5-s sprints similar to those performed here (16).

Values are expressed as mean ± SD. Two-way repeated-measures analysis of variance (ANOVAs) [Condition (SL, MH and SH) × Time (Sprint number 1, 2, 3, 4, 5, 6, 7 and 8)] were used to compare physiological/perceptual, running performance and mechanical responses. Outcome variables were tested using Mauchly's procedure for sphericity. Whenever the data violated the assumption of sphericity, p values and adjusted degrees of freedom based on Greenhouse-Geisser correction were reported instead. Where significant effects were established, pairwise differences were identified using the Bonferroni post hoc analysis procedure adjusted for multiple comparisons. All statistical calculations were performed using SPSS statistical software V.21.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $p<0.05$.

Results

Blinding procedure. Observations that a) only 4 out of 13 participants assumed that they were exposed to SL, MH or SH at the end of the first, second and third experimental session (*i.e.*, treatment order) and b) only 12 out of a possible 39 sessions were correctly identified, together indicate that the blinding procedure was successful.

Responses to exercise. When reporting to the laboratory, subjects displayed similar resting HR (pooled values: 67±11 bpm; P = 0.98), SpO_2 (97.3±1.2%; P = 0.72) and [La] (1.4±0.4 $mmol.L^{-1}$; P = 0.89) values across visits. At the end of the warm-up procedure (5 min after breathing the hypoxic mixture), HR tended to be higher under hypoxic conditions (SL: 94±15 bpm; MH: 102±12 bpm; SH: 102±17 bpm; P = 0.06), whereas SpO_2 was significantly (P < 0.001) reduced under SH (88.8±2.9%) compared with both SL (95.5±1.1%) and MH (94.5 ± 1.2%), with also no significant (P = 0.07) difference between SL and MH.

HR increased significantly from the first to the fifth repetition (P < 0.001), with no further change thereafter, independently of the conditions (figure 1).

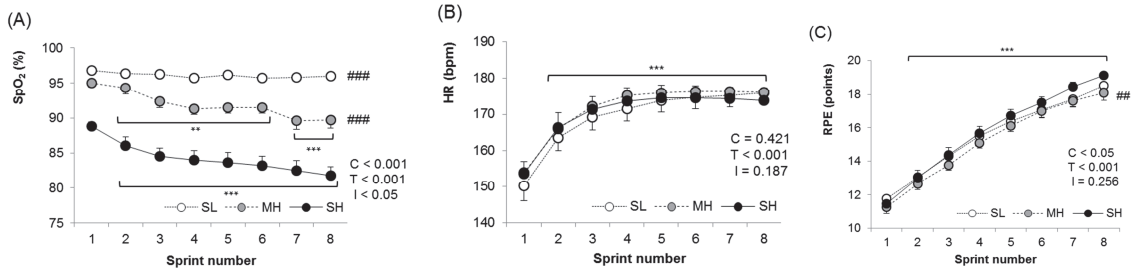

Figure 1. Pulse oxygen saturation (SpO_2; A), heart rate (HR; B) and perceptual (RPE; C) responses during the repeated-sprint ability test. Values are mean ± SD, N = 13. Data are presented for normoxia (SL; FiO_2 = 20.9%), moderate (MH; FiO_2 = 16.8%) and severe normobaric hypoxia (SH; FiO_2 = 13.3%). C, T and I for condition, time and interaction effects. ** Significantly different from sprint 1, P < 0.01 and *** P < 0.001. ## Significantly different from SH, P < 0.01 and ### P < 0.001.

With every increase in hypoxia severity corresponded significantly lower (P < 0.001) SpO_2 values (figure 1). Whereas fluctuations of SpO_2 values for SL condition remained within 1% of sprint 1 (P = 0.97), SpO_2 values decreased significantly from the first to the last repetition in both MH and SH trials (-5.5±4.9% and -7.9±4.2%, respectively; both P < 0.05). RPE increased significantly (P < 0.001) across all conditions (figure 1), but significantly higher (P < 0.01) RPE values were rated for sprint 8 in SH (19.1±0.8) compared with SL (18.5±1.2) and MH (18.1±1.6). Exercise-induced increase in [La] (P < 0.001) was not different (P = 0.40) between conditions with values of 9.9±1.7, 10.4±1.8 and 10.7±2.1 mmol.L-1 reached 2 min after the RSA test in SL, MH and SH, respectively.

RSA and other running performance outcomes. During the RSA test, the 95% criterion score was satisfied by all participants in the three testing conditions (SL: 99.5±2.0%; MH: 101.0±5.9%; SH: 100.7±4.2%). This corresponded to a similar V and P_P during the first sprint in all three trials (SL: 6.39±0.28 m.s^{-1} and 13.39±2.18 W.kg^{-1}; MH: 6.44±0.25 m.s^{-1} and 13.15±1.86 W.kg^{-1}; SH: 6.35±0.33 m.s^{-1} and 12.73±1.95 W.kg^{-1}; P = 0.71 and 0.70, respectively). Subsequently, progressive reduction in V (P < 0.001) and P_P (P < 0.001) occurred from sprint 1 to 8, irrespective of the conditions, yet with larger decrease in SH (-11.2±4.1% and -22.9±11.6%, respectively) compared with SL (-8.5±4.0%, P < 0.05 and -14.9±14.7%, P < 0.001) and MH (-11.1±4.1%, P < 0.05 and -15.8±7.5%, P < 0.01) (figure 2). Cumulated P_P was lower in SH (88.28±16.13 W.kg^{-1}) compared with SL (95.31±17.06 W.kg^{-1}, -7.3±5.5%, P < 0.001) and MH (94.76±14.77 W.kg^{-1}, -7.1±5.9%, P < 0.01), with no difference between SL and MH (+0.1±8.0%, P = 1.00). Sprint decrement score did not differ significantly (P = 0.49) between SL (-10.9±8.1%), MH (-9.8±8.0%) and SH (-13.5±7.8%).

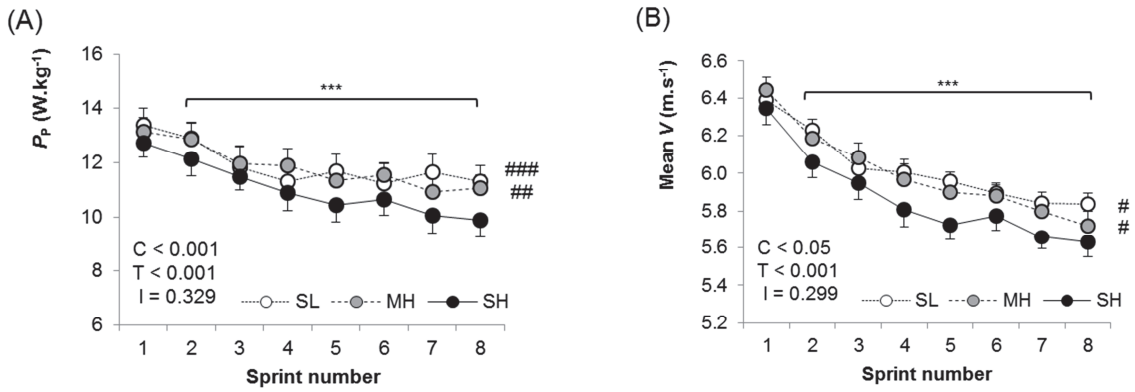

Figure 2. Propulsive power (P_P, A) and mean velocity (V, B) during the repeated-sprint ability test. Values are mean ± SD, N = 13. Data are presented for normoxia (SL; FiO_2 = 20.9%), moderate (MH; FiO_2 = 16.8%) and severe normobaric hypoxia (SH; FiO_2 = 13.3%). C, T and I for condition, time and interaction effects. *** Significantly different from sprint 1, P < 0.001. # Significantly different from SH, P < 0.05; ## P < 0.01 and ### P < 0.001.

Running kinetics and kinematics. F_V, F_H and F_{Tot} decreased significantly (P < 0.001) from the first to the last repetition in all conditions (pooled values: -2.4±1.9%, -8.6±6.5% and -2.4±1.9%, respectively) (figure 3). F_H was significantly lower in SH compared with SL and

MH (-4.9±5.9% and -5.2±5.4%, respectively; P < 0.01), while non-significant trends were observed for F_V (P = 0.09) and F_{Tot} (P = 0.07) (figure 3).

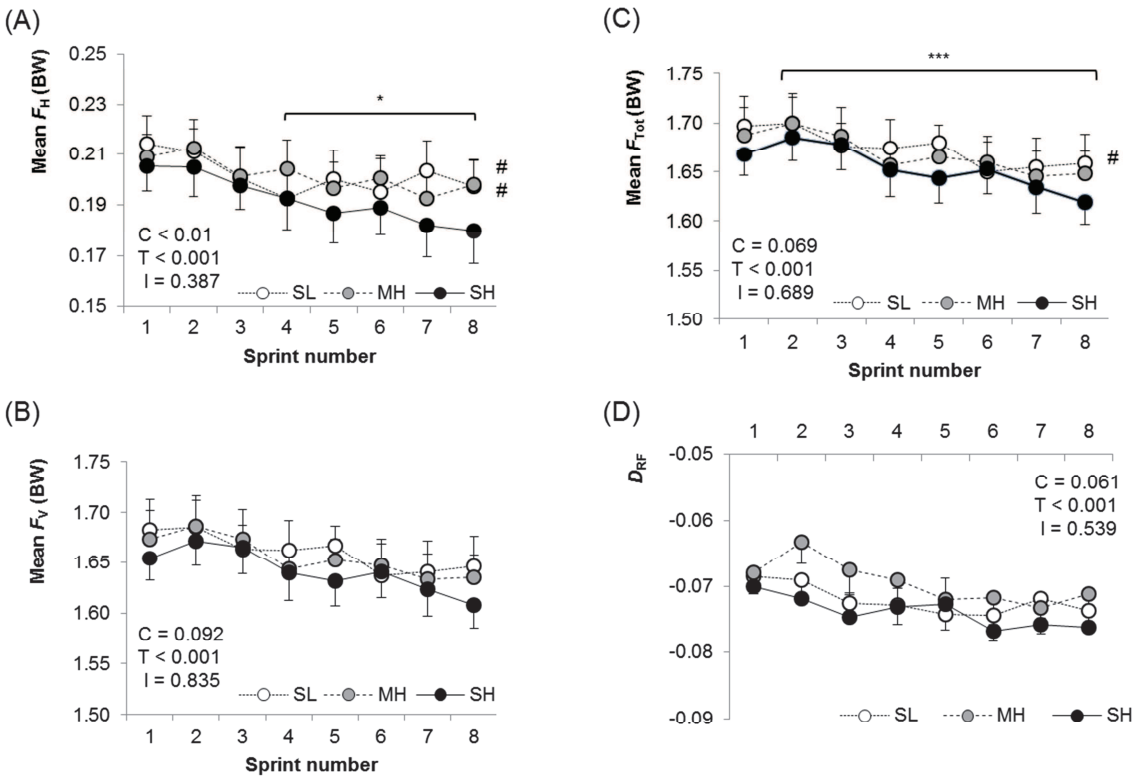

Figure 3. Kinetic characteristics [net horizontal forces (F_H; A), mean vertical forces (F_V; B), mean total forces (F_{Tot}; C)] and index of force application technique (D_{RF}; D) during the repeated-sprint ability test. Values are mean ± SD, N = 13. Data are presented for normoxia (SL; FiO_2 = 20.9%), moderate (MH; FiO_2 = 16.8%) and severe normobaric hypoxia (SH; FiO_2 = 13.3%). C, T and I for condition, time and interaction effects. * Significantly different from sprint 1, P < 0.05; ** and *** P < 0.001. # Significantly different from SH, P < 0.05.

D_{RF} significantly decreased across repetitions (pooled value: -7.7±9.7%, P < 0.001) so that the RF-*V* relationship became steeper with fatigue, while these changes tended to differ (P = 0.06) between SL (-8.6±14.2%), MH (-5.0±12.0%) and SH (-9.5±15.3%) (figure 3).

t_c was the only mechanical variable displaying a significant interaction between time and condition with a larger magnitude of fatigue-induced changes seen in SH compared with SL and MH (+4.0±2.9%, P < 0.001 and +3.3±3.6%, P < 0.05; respectively). Compared with SL and MH, greater alterations in SH were found for t_{swing} (+1.5±2.5% and +2.1±2.7%, respectively; both P < 0.05) and S_F (-2.3±2.0%, P < 0.01 and -2.3±2.8%, P < 0.05;

respectively) but not for t_a (+0.1±5.4% and +1.1±4.9%, respectively; P = 0.52) and S_L (-0.6±3.8% and +0.1±2.9%, respectively; P = 0.64) (figure 4).

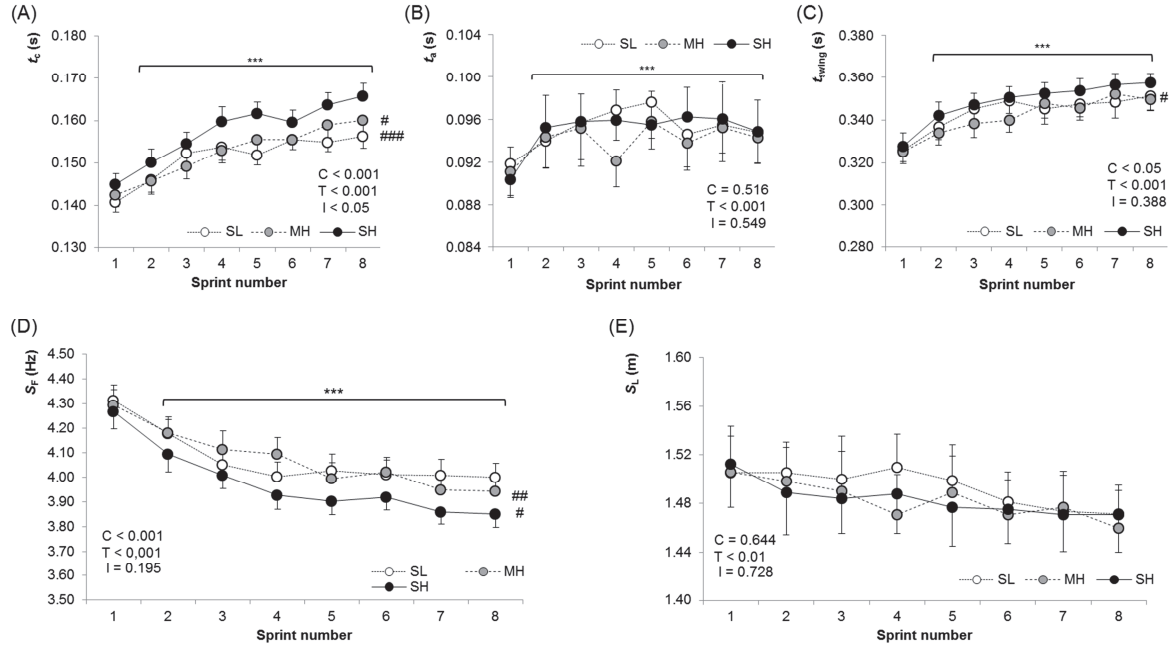

Figure 4. Kinematic characteristics [contact time (t_c; A), aerial time (t_a; B), swing time (t_{swing}; C), Step frequency (S_F;D) and Step length (S_L; E)] during the repeated-sprint ability test. Values are mean ± SD, N = 13. Data are presented for normoxia (SL; FiO_2 = 20.9%), moderate (MH; FiO_2 = 16.8%) and severe normobaric hypoxia (SH; FiO_2 = 13.3%). C, T and I for condition, time and interaction effects. *** Significantly different from sprint 1, P < 0.001. # Significantly different from SH, P < 0.05; ## P < 0.01 and ### P < 0.001.

Spring-mass parameters. Whereas F_{Zmax} significantly (-2.9±1.9%, P < 0.001) decreased, both Δ_Z and Δ_L significantly increased (conditions pooled: +19.8±6.2%, P < 0.001 and +5.3±3.5%, P < 0.05, respectively) from the first to the last sprint (Figure 5). Consequently, K_{vert} and K_{leg} significantly (all conditions pooled: -18.5±5.0% and -7.4±4.1%, respectively; both P < 0.001) decreased with fatigue. In reference to SL and MH, Δ_Z values were larger (+5.1±4.4%, P < 0.01 and +5.2±6.4%, P < 0.05, respectively) and K_{vert} smaller (-6.0±3.9%, P < 0.001 and -5.1±5.7%, P < 0.01, respectively) in SH, while only a tendency for lower F_{Zmax} values (-1.5±4.4% and -0.8±6.4%, respectively; P = 0.051) was observed (figures 5 and 6). Figure 6 shows the comparison of the main averaged running mechanical data for the eight sprints in MH and SH in reference to SL.

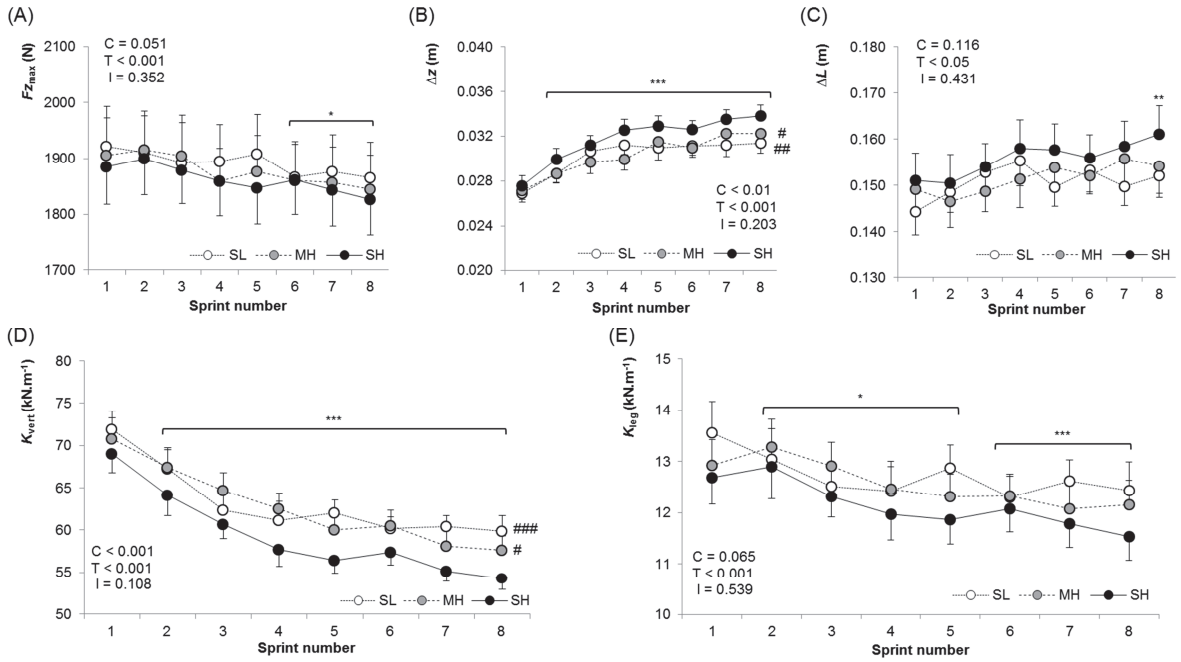

Figure 5. Spring-mass behavior [peak vertical forces (F_{Zmax}; A), vertical maximal displacement of CM (Δ_Z; B), maximum leg spring compression (Δ_L; C), vertical stiffness (K_{vert}; D) and leg stiffness (K_{leg}; E)] during the repeated-sprint ability test. Values are mean ± SD, N = 13. Data are presented for normoxia (SL; FiO_2 = 20.9%), moderate (MH; FiO_2 = 16.8%) and severe normobaric hypoxia (SH; FiO_2 = 13.3%). C, T and I for condition, time and interaction effects. * Significantly different from sprint 1, P < 0.05; ** P < 0.01 and *** P < 0.001. # Significantly different from SH, P < 0.05; ## P < 0.01 and ### P < 0.001.

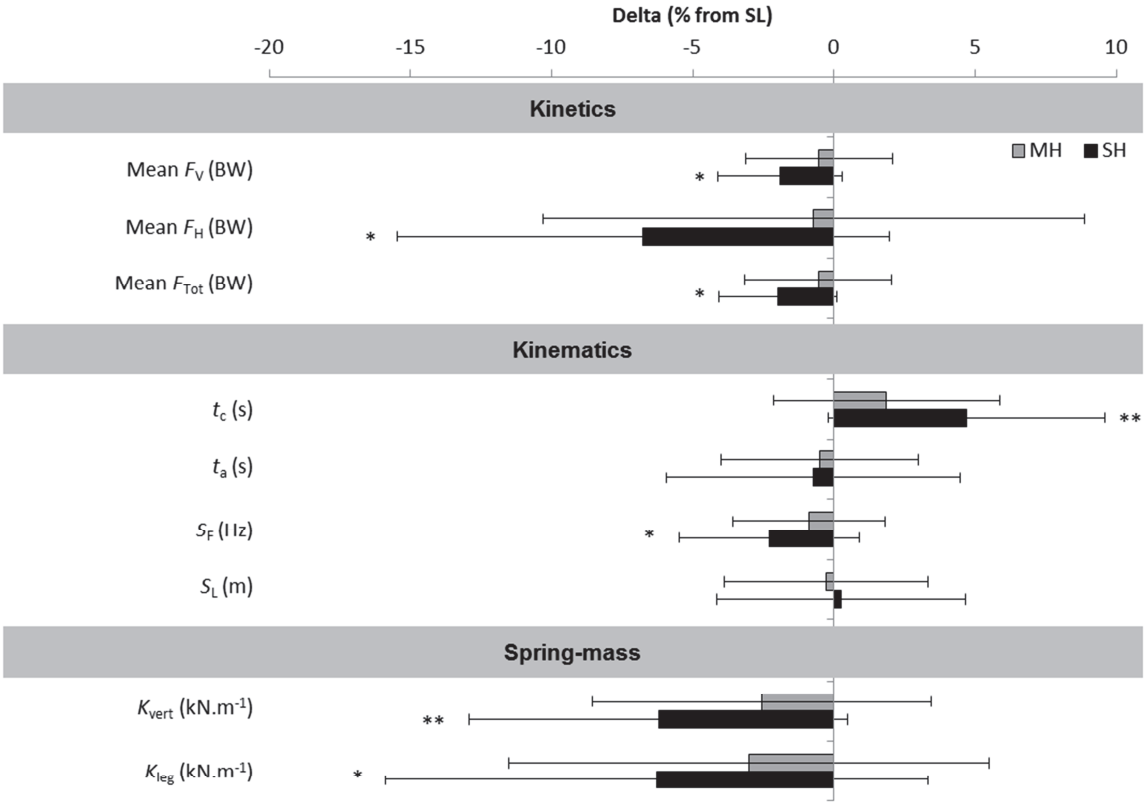

Figure 6. Comparison of averaged running mechanical data for the eight sprints in MH and SH in reference to SL. Values are mean ± SD, N = 13. * Significantly different from SL, P < 0.05 and ** P< 0.01.

Discussion

The present study specifically examined how the level of normobaric hypoxia severity impacts performance of repeated treadmill sprints and associated mechanical parameters. The main results were that in severe (FiO_2 = 13.3%; ~3600 m), but not moderate (FiO_2 = 16.8%; ~1800 m), normobaric hypoxia, impairments in RSA and in accompanying kinetics/kinematics and spring-mass characteristics exceed those observed at sea level (FiO_2 = 20.9%).

In this study, both MH and SH had no detrimental effect on the initial sprint performance compared with SL. This was expected since performance of single sprint of short-duration (≤10 s) largely depends on neuromuscular recruitment/activation and intramuscular energy stores (2, 43). However, in partial agreement with previous studies (5, 23), P_P was lower in SH, but not in MH, compared with SL, and resulted in shorter cumulated P_P and larger sprint decrement score during the set of repeated sprints in SH compared with SL and MH. While it remains unclear how the present data would fit with elite team-sport athletes (38) since they may experience a larger O_2 desaturation in hypoxia (31) that would exacerbate performance alteration for a given simulated altitude, our results (from recreational athletes) suggest that RSA performance decrements with increased hypoxia severity did not follow a monotonic (*i.e.*, linear) pattern. Given the importance to maintain maximal P_P levels across sprints repetition during a RSA protocol (19), one may postulate that SH is less suitable than MH for training purpose, even though larger physiological effects associated with SH *vs*. MH cannot be ruled out. In this view, a notable finding is that most of the alterations in sprint performance and accompanying running mechanics (discussed below) occurred within the first half (*i.e.*, sprints 1 to 4) of the RSA test with smaller changes during the second part (*i.e.*, sprints 5 to 8). This phenomenon is particularly notable in SH condition. This appears in line with the biphasic profile (*i.e.*, a gradual decrease in power output during the first sprints followed by a plateau-like phase) previously observed during repeated cycling sprints (*i.e.*, 10

× 10 s 'all-out' sprints with 30 s of passive recovery) (26). Of interest is that SpO_2 values remained >90% in MH whereas initial SpO_2 values were <90% in SH and decreased to ~82% (ranging 76-89%) across RSA test repetitions. These values are above the 70-75% SpO_2 values suggested as a critical 'threshold' (derived from self-paced exercises) where central nervous system hypoxia would primarily (*e.g.*, over peripheral muscle fatigue) influence exercise performance (1). Recent evidence using manipulations of preexisting fatigue levels (26) and hypoxia severity (4) would support the view that power output and EMG are adjusted during repeated-sprinting for the purpose of limiting the development of peripheral fatigue beyond a constant threshold.

It is generally accepted that, with an acute reduced O_2 availability (such as in normobaric hypoxia), there is an increased reliance on non-oxidative glycolysis (5) and PCr hydrolysis rate (24). As such, cycling (2) and treadmill (5, 23) hypoxia-induced (FiO_2 ranging 14-16%) RSA performance decrements were previously associated with higher [La] (2, 5, 23), HR (2, 5) and RPE (23). Here, the responses (*i.e.*, [La], HR and RPE) to exercise followed a similar trend, albeit [La] and end-exercise HR values were not significantly different between conditions, as previously demonstrated during RSA tests (2, 23). Of note, in line with the above mentioned performance measures, HR displayed a plateau-like phase from sprint 5. Furthermore, RPE was only significantly higher in SH compared with other conditions during the last sprint repetition. This, despite subjects being constantly remained during between-sprint recoveries of each of the three conditions that subjective awareness of effort expended during the next sprint bout must be maximal. Importantly, this larger perceived peripheral discomfort (RPE) in the more severe hypoxic condition occurred despite lower fatigue resistance. Fatigue during RSA is a disabling symptom in which physical and cognitive functions are limited by interactions between objective (performance) and subjective (effort perception) signals with hypoxia severity influencing the nature of this relationship.

Mechanical alterations during normoxic RSA tests have been well described in recent studies [treadmill: (32, 34, 36); force plate: (27, 29, 37, 41); over-ground: (7, 20, 21, 25)]. Briefly, alterations in sprinting mechanics (*i.e.*, kinetics: reduction in FH production; kinematics: lengthening in t_c and t_{swing}, decrease in S_F; spring-mass characteristics: increase in Δ_Z, decline in K_{vert}) and force application technique (*i.e.*, decrease in D_{RF}) occur concomitantly with a reduced performance (*i.e.*, decreases in P_P, V and sprint decrement score) during repeated sprints. With similar adjustments occurring irrespectively from the conditions, our results confirm a deteriorated ability to tolerate ground impact/stretch loads as fatigue develops with sprint repetitions. Of particular relevance, and because (i) increasing V can be achieved by pushing on the ground more forcefully (S_L), more frequently (S_F) or by combining both schemes (42); (ii) a more forceful ground contact results in a longer S_L (11) and (iii) S_L is inversely proportional to S_F (25), our results clearly demonstrate that t_c is the main kinematic variable explaining the fatigue-induced reduction in S_F (and to a lesser extent S_L).

Regarding the impact of hypoxia severity, SH further worsened stride efficiency compared with SL and MH. With the exception of t_c which displayed an interaction effect, all other mechanical variables displayed only a main effect of the condition. Of note, the magnitude of sprint 1 to 8 reductions for F_H was three times larger than for F_{Tot} in SH compared with SL and MH, in agreement with previous RSA studies conducted at SL (20, 36). This was also accompanied by a deteriorated D_{RF} across sprint repetitions and exacerbated by hypoxia severity, thereby indicating progressively shorter and less effective acceleration phases across repetitions. This, added to the fact that vertical impulse was not related to acceleration performance in highly-trained sprinters (37), is of particular relevance as applying ground reaction impulse in a more horizontal direction is crucial for an effective ability to accelerate from a standing start (34, 36) and explains 44% and 61% of the variance of V at 8 m (27) and 16 m (25) from the start. Altogether, these findings therefore corroborate the biomechanical assumption that applying forward-oriented total force against the ground is a key determinant

of repeated sprinting (20, 36). This reliance on F_H production during "all-out" sprinting (29) has recently been confirmed in elite sprinters using both treadmill (32) and force plate protocols (41). While during the initial steps of sprinting (acceleration phase), the biomechanical objective is to maximize the propulsive component of the GRF (39), such observations do lend some support to the proposed combination of ground force production and S_F (42) or vice versa.

Although it has been recently challenged at top running velocities in elite individuals (10), modeling the spring-mass behavior remains valid and reliable (17) at slower maximal running velocities than those reached by world-class sprinters (41). This model remains an interesting descriptor of the stance-limb mechanics during our RSA protocol in a group of recreational team-sport athletes. While the musculotendinous stiffness is believed to be a vital component of setting S_F during sprinting (12), the substantial impairment of the main kinematics and spring-mass factors of stride efficiency (*i.e.*, t_c, t_{swing}, Δ_Z and K_{vert}) induced by SH, compared with SL and MH, confirms that the maintenance of K_{vert} values under fatigue is paramount to minimize the increase in t_c and concomitant decrease in S_F (7, 20, 21). To which extent a stiffer musculotendinous system allows for preservation of efficient elastic energy contribution at maximal sprinting with exacerbated fatigue (*i.e.*, SH) (9), thereby maintaining force production during the concentric phase of the movement (9), needs to be ascertained.

Several potential limitations deserve attention. First, using step changes in FiO_2 as hypoxic stimulus is associated with larger inter-individual variability in the degree of arterial hypoxemia (and related stimulus at the muscle level) as opposed to pre-determined values of SpO_2 (8). That said, whether clamping SpO_2 will cause better heterogeneity in the oxygenation response and similar hypoxia-induced neuro-mechanical alterations compared with the 'classical' approach using fixed FiO_2 values is unknown. Second, it remains also to be verified whether hypobaric and normobaric hypoxia holds the same potential for alterations in (repeated) sprint performance and shares similar underlying neuro-mechanical

mechanisms (30). Third, with "repeated sprints in hypoxia" undergoing unprecedented popularity, quantifying neuro-mechanical responses of training sessions that include 3-5 sets of repeated sprints as performed here with different combinations of exercise-to-rest ratios (36) is needed to appropriately manage residual fatigue. Last, it may also be warranted to investigate the impact of an increase in muscle activation strategies through kinematic compensation (*i.e.*, joint actions and neuromuscular coordination) arising from team-sport specific training (*e.g.*, resistance or repeated sprints in hypoxia) and the effect that this may have during accelerated runs in field conditions.

To conclude, in severe normobaric hypoxia (FiO_2 = 13.3%; ~3600 m), impairments in repeated-sprint ability and in accompanying kinetics/kinematics and spring-mass characteristics exceed those observed near sea level (FiO_2 = 20.9%) and in moderate normobaric hypoxia (FiO_2 = 16.8%; ~1800 m). Specifically, severe hypoxia accentuates the RSA fatigue-related inability to effectively apply forward-oriented ground reaction force and to maintain vertical stiffness and stride frequency.

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The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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Chapter 9

Article 4 – High hypoxia increases alteration in maximal torque but not in rapid torque development in knee extensors after repeated treadmill sprinting

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High Altitude Increases Alteration in Maximal Torque but Not in Rapid Torque Development in Knee Extensors after Repeated Treadmill Sprinting

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We assessed knee extensor neuromuscular adjustments following repeated treadmill sprints in different normobaric hypoxia conditions, with special reference to rapid muscle torque production capacity. Thirteen team- and racquet-sport athletes undertook 8 × 5-s "all-out" sprints (passive recovery = 25 s) on a non-motorized treadmill in normoxia (NM; FiO_2 = 20.9%), at low (LA; FiO_2 = 16.8%) and high (HA; FiO_2 = 13.3%) normobaric hypoxia (simulated altitudes of ∼1800 m and ∼3600 m, respectively). Explosive (∼1 s; "fast" instruction) and maximal (∼5 s; "hard" instruction) voluntary isometric contractions (MVC) of the knee extensors (KE), with concurrent electromyographic (EMG) activity recordings of the *vastus lateralis* (VL) and *rectus femoris* (RF) muscles, were performed before and 1-min post-exercise. Rate of torque development (RTD) and EMG (i.e., Root Mean Square or RMS) rise from 0 to 30, −50, −100, and −200 ms were recorded, and were also normalized to maximal torque and EMG values, respectively. Distance covered during the first 5-s sprint was similar ($P > 0.05$) in all conditions. A larger ($P < 0.05$) sprint decrement score and a shorter ($P < 0.05$) cumulated distance covered over the eight sprints occurred in HA (−8 ± 4% and 178 ± 11 m) but not in LA (−7 ± 3% and 181 ± 10 m) compared to NM (−5 ± 2% and 183 ± 9 m). Compared to NM (−9 ± 7%), a larger ($P < 0.05$) reduction in MVC torque occurred post-exercise in HA (−14 ± 9%) but not in LA (-12 ± 7%), with no difference between NM and LA ($P > 0.05$). Irrespectively of condition ($P > 0.05$), peak RTD (−6 ± 11%; $P < 0.05$), and normalized peak RMS activity for VL (−8 ± 11%; $P = 0.07$) and RF (−14 ± 11%; $P < 0.01$) muscles were reduced post-exercise, whereas reductions ($P < 0.05$) in absolute RTD occurred within the 0–100 (−8 ± 9%) and 0–200 ms (−10 ± 8%) epochs after contraction onset. After normalization to MVC torque, there was no difference in RTD values. Additionally, the EMG rise for VL muscle was similar ($P > 0.05$), whereas it increased ($P < 0.05$) for RF muscle during all epochs post-exercise, independently of the conditions. In summary, alteration in repeated-sprint ability and post-exercise MVC decrease were greater at high altitude than in normoxia or at low altitude. However, the post-exercise alterations in RTD were similar between normoxia and low-to-high hypoxia.

Keywords: repeated-sprint ability, hypoxia, rapid torque development, neural drive, voluntary force production

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INTRODUCTION

Intense physical efforts performed at or near maximal speed and the ability to recover from it are important markers of successful in-game performance in high-intensity, intermittent sports (Spencer et al., 2005). Team- and racquet-sport players implement training methods, based on the repetition of maximal efforts in normoxia (e.g., repeated sprints, Bishop et al., 2011) or in hypoxia (e.g., repeated sprints in hypoxia; Brocherie et al., 2015a), for eliciting neuromuscular adaptations (e.g., enhanced muscle oxygenation and activation responses) and improving cardiovascular and metabolic function, in turn maximizing their physical performance. Although the physiological responses and potential metabolic limiting factors (i.e., limitations in energy supply, metabolite accumulation) associated with the completion of one repeated-sprint set have largely been described, there is comparatively less data on the neuromuscular consequences (Girard et al., 2011).

Neuromuscular fatigue is an exercise-induced reduction in the maximal isometric voluntary contraction (MVC) force/torque or power of a muscle group, which potentially involves alterations at any levels from the brain to skeletal muscles (Gandevia, 2001). Over the past decade, an increasing number of studies have quantified neuromuscular fatigue following repeated running (Perrey et al., 2010) or cycling (Racinais et al., 2007; Billaut et al., 2013; Girard et al., 2013; Hureau et al., 2014) sprints by comparing pre- to post-sprint values of force/torque, voluntary activation, electromyogram (EMG), and twitch responses. Recent studies assessed the neuromuscular function during the actual repeated-sprint sets (Goodall et al., 2015; Pearcey et al., 2015). With regard to MVC, however, it is important to note that maximal force/torque production capacity is most often obtained when exceeding 300 ms following contraction onset (Thorstensson et al., 1976). This is contrasting to the characteristics of muscular contraction occurring in several sporting events, where muscle force needs to be developed in less than 250 ms (e.g., sprints: Kuitunen et al., 2002; jumps: Luhtanen and Komi, 1979). Therefore, the ability to rapidly generate force/torque, i.e., the rate of force/torque development (RTD) within the initial (i.e., <250 ms) phase of an MVC which in turn correlates with sprint performance (Tillin et al., 2013), likely constitutes a more functional outcome measure (Girard and Millet, 2009). Therefore, RTD as a surrogate for explosive strength should be assessed for a better understanding of the acute neuromuscular adjustments to repeated sprinting.

Repeated-sprint ability is more altered at high (>3000–3500 m or FiO_2 below 13–14%) than lower altitudes, either normoxia or low-to-moderate (<3000 m or FiO_2 above 14%) altitude (Bowtell et al., 2014; Goods et al., 2014). Not only acute hypoxic exposure decreases convective O_2 transport (i.e., reduction in arterial O_2 saturation values or SpO_2), but also challenges multiple regulatory systems by increasing cardiorespiratory (i.e., higher heart rate, minute ventilation, O_2 debt), metabolic (i.e., slower muscle re-oxygenation responses) and/or neuromuscular (i.e., incomplete muscle activation) requirements during sprinting or subsequent recovery periods (Balsom et al., 1994; Billaut et al., 2013; Bowtell et al., 2014). Compared to normoxia, the completion of a repeated-sprint cycling protocol (15 × 5-s efforts, 25-s rest) in high hypoxia (FiO_2 = 14%) led to ∼8% lower total mechanical work as a result of impaired muscle activation, which was also accompanied by ∼6% lower post-exercise MVC (Billaut et al., 2013). However, these authors did not include any measure of explosive strength in their study.

To our knowledge, only one study has investigated the effect of repeated sprinting (i.e., 10 × 6-s "all out" cycling sprints, followed, after 6 min of passive rest, by 5 × 6-s sprints; recoveries = 30 s) performance on post-exercise alterations in rapid muscle torque production capacity of the knee extensors (KE) (Girard et al., 2013). MVC (−12%) and RTD (−15 to −26% from the 0–30 to 0–200 ms epochs after contraction onset) decreased during brief (i.e., 5 s) contractions after (i.e., 3 min) the repeated-sprint exercise. From this report, however, it is not entirely clear to which extent differences in RTD actually resulted from maximal voluntary strength adjustments, since RTD results were not normalized to MVC torque. Hence, whereas MVC torque losses following prolonged match-play tennis accounted for the dampened RTD values (Girard et al., 2014), fatigue induced by 10 sets of voluntary maximal explosive contractions exerted a more rapid and pronounced effect (particularly during the initial 50 ms of contraction) on explosive strength than MVC torque (Buckthorpe et al., 2014). Consequently, although repeated sprinting decreases RTD (Girard et al., 2013), the question of whether RTD at early and/or late intervals is more pronouncedly impaired than MVC has not been specifically addressed.

The analysis of EMG amplitude throughout the rising force-time curve can reveal how voluntary neural drive to skeletal muscle underlies the post-exercise decreased RTD. Hence, the rate of muscle activation is related to muscle shortening velocity (Nelson, 1996), a factor directly influencing RTD (Harridge et al., 1996). Furthermore, the determinants of explosive force production appear to change throughout the rising force-time curve (Folland et al., 2014) and fatigue may differentially affect the development of force throughout the time course of an explosive contraction. After repeated sprinting, non-significant reductions in *vastus lateralis* (VL) Root Mean Square (RMS) activities have been shown to accompany deteriorated RTD values (Girard et al., 2013). However, because the delay between exercise termination and post-exercise neuromuscular testing was 3 min in the aforementioned study, any meaningful changes in the central nervous performance may have already recovered. Furthermore, it has not been investigated if the early and later RTD time intervals (and associated rate of EMG activity rise) are in fact modified differently by performing repeated-sprint in various hypoxia severity levels. This question of the alteration in explosive strength post-hypoxic exposure/training is of high practical relevance in team- and racquet-sports but remains unclear: Hence, while countermovement jump performance increased to a similar extent after repeated-sprint training in normoxia vs. hypoxia (Brocherie et al., 2015a), movement velocity and power during the execution of a force-velocity in bench-press are improved when exposed to hypobaric vs. normobaric hypoxia in reference to normoxia (Feriche et al., 2014).

The aim of this study was therefore to assess the effects of repeated sprinting in different levels of normobaric hypoxia on the alterations in RTD and neuromuscular activity of KE. Given that there is "*extraordinarily little that changes with regard to maximal force-generating capacity with acute hypoxia*" (Perrey and Rupp, 2009), it was hypothesized that the already-known decrease in repeated-sprint ability (i.e., lower fatigue resistance) under high hypoxic conditions would not be associated with more pronounced alterations in explosive force production (i.e., RTD) compared to normoxia or low hypoxia.

METHODS

Participants

Thirteen male recreational team- (i.e., football, rugby, basketball) and racket- (i.e., tennis, squash) sport athletes (Mean ± SD: 31.2 ± 4.8 years; 178.4 ± 6.6 cm; 74.3 ± 8.2 kg) participated in the study. All participants were born and raised at <1000 m and had not traveled to elevations >1000 m in the 3 months prior to investigation. They gave their informed, written consent preceding the commencement of the experiment. Experimental protocol was conducted according to the Declaration of Helsinki for use of Human Subjects and approved by the Ethics Committee of *Shafallah Medical Genetics Center*.

Study Design

Elements have previously been reported in Girard et al. (2015a). About a week prior to testing, participants undertook a complete preliminary session where they performed short (<5 s) treadmill sprints at increasing intensities wearing a facemask for habituation (i.e., with the hypoxic system turned off), with full recovery and until being comfortable with the running technique required (which generally necessitated 7–10 trials). Then they performed three maximal 5-s single sprints, separated by 2 min of passive recovery, and after 5 min of rest, the repeated-sprint exercise test in full. All of them satisfied the criteria of having a coefficient of variation < 2.2% for distance covered across three successive trials (Girard et al., 2015b). Strong verbal encouragement was given during all maximal efforts. Participants were also thoroughly familiarized with the neuromuscular function assessment protocol (see *Neuromuscular Function*) until they felt accustomed with the equipment (i.e., coefficient of variation in three successive KE trials for peak RFD and maximal torque with "fast" and "hard" instructions lower than 5 and 3%, respectively).

Participants reported to the laboratory (well-ventilated at a constant temperature of ∼25°C and 40% relative humidity) on three different occasions (∼1 h; counterbalanced randomized crossover design in double-blind fashion) at least 3–4 days apart to complete an experimental session. This involved performing a repeated-sprint running protocol on a sprint treadmill (ADAL3D-WR, Medical Development – HEF Tecmachine, Andrézieux-Bouthéon, France), allowing participants to produce realistic acceleration and high running velocities (Morin et al., 2010). Participants performed their trials at the same time of the day (± 1 h) and wore similar sports gear (running shoes, short, and T-shirt). They were instructed to maintain their normal diet (i.e., avoiding any nutritional supplements or alcohol consumption), sleeping (i.e., ≥7 h/night) and training (i.e., avoiding vigorous exercise 24 h before every trial) habits during the 1–2 weeks period of testing to prevent any possible interference on their sprinting abilities. Participants were instructed to drink 4–6 mL of water per kilogram of body mass every 2.5 h on the day before each experimental session to ensure euhydration at the start of exercise. They were permitted to drink *ad libitum* during the warm-up procedure.

Experimental Protocol

Upon arrival on testing days, participants were instrumented and pre-exercise (Pre-tests) neuromuscular function assessment (see *Neuromuscular Function*) was conducted in normoxia. Thereafter, they completed a running warm-up (i.e., on the sprint treadmill with participants breathing ambient air) consisting of 5 min of running at 10 km.h^{-1}, followed by 10 min of sprint-specific muscular warm-up exercises [i.e., 3 × (skipping, high knee, butt-kick, high heels for ∼10 s with 30-s walking in between), followed by 3 × (3 steps accelerations at a subjective "sense of effort" of 7, 8, and 9 on a modified Borg 10 scale), then by 2 × (3-s sprints at a subjective "sense of effort" of 8 and 9)] (Christian et al., 2014). Afterwards, three maximal 5-s single sprints, separated by 2 min of passive recovery, were completed. After a facemask connected to a portable hypoxic generator (Altitrainer, SMTEC SA, Nyon, Switzerland) had been attached on participants, they were allowed 5-min of free cool-down prior to the repeated-sprint protocol. This exercise consisted of performing eight, 5-s "all-out" sprints interspersed with 25 s of passive rest and was randomly conducted in normoxia (NM; FiO_2 = 20.9%), in low and high simulated altitudes (normobaric hypoxia) of ∼1800 m (LA; FiO_2 = 16.8%) and ∼3600 m (HA; FiO_2 = 13.3%), respectively. Normobaric hypoxia was obtained by mixing nitrogen into ambient air under control of FiO_2. During recovery periods, participants stood quietly on the treadmill. Repeated-sprint ability was assessed from covered distance data using three scores: the largest (i.e., during the first sprint in all cases) distance ran, the cumulated distance covered over the eight sprints (i.e., sum of the eight sprints) and the sprint decrement score [i.e., ([cumulated distance/(largest distance × 8)]–1) × 100] (Girard et al., 2011). Finally, the neuromuscular function assessment was repeated (Post-tests) in normoxia (i.e., participants took off the facemask 25 s after completion of the last sprint) and was started exactly 1 min after the repeated-sprint exercise protocol ended.

Responses to Exercise

Heart rate and SpO_2 were monitored and estimated, respectively, via a Polar transmitter-receiver (Wearlink T-31, Polar Electro Oy, Kempele, Finland) and non-invasive pulse oximetry using a finger probe (Palmsat, 2500, NONIN Medical Inc., Plymouth, MI, USA). Together with heart rate and SpO_2, ratings of perceived exertion were recorded using the Borg 6–20 scale (i.e., 6 = no exertion at all, 20 = maximal exertion) exactly 10 s following each sprint (i.e., peak values likely to be obtained). Additionally, SpO_2 was recorded between before the warm-up

and 4 min after the last sprint. These time points corresponded to the end of pre- and post-tests.

Neuromuscular Function

Neuromuscular test sessions began by the completion of three successful MVCs, all brief (∼5 s) and separated by ≥30 s of rest, with a twitch delivered over the isometric plateau. Participants were instructed to increase torque production over a 1-s period, hold it for 3–4 s and then relax before completing the next contraction. Thereafter, participants were instructed to perform "explosive" MVCs (separated by ≥20 s). During all brief MVCs trials the participants were carefully instructed to contract "as fast as possible" for ∼1 s from a fully relaxed state, in an attempt to achieve at least 90% of their MVC torque. Participants were asked to avoid any countermovement before torque onset; i.e., they were reminded not to flex the knee immediately prior to KE. They were strongly encouraged with verbal feedback and a visual display of the torque production. Contractions that had any discernable countermovement or pre-tension (i.e., change of baseline torque of >1.5 Nm during the 100 ms before contraction onset; Girard et al., 2014) were discarded and another attempt was made. To provide biofeedback on whether a countermovement had occurred, the resting torque level was displayed on a sensitive scale. The slope of the torque–time curve (10 ms time constant) was displayed throughout testing and the peak slope was used to provide visual performance feedback to participants after each contraction. Pre-tests assessment was preceded by a warm-up consisting of 10 isometric contractions of ∼3–5 s in duration interspaced with ∼10–20 s of recovery. Contraction intensity was progressively self-adjusted by the participant to attain maximal torque in the last three contractions.

Recordings

Torque Measurements

KE torque was measured with participants seated upright on a custom-built adjustable chair with the hips and knees flexed at 90°. Restraining straps placed across the chest and hips secured the participants in the chair to prevent extraneous movement, while the dynamometer (Captels, St Mathieu de Treviers, France) was attached 3–5 cm above the tip of the lateral malleoli. During all contractions the torque signals were amplified, sent through an A/D board and sampled at 2000 Hz by commercially available hardware and software (MP35 and BSL Pro Version 3.6.7, Biopac Systems Inc., Santa Barbara, USA).

Electromyography

The EMG activity of the VL and *rectus femoris* (RF) muscles was recorded via bipolar Ag/AgCl electrodes (Ambu Blue sensor T, Ambu A/S, Denmark; diameter = 9 mm; inter-distance electrode = 30 mm) fixed longitudinally over the muscle bellies. The reference electrode was attached to the right wrist. Low impedance between the two electrodes was obtained by abrading the skin with emery paper and cleaning with alcohol. The position of the electrodes was marked for consistent placement. EMG signals were amplified (gain = 1000), filtered (band-width frequency 30–500 Hz) and recorded (sampling frequency = 2000 Hz) by commercially available hardware (Biopac MP35, systems Inc., Santa Barbara, CA) and software (Acqknowledge 3.6.7, Biopac Systems Inc., Santa Barbara, CA).

Motor Nerve Stimulation

Femoral nerve stimulations (400 V, rectangular pulse of 0.2 ms) were delivered by a high-voltage stimulator (Digitimer DS7AH; Digitimer, Hertfordshire, UK) via a cathode electrode (diameter of 5 mm) placed in the inguinal crease and an anode (5 × 10 cm; Medicompex, SA, Ecublens, Switzerland) in the gluteal fold. The intensity of stimulation was determined during the familiarization test session using a passive isometric recruitment curve (Racinais et al., 2013). Briefly, the stimulation intensity was increased by 10-mA increments until a maximal peak twitch torque was achieved and then a further increased by 50% to ensure constant supramaximal stimulation throughout the protocol.

Data Analysis

All analyses were performed using Spike 2 Software (Cambridge Electronic Design, Cambridge, UK). The MVC torque was defined as the maximum value recorded for 1 s when the torque had reached a plateau (before the superimposed twitch), and the RMS of the EMG activity was computed during the same 1-s period (RMS_{MAX}). Similarly, the peak-to-peak amplitude of superimposed maximum compound action potential (M-wave) responses was measured for each agonist muscle, and RMS_{MAX} was divided by M-wave to give a ratio RMS_{MAX}/M-wave.

The contractile RTD (expressed as $Nm.s^{-1}$) was derived from the "explosive" MVC measurements, as the average slope of the initial time phase of the torque-time curve at 0–30, 0–50, 0–100, and 0–200 ms, relative to the onset of contraction (Aagaard et al., 2002; Suetta et al., 2004; Thorlund et al., 2009) using a custom written program (Spike 2 Software, Cambridge Electronic Design, Cambridge, UK). The onset of muscle contraction was defined as the time point at which the torque curve exceeded baseline by >4.5 Nm, corresponding to ∼2.5% of MVC torque values (Andersen et al., 2010). The peak RTD was defined as the peak Δtorque/Δtime achieved during the initial 200 ms of the isometric contraction (de Oliveira et al., 2013; Girard et al., 2014). In addition, the rate of muscle activation (expressed as $mV.s^{-1}$) was measured as the raw RMS activity increase obtained at similar time intervals relative to onset integration (i.e., activity). The onset of EMG integration was shifted 50 ms before the onset of contraction to account for the presence of electromechanical delay (Aagaard et al., 2002; Girard et al., 2013). The RTD and EMG rise were also normalized relative to maximal MVC torque (%MVC) and maximal EMG activity (%RMS_{MAX}/M-wave). The mean over three trials was used for further analysis for each parameter.

Statistical Analysis

Values are expressed as means ± SD. Two-way repeated-measures ANOVAs [Time (Pre-tests vs. Post-tests) × Condition (NM, LA vs. HA)] were used to compare torque and muscle activation data for each time window (0–30, 0–50, 0–100, and 0–200 ms) independently for absolute and relative changes.

Outcome variables were tested using Mauchly's procedure for sphericity. Whenever the data violated the assumption of sphericity, *P*-values and adjusted degrees of freedom based on Greenhouse-Geisser correction were reported instead. Where significant effects were established, pairwise differences were identified using the Bonferroni *post-hoc* analysis procedure adjusted for multiple comparisons. For each ANOVA, partial eta-squared was calculated as measures of effect size. Values of 0.01, 0.06, and above 0.14 were considered as small, medium, and large, respectively. All statistical calculations were performed using SPSS statistical software V.21.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $P < 0.05$.

RESULTS

Repeated-Sprint Ability and Responses to Exercise

Distance covered during the first 5-s sprint was similar (24.2 ± 1.4, 24.5 ± 1.5, and 24.4 ± 1.7 m for NM, LA, and HA, respectively; $P > 0.05$) across conditions (**Figure 1**). In reference to sprint 1, distance covered decreased from sprint 2 onwards ($P < 0.001$), independently of the condition ($P = 0.324$). The averaged values of distance covered for sprints 1–8 were lower in HA (22.3 ± 1.3 m) compared to NM (22.9 ± 1.2 m; $P = 0.044$) but not LA (22.7 ± 1.3 m; $P = 0.183$), with also no difference between NM and LA ($P = 0.710$). A larger sprint decrement score occurred in HA (−7.8 ± 3.6%) vs. NM (−5.3 ± 1.9%; $P = 0.015$) but not LA (−6.3 ± 3.5%; $P = 0.060$), with also no difference between NM and LA ($P = 0.237$). Compared to NM (183.2 ± 9.3 m), the cumulated distance covered over the eight sprints was shorter in HA (178.5 ± 10.7 m; $P = 0.014$) but not in LA (181.4 ± 10.3 m; $P = 0.056$), with also no difference between NM and LA ($P = 0.240$).

Whereas, it did not change in NM (96.8 ± 0.8 vs. 96.0 ± 1.6%; $P = 0.455$), SpO_2 values decreased from the first to the last sprint in LA (95.0 ± 2.0 vs. 89.7 ± 4.0%; $P = 0.050$) and HA (88.8 ± 2.5 vs. 81.8 ± 4.5%; $P = 0.001$) (**Table 1**). Compared to pre-tests (96.9 ± 0.4%), SpO_2 values were not different among conditions during post-tests after completion of the repeated-sprint ability protocol (96.2 ± 0.5%; all conditions pooled, $P > 0.05$). Heart rate (NM: 150 ± 15 vs. 176 ± 12 bpm; LA: 154 ± 9 vs. 176 ± 9 bpm; HA: 154 ± 12 vs. 174 ± 13 bpm, all $P < 0.001$) and ratings of perceived exertion (NM: 11.8 ± 1.4 vs. 18.5 ± 1.2 points; LA: 11.3 ± 1.4 vs. 18.1 ± 1.6 points; HA: 11.5 ± 1.8 vs. 19.1 ± 0.8 points, all $P < 0.001$) increased from sprint 1 to sprint 8, irrespective of the environmental condition ($P = 0.256$). Higher ratings of perceived exertion values were recorded for the average of eight sprints in HA (15.8 ± 0.3 points) vs. LA (15.2 ± 0.3 points; $P = 0.006$), but not NM (15.5 ± 0.3 points; $P = 0.452$).

Maximal Strength

Compared to NM (−9 ± 7%), a larger ($P < 0.05$) reduction in MVC torque occurred from pre- to post-exercise in HA (−14 ± 9%; $P = 0.021$) but not in LA (−12 ± 7%; $P = 0.270$), with

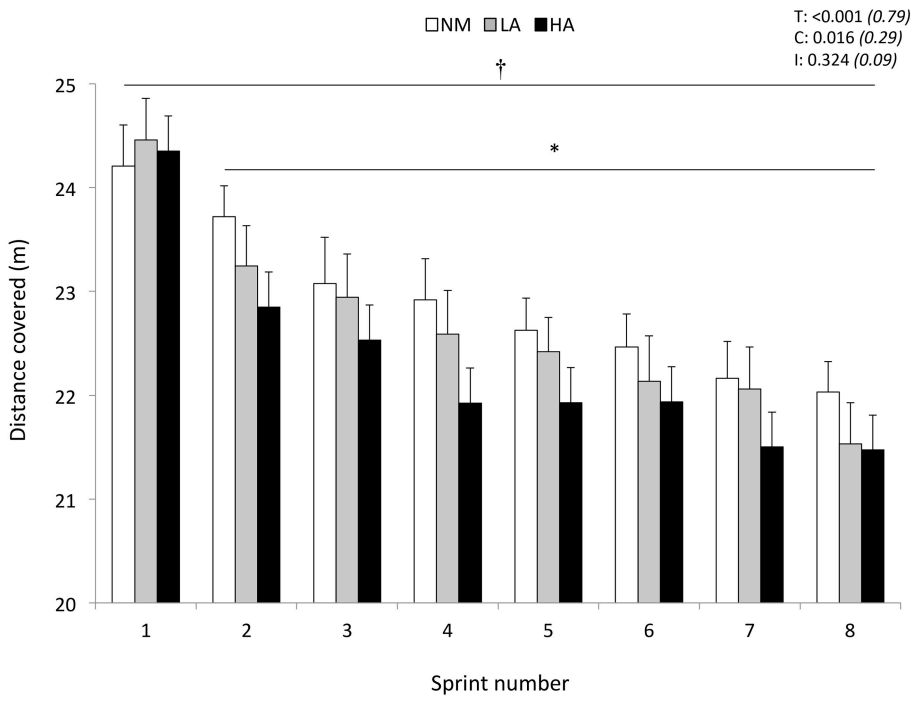

FIGURE 1 | Distance covered during the repeated-sprint ability test. Data are presented in normoxia (NM; $FiO_2 = 20.9\%$), at low (LA; $FiO_2 = 16.8\%$), and high (HA; $FiO_2 = 13.3\%$) normobaric hypoxia. Values are mean ± SD ($n = 13$). T, C, and I respectively refer to ANOVA main effects of time, condition, and interaction between these two factors with *P*-value and partial eta-squared in parentheses. *Significantly different from sprint 1 (all conditions pooled), $P < 0.05$. †NM different from HA (all sprints pooled), $P < 0.05$.

also no differences between LA and HA ($P = 0.340$) (**Table 2**). During MVCs, raw EMG signals of both VL and RF muscles were lower at post- relative to pre-, with no difference between conditions. Peak-to-peak M-wave amplitudes for both VL and RF muscles did not change. A global reduction of the RMS_{MAX}/M-wave ratio occurred from pre- to post-exercise for the RF ($-14 \pm 11\%$; $P = 0.002$), while failing to reach statistical significance for the VL ($-8 \pm 11\%$; $P = 0.075$).

Rapid Muscle Characteristics

Peak RTD (all conditions pooled: $-6 \pm 11\%$; $P = 0.031$) was significantly reduced from pre- to post-exercise (**Table 2**). Reduction in RTD (absolute values) occurred within the 0–100 ($-8 \pm 9\%$; $P = 0.011$) and 0–200 ms ($-10 \pm 8\%$; $P < 0.001$) epochs after contraction onset, independent of the condition ($P > 0.23$; **Figure 2**). No differences in RTD (relative values) were observed after normalization to MVC torque ($P > 0.197$; **Figure 3**). Furthermore, the relative rates of EMG rise for VL muscle for any epochs throughout the experimental protocol were not different between conditions ($P > 0.49$) and there were no interaction ($P > 0.42$) or time effects ($P > 0.12$) (**Figure 4**). The rate of EMG rise for RF muscle increased during the periods 0–30 ($+22 \pm 26\%$; $P = 0.006$), 0–50 ($+25 \pm 28\%$; $P = 0.002$), 0–100 ($+27 \pm 27\%$; $P < 0.001$), and 0–200 ms ($+23 \pm 2\%$; $P = 0.002$) post-exercise, independently of the condition ($P > 0.23$; **Figure 5**).

DISCUSSION

Repeated-Sprint Performance and Responses to Exercise

Distance ran during the first 5-s sprint was similar in all conditions, as an enhanced anaerobic energy release can compensate for the reduced aerobic ATP production during short maximal efforts in hypoxic conditions (Calbet et al., 2003). Compared to NM, a larger sprint decrement score and a shorter cumulated distance covered from sprint 1 to 8 occurred in HA, but not in LA, with also no difference between NM and LA. Consistent with previous repeated sprinting literature (Bowtell et al., 2014), our data therefore show that performance fatigability was significantly exacerbated relative to NM only under our severer hypoxic condition. The question of whether the same is true when completing identical repeated sprinting protocol at natural altitude (i.e., hypobaric hypoxia), known to induce severer physiological responses (Millet et al., 2012) and eventually larger neuromuscular alterations, has not been specifically addressed. Hence, the decrease in air density upon ascent to terrestrial altitude reduces air resistance, which is likely to decrease the energy cost of running at high velocities, and thereby improve single sprint performance (Levine et al., 2008). When sprints are repeated, however, hypobaric hypoxia would induce higher work of breathing responses and more detrimental neuromuscular consequences than exposure to gas mixtures lowering FiO_2. Despite lower SpO_2 values in the severer hypoxic condition, it is interesting to observe in this study that sensations that regulated the integrity of the performer (ratings of perceived exertion or perceived fatigability) and associated heart rate responses did not differ between SL and HA.

Neuromuscular Parameters during Maximal Contractions

Along with lower distance covered during the repeated-sprint test, post-exercise reduction in maximal KE torque, as measured from brief MVCs, was ∼5% larger in HA (−14%) compared to NM (−9%). This later result is in line with a previous study where the decrease in KE MVC torque under hypoxia ($FiO_2 = 14\%$) was 6% larger in reference to normoxia after the completion

TABLE 1 | Changes in responses to exercise during the repeated-sprint ability test in normoxia (NM; $FiO_2 = 20.9\%$), under low (LA; $FiO_2 = 16.8\%$) and high (HA; $FiO_2 = 13.3\%$) normobaric hypoxia.

Variables	Sprint number								ANOVA *(Partial eta-squared)*
	1	2	3	4	5	6	7	8	
SpO_2 (%)									
NM	96.8 ± 0.8	96.4 ± 1.9	96.3 ± 1.9	95.8 ± 2.0	96.2 ± 2.1	95.8 ± 2.0	95.8 ± 1.5	96.0 ± 1.6	T < 0.001 *(0.60)*
LA#	95.0 ± 2.0	94.3 ± 3.2	92.4 ± 3.4*	91.3 ± 2.9*	91.5 ± 3.0*	91.5 ± 3.0	89.6 ± 4.4*	89.7 ± 4.0*	C < 0.001 *(0.85)*
HA#,†	88.8 ± 2.5	86.1 ± 4.5	84.5 ± 4.1*	84.0 ± 4.9*	83.6 ± 5.2*	83.2 ± 4.6*	82.5 ± 5.3*	81.8 ± 4.5*	I = 0.016 *(0.24)*
HR (bpm)									
NM	150 ± 15	164 ± 13*	169 ± 13*	171 ± 12*	174 ± 12*	175 ± 11*	175 ± 11*	176 ± 12*	T < 0.001 *(0.92)*
LA	154 ± 9	166 ± 11*	172 ± 9*	175 ± 10*	176 ± 10*	176 ± 11*	176 ± 10*	176 ± 9*	C = 0.427 *(0.07)*
HA	154 ± 12	166 ± 15*	171 ± 13*	174 ± 14*	174 ± 13*	175 ± 12*	174 ± 12*	174 ± 13*	I = 0.187 *(0.12)*
RPE (POINTS)									
NM	11.8 ± 1.4	13.0 ± 1.1*	14.3 ± 1.4*	15.5 ± 1.5*	16.4 ± 1.7*	17.1 ± 1.6*	17.7 ± 1.5*	18.5 ± 1.2*	T < 0.001 *(0.95)*
LA	11.3 ± 1.4	12.7 ± 1.1*	13.8 ± 1.2*	15.1 ± 1.1*	16.1 ± 1.2*	17.0 ± 1.5*	17.6 ± 1.4*	18.1 ± 1.6*	C = 0.034 *(0.25)*
HA†	11.5 ± 1.8	13.0 ± 1.6*	14.4 ± 1.6*	15.7 ± 1.6*	16.7 ± 1.4*	17.5 ± 1.3*	18.4 ± 1.1*	19.1 ± 0.8*	I = 0.256 *(0.10)*

Mean ± SD (n = 13).

SpO_2, arterial O_2 saturation; HR, heart rate; RPE, ratings of perceived exertion.

**Significantly different from sprint 1 ($P < 0.05$). #and †significantly different from NM and LA, respectively ($P < 0.05$).*

TABLE 2 | Neuromuscular parameters recorded during brief explosive maximal knee extension before (Pre-tests) and after (Post-tests) repeated sprinting in normoxia (NM; FiO_2 = 20.9%), under low (LA; FiO_2 = 16.8%) and high (HA; FiO_2 = 13.3%) normobaric hypoxia.

Variables	Pre-tests			Post-tests			ANOVA *(Partial eta-squared)*		
	NM	LA	HA	NM	LA	HA	Time	Condition	Interaction
MVC torque (Nm)	271.0 ± 45.6	269.5 ± 41.9	270.1 ± 42.3	246.5 ± 35.4*	237.6 ± 39.0*	232.3 ± 40.8*#	<0.001 *(0.77)*	0.085 *(0.19)*	0.021 *(0.28)*
Peak RTD (Nm.s)	1018 ± 122	1074 ± 208	986 ± 195	965 ± 133*	948 ± 152*	942 ± 199*	0.031 *(0.33)*	0.024 *(0.11)*	0.040 *(0.24)*
RMS_{MAX}_VL (mV)	0.604 ± 0.112	0.599 ± 0.110	0.597 ± 0.111	0.568 ± 0.107	0.524 ± 0.093	0.553 ± 0.110	<0.001 *(0.59)*	0.159 *(0.14)*	0.117 *(0.16)*
RMS_{MAX}_RF (mV)	0.449 ± 0.173	0.451 ± 0.158	0.449 ± 0.160	0.403 ± 0.162	0.398 ± 0.147	0.395 ± 0.169	<0.001 *(0.65)*	0.927 *(0.06)*	0.777 *(0.13)*
M-wave_VL (mV)	11.4 ± 4.5	12.0 ± 4.5	11.4 ± 4.6	11.5 ± 4.5	11.3 ± 4.4	11.9 ± 4.9	0.903 *(0.01)*	0.905 *(0.08)*	0.176 *(0.15)*
M-wave_RF (mV)	12.4 ± 2.0	12.6 ± 3.2	12.4 ± 2.0	13.0 ± 2.1	12.5 ± 1.4	12.8 ± 1.7	0.436 *(0.051)*	0.914 *(0.07)*	0.570 *(0.05)*
RMS_{MAX}/M-wave_VL (mV)	0.054 ± 0.027	0.052 ± 0.025	0.056 ± 0.030	0.051 ± 0.025	0.049 ± 0.029	0.048 ± 0.022	0.075 *(0.24)*	0.643 *(0.04)*	0.395 *(0.07)*
RMS_{MAX}/M-wave_RF (mV)	0.038 ± 0.018	0.039 ± 0.020	0.037 ± 0.017	0.033 ± 0.016	0.033 ± 0.013	0.031 ± 0.015	0.002 *(0.57)*	0.053 *(0.05)*	0.813 *(0.02)*

Mean ± SD (n = 13).

MVC torque, maximal voluntary contraction torque; Peak RTD, peak rate of torque development; RMS_{MAX}, M-wave and RMS_{MAX}/M-wave represent the average of root mean square, maximal M-waves, and normalized electromyogram activity of vastus lateralis (VL) and rectus femoris (RF) muscles.

**Significantly different from Pre-tests ($P < 0.05$). #Significantly different from NM ($P < 0.05$).*

of 15 5-s cycling sprints interspersed with 25-s of rest (Billaut et al., 2013). Despite not supported by statistical analysis, our novel finding was that a graded effect of hypoxia was visible for strength losses post-repeated sprinting with progressively larger values (−9, −12, and −14%) with increasing severities of acute hypoxia. This extends similar findings following maximal intermittent dynamic leg extension where KE MVC torque losses increased (yet non-significantly) with hypoxia severity (−18, −19, and −27% in FiO_2 = 21, 14, and 10%, respectively) (Christian et al., 2014). Contrastingly, performing sets of intermittent, isometric, quadriceps contractions at 60% of MVC force to task failure in normoxia, mild hypoxia, moderate hypoxia and severe hypoxia (FiO_2 = 21, 16, 13, and 10%) resulted in ∼30% declines in MVC force in all conditions, despite large differences in time-to-task failure (24.7 vs. 15.9 min in FiO_2 = 21 vs. 10%) (Goodall et al., 2010). Potentially, differences in exercise mode/duration/intensity and individuals' tolerance to hypoxic stress, in turn affecting the magnitude of reduction in convective O_2 transport, may explain aforementioned diverging results on MVC torque and further confirm that fatigue is task-dependent.

Also worth noticing, the effect of fatigue on M-wave changes during repeated sprinting is not clear-cut in literature, with reports of decreased (Perrey et al., 2010), unchanged (Girard et al., 2013), and increased (Racinais et al., 2007) amplitudes. Consequently, normalizing the raw EMG amplitude to a maximum compound action potential (M-wave) is a methodological requirement allowing control for any changes in neuromuscular junction and sarcolemma excitation, and hence, enhancing the sensitivity of EMG amplitude measurements. In this study, maximal normalized EMG activity (RMS_{MAX}/M-wave ratio) was dampened during MVCs in VL (−8%; albeit not significantly different) and RF (−14%) muscles. This suggests that the magnitude of the efferent motor outflow reaching the KE was adversely affected by the repetition of eight maximal sprints, confirming previous observations (Girard et al., 2015a; Brocherie et al., 2015b), while severity of hypoxic exposure had only minimal effect.

Rapid Muscle Force Characteristics

In addition to the post-exercise decrement in MVC torque, this study is unique as far as we are aware in reporting that the altitude severity (at least to 3600 m) did not modify the post-exercise RTD responses: the early phase RTD (30 and 50 ms) did not change, whereas the decline in peak RTD and absolute RTD values in the late phase (100 and 200 ms) of muscle contraction was similar between the normoxic and hypoxic conditions. In hot and cool conditions, a global (i.e., at all time intervals) downward-shift in the contractile RTD occurred after repeated cycling sprints (Girard et al., 2013). As it was unrelated to environmental conditions (i.e., modest hyperthermia), this was taken to reflect an overall fatigue-induced reduction in rapid muscle force characteristics. In the present study adopting a running mode, the difference between early (unchanged) and late (decreased) phases absolute RTDs may relate to the relative influence of passive stiffness in serial/lateral force transmission structures, myofiber cross-bridge kinetics and neural drive (Edman and Josephson, 2007).

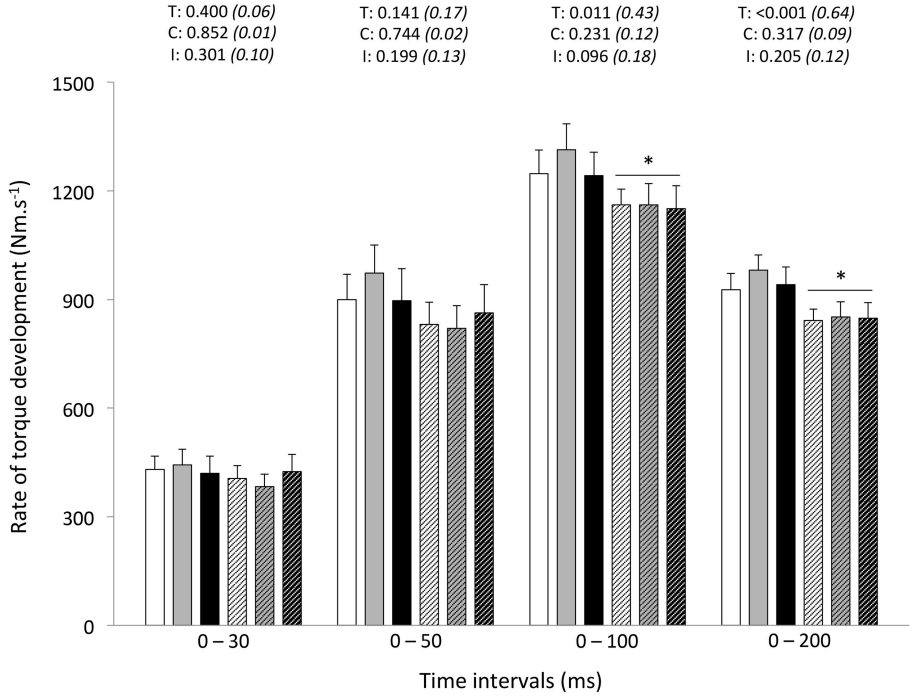

FIGURE 2 | Rate of torque development (absolute values) during explosive isometric knee extension obtained at 0–30, −50, −100, and −200 ms prior to (Pre-tests; full bars) and following (Post-tests; dashed bars) repeated sprinting in normoxia (NM; FiO_2 = 20.9%; white bars), at low (LA; FiO_2 = 16.8%; gray bars) and high (HA; FiO_2 = 13.3%; black bars) normobaric hypoxia. Values are mean ± SD (n = 13). T, C, and I respectively refer to ANOVA main effects of time, condition, and interaction between these two factors with P-value and partial eta-squared in parentheses. *Significantly different from Pre-tests, $P < 0.05$.

With late phase RTD more likely be affected by the fiber type composition (Penailillo et al., 2014), a more pronounced fatigue of faster fiber types after repeated running sprinting (Girard et al., 2015a), in turn associated with slower cross-bridge kinetics (Hamada et al., 2003), may dictate observed magnitude of post-exercise alterations in absolute RTD. Importantly, the exercise-related decline seen for the late-phase absolute RTD values occurred regardless of the environmental condition. In line with these findings, we have recently reported that heat stress does not exacerbate alterations in rapid muscle torque production capacity of KE neither after repeated cycling sprints (Girard et al., 2013) nor prolonged tennis playing (Girard et al., 2014).

In this study, when controlling for fatigue-induced reduction in maximal strength, we failed to demonstrate significant reductions in explosive strength post-exercise at any time interval. In fact, RTD is increasingly related to maximal muscle force, and reliance on muscle contractile properties decreases, as the time (mostly from 90 ms) from the onset of contraction increases (Andersen and Aagaard, 2006). The time interval in which RTD is determined influences the nature of the association between maximal muscle force and RTD and, in turn, the nature of fatigue-induced responses. By using different experimental designs (i.e., resistance training and verbal instructions during protocol), however, others have questioned the direct relationship between maximal force and RTD (Griffin and Cafarelli, 2005; Holtermann et al., 2007). Regardless, our data show the influence of maximal strength in maintaining normalized RFD values at all time intervals after repeated sprinting in differing hypoxic conditions.

Muscle Activation Rise

Whereas maximal VL muscle activation capacity (RMS_{MAX}/M-wave ratio) was impaired by sprints repetition, the ability to rapidly activate the VL muscle, as inferred from the rate of EMG amplitude rise, did not change in our study. Consistently, whole-body exercise studies, for instance following simulated team- (football: Thorlund et al., 2009; handball: Thorlund et al., 2008) and racket- (tennis: Girard et al., 2014) sport matches or a repeated cycling sprint protocol (Girard et al., 2013), show that reductions in RTDs are generally not associated with significant decreases in EMG values of quadriceps muscles. Opposite results have been reported by Morel et al. (2015) after the completion of 20 sets of 6-s isokinetic maximal KE at 240° s^{-1}, starting every 30 s. In their study, reductions of RTD were associated with a dampened activation capacity of the VL muscle in the early phase of muscle contraction, whereas participants were capable of producing similar maximal activation levels (Morel et al., 2015). Methodological differences (e.g., various time intervals, manual vs. automated methods to detect contraction onset; instructions to the participants during the contraction execution) preclude meaningful comparisons of EMG responses between studies.

In our study, the fact that RF muscle activation rise increased (at all time intervals) from pre- to post-exercise, despite

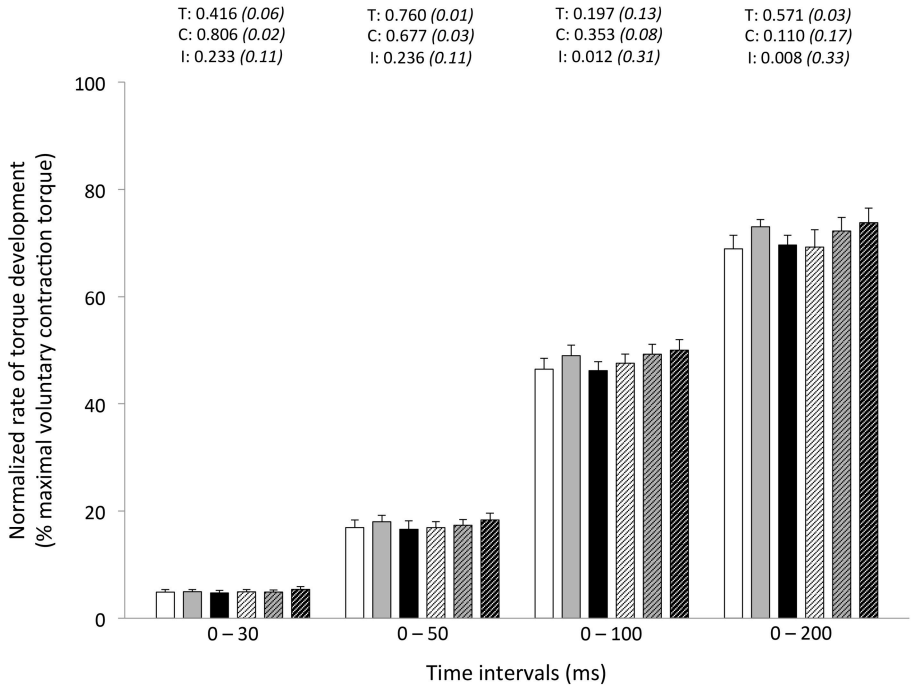

FIGURE 3 | Normalized rate of torque development (% maximal voluntary contraction torque) during explosive isometric knee extension obtained at 0–30, −50, −100, and −200 ms prior to (Pre-tests; full bars) and following (Post-tests; dashed bars) repeated sprinting in normoxia (NM; FiO_2 = 20.9%; white bars), at low (LA; FiO_2 = 16.8%; gray bars) and high (HA; FiO_2 = 13.3%; black bars) normobaric hypoxia. Values are mean ± SD (n = 13). T, C, and I respectively refer to ANOVA main effects of time, condition and interaction between these two factors with P-value and partial eta-squared in parentheses.

significant alteration of the ability to maximally activate this muscle, is an interesting observation. Considering the different activation sequences of this bi-articular muscle during sprinting (Morin et al., 2015) and the observation of reductions in RF RMS activity levels over sprints repetition (Brocherie et al., 2015b), it cannot be ruled out that this finding may partly depend on our non-specific testing position (i.e., marked hip extension while seating). Potentially, different results would occur when adopting another posture (i.e., when lying down) since knee position (RF muscle length) significantly affects quadriceps activation strategies (Krishnan et al., 2011). While this result was unexpected, larger activation in the *soleus* muscle has also been observed +24-h post-football game in hot vs. neutral environment (Girard et al., 2015c). Explosive force production depends on muscle fascicle shortening velocity and the tendon's elastic energy storage capacity, with tendon stiffness in turn affecting the time lag between muscle activation and muscle force production (i.e., electromechanical delay) (Proske and Morgan, 1987). In our study, a fixed electromechanical delay was used to determine EMG onset rise. As such, the role of tension-sensitive mechanoreceptors located in the muscle (e.g., Golgi tendon organs and muscle spindles) in influencing the tendon's stiffness, and thereby length change of the muscle fibers during explosive KE through proprioceptive feedback, probably did not change. A further result was to demonstrate that hypoxia severity had no effect on post-exercise adjustments in rapid muscle activation capacity. Potentially, longer sprints, shorter recoveries or combination of both (more intense exercise-to-rest ratios) and/or the use or severer hypoxic conditions might yield more unfavorable results with respect to RTD adjustments resulting directly to decreases in muscle activation rates if greater fatigue levels could be attained.

Additional Considerations

Importantly, a marked reduction in the intrinsic contractile capacity for explosive force production cannot be ruled out, as substantial peripheral locomotor muscle fatigue development (i.e., twitch torque decrease) usually occur as a result of repeated sprinting, independently of hypoxic severity (Billaut et al., 2013). Assessment of electrically evoked RTD can give insight into the intrinsic capacity of the muscle-tendon unit for explosive force production without the influence of voluntary control. This can be investigated by examining the response to a single or ideally high frequency contractions, such as an evoked octet (e.g., eight pulses at 300 Hz; Buckthorpe et al., 2012), to reliability evoke the maximum capacity for RTD.

In our study, all pre- and post-neuromuscular assessments were performed in normoxia with similar SpO_2 values of 96–97%. Reportedly, SpO_2 recovery response after an acute exposure to normobaric hypoxia (FiO_2 = 10%) decreasing SpO_2 to 85% is ∼2 min (Krivoshchekov et al., 2014). With hypoxic simulation applied during the repeated sprint exercise through the use of a facemask, participants were switched

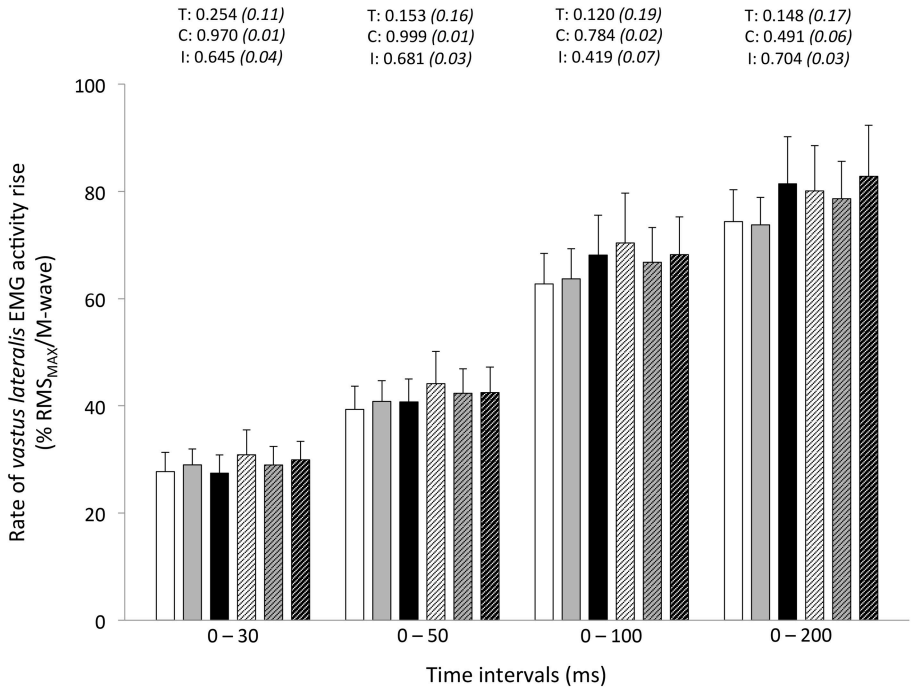

FIGURE 4 | Rate of *vastus lateralis* EMG activity rise (% RMS_{MAX}/M-wave) during explosive isometric knee extension obtained at 0–30, −50, −100, and −200 ms prior to (Pre-tests; full bars) and following (Post-tests; dashed bars) repeated sprinting in normoxia (NM; FiO_2 = 20.9%; white bars), at low (LA; FiO_2 = 16.8%; gray bars) and high (HA; FiO_2 = 13.3%; black bars) normobaric hypoxia. Values are mean ± SD (n = 13). T, C, and I respectively refer to ANOVA main effects of time, condition and interaction between these two factors with P-value and partial eta-squared in parentheses.

to normoxic breathing immediately (within seconds) after exercise cessation and seated on the chair, located near by the treadmill, for post-test assessment that started exactly 1 min after the last sprint completion. Whether this maneuver induced a faster recovery of neuromuscular function parameters, compared to situations where individuals continued breathing a hypoxic mixture (i.e., similar to exercise conditions), is unknown.

When evaluating RTD, most of the studies have used the same contraction, with a "hard and strong" instruction, to evaluate explosive and maximal voluntary strength capacities. The potential problem associated with this practice, however, is that only ballistic contractions (i.e., force production as fast as possible followed by muscle relaxation as soon the target force is reached) allow a careful evaluation of the maximal discharge rate of motor neurons (Duchateau and Baudry, 2014). Compared with a "hard-and-fast instruction," the steeper force development with a "fast" only instruction relates to a better activation of the agonist muscles at contraction onset (Sahaly et al., 2003). Although using this methodological precaution required a greater number of contractions (and potentially may have induced some recovery in neuromuscular function), we felt that it was a necessary prerequisite not to underestimate the true rate of muscle activation of our participants.

Because elite players are more accustomed to repeated-sprint activities, one can assume that, in comparison to recreational team-sport participants involved here, they may have been able to better resist fatigue. In a group of 17 healthy recreationally active individuals, those facing the greatest RTD reductions also experienced the largest fatigue rate during a Wingate cycle ergometer test and greater fatigue during an electrical stimulation protocol (Morris et al., 2010). Along the same line, only males were studied here, while females are generally less fatigable compared with men during repeated-sprint protocols (Billaut and Bishop, 2009). Whether performance level and/or gender differences exist regarding neuromuscular consequences (with special references to explosive strength), when completing repeated-sprint exercises at different altitudes, warrant further investigation.

CONCLUSION

In summary, alteration in repeated-sprint ability and post- KE MVC was greater under high altitude than in normoxia or at low altitude. In the KE, peak and late phase (>100 ms) contractile RTD decreased post-exercise to the same extent between conditions. However, contractile RTDs were not different after normalization to MVC torque, indicating that post-exercise strength losses accounted for the decrease in RTD. Additionally, we reported that repeated running sprints do not negatively influence the capacity of the central nervous

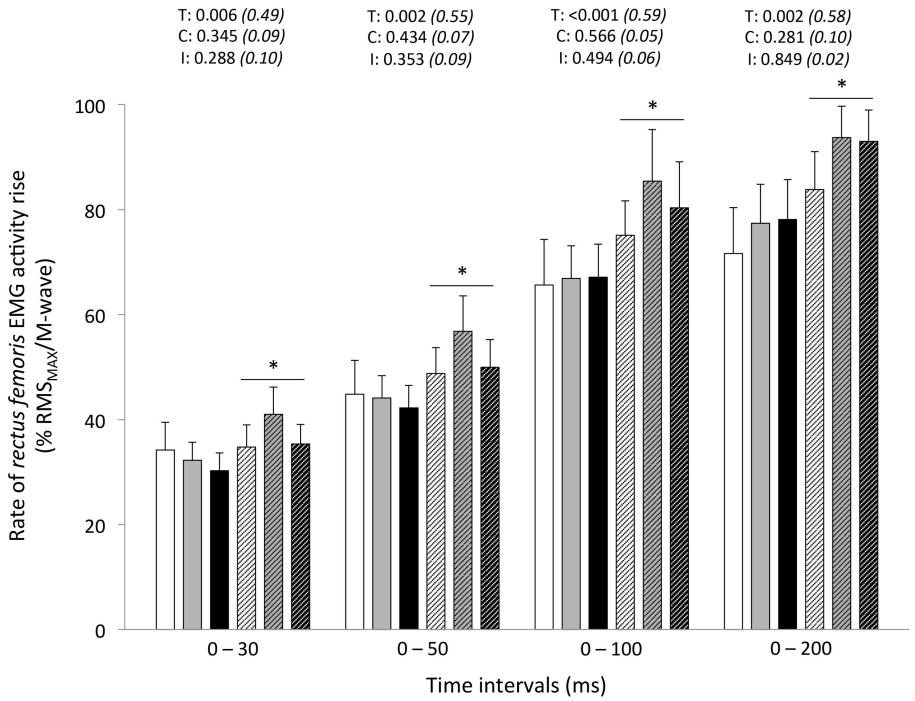

FIGURE 5 | Rate of *rectus femoris* EMG activity rise (% RMS_{MAX}/M-wave) during explosive isometric knee extension obtained at 0–30, −50, −100, and −200 ms prior to (Pre-tests; full bars) and following (Post-tests; dashed bars) repeated sprinting in normoxia (NM; FiO_2 = 20.9%; white bars), at low (LA; FiO_2 = 16.8%; gray bars) and high (HA; FiO_2 = 13.3%; black bars) normobaric hypoxia. Values are mean ± SD (n = 13). T, C, and I respectively refer to ANOVA main effects of time, condition and interaction between these two factors with P-value and partial eta-squared in parentheses. *Significantly different from Pre-tests, $P < 0.05$.

system to rapidly activate the VL (unchanged) and RF (improved) muscles during the first 200 ms, whereas maximal activation was dampened later during the contraction. Finally, normobaric hypoxia exposure had no additional influence on post-exercise alterations in rapid muscle torque production of the KE.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: OG, FB, GM. Performed experiments: OG, FB. Analyzed data: OG. Interpreted results of research: OG. Drafted manuscript and prepared tables/figures: OG. Edited, critically revised paper, and approved final version of manuscript: OG, FB, GM.

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Chapter 10

Article 5 – High-intensity intermittent training in hypoxia: a double-blinded, placebo-controlled field study in youth football players

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High-Intensity Intermittent Training in Hypoxia: A Double-Blinded, Placebo-Controlled Field Study in Youth Football Players

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Abstract

Brocherie, F, Girard, O, Faiss, R, and Millet, GP. High-intensity intermittent training in hypoxia: A double-blinded, placebo-controlled field study in youth football players. *J Strength Cond Res* 29(1): 226–237, 2015—This study examined the effects of 5 weeks (~60 minutes per training, 2 $d \cdot wk^{-1}$) of run-based high-intensity repeated-sprint ability (RSA) and explosive strength/agility/sprint training in either normobaric hypoxia repeated sprints in hypoxia (RSH; inspired oxygen fraction [F_IO_2] = 14.3%) or repeated sprints in normoxia (RSN; F_IO_2 = 21.0%) on physical performance in 16 highly trained, under-18 male footballers. For both RSH (n = 8) and RSN (n = 8) groups, lower-limb explosive power, sprinting (10–40 m) times, maximal aerobic speed, repeated-sprint (10 × 30 m, 30-s rest) and repeated-agility (RA) (6 × 20 m, 30-s rest) abilities were evaluated in normoxia before and after supervised training. Lower-limb explosive power (+6.5 ± 1.9% vs. +5.0 ± 7.6% for RSH and RSN, respectively; both $p < 0.001$) and performance during maximal sprinting increased (from −6.6 ± 2.2% vs. −4.3 ± 2.6% at 10 m to −1.7 ± 1.7% vs. −1.3 ± 2.3% at 40 m for RSH and RSN, respectively; p values ranging from <0.05 to <0.01) to a similar extent in RSH and RSN. Both groups improved best (−3.0 ± 1.7% vs. −2.3 ± 1.8%; both $p \leq 0.05$) and mean (−3.2 ± 1.7%, $p < 0.01$ vs. −1.9 ± 2.6%, $p \leq 0.05$ for RSH and RSN, respectively) repeated-sprint times, whereas sprint decrement did not change. Significant interactions effects ($p \leq 0.05$) between condition and time were found for RA ability–related parameters with very likely greater gains ($p \leq 0.05$) for RSH than RSN (initial sprint: 4.4 ± 1.9% vs. 2.0 ± 1.7% and cumulated times: 4.3 ± 0.6% vs. 2.4 ± 1.7%). Maximal aerobic speed remained unchanged throughout the protocol. In youth highly trained football players, the addition of 10 repeated-sprint training sessions performed in hypoxia vs. normoxia to their regular football practice over a 5-week in-season period was more efficient at enhancing RA ability (including direction changes), whereas it had no additional effect on improvements in lower-limb explosive power, maximal sprinting, and RSA performance.

Key Words normobaric hypoxia, hypoxic training, repeated-sprint ability, agility, football (soccer)

Introduction

In football (soccer), players must possess well-developed technical and tactical skills and a high fitness to cope with the physical demands of the game at the professional level (54). Despite a large number of tests and the growing scientific debate regarding the tradeoffs between laboratory and field evaluation, valid and reliable field tests (50) are usually preferred for performance evaluation to better reflect football specificity (54). Such field tests should include an evaluation of aerobic endurance, muscular strength, peak speed and power, acceleration, repeated-sprint ability (RSA), and the ability to change direction (COD) to reflect the complexity of the game. Crucial determinants of match-winning situations include short-sprint accelerations, jumps, and football-specific skills (e.g., dribbling, short-passing ability, shooting), requiring well-developed muscle strength capacity and neuromuscular control strategies (43,49).

To enhance these run-based abilities, diverse training strategies focusing on important physical aspects of the game (i.e., aerobic, sprint, repeated sprint and resistance training) have been proposed (3,6). Considering the physiological requirements and high energetic demands of football match play (i.e., sustained [90 minutes] high-intensity intermittent activity with mean and peak heart rates of approximately 85 and 98% of maximal values) (28), training modalities that specifically enhance the capacity to repeat

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Table 1. Subjects' anthropometric characteristics.*†

	RSN ($n = 8$)	RSH ($n = 8$)	RSH-RSN (Cohen's $d \pm 95\%$ CL)
Age, y	17.1 ± 0.2 (16.7–17.5)	17.1 ± 0.3 (16.6–17.4)	0.26 ± 1.21
Weight, kg	56.1 ± 9.1 (45.2–69.1)	62.4 ± 6.1 (51.7–68.2)	0.62 ± 1.66
Height, cm	170.0 ± 5.5 (162.8–177.6)	172.5 ± 3.0 (168.7–175.7)	0.69 ± 1.11
BMI, $kg \cdot m^{-2}$	19.3 ± 2.4 (16.1–21.9)	21.0 ± 1.7 (18.2–22.8)	0.47 ± 1.89
Sum of 7 skinfolds, mm	41.4 ± 4.6 (35.4–48.7)	48.2 ± 13.7 (31.3–72.9)	0.87 ± 2.18

*RSN = repeated-sprint in normoxia; RSH = repeated-sprint in hypoxia; CL = confidence limits.
†Values are given as mean ± *SD* (range), between-condition differences (Cohen's $d \pm 95\%$ CL).

high-intensity efforts have clear benefits for these athletes (12,53), with a likely direct transfer to the playing field (28). For instance, both high-intensity intermittent exercise and RSA are complex fitness components that depend on both muscular (e.g., oxidative capacity, phosphocreatine recovery, and H+ buffering) and neural (e.g., muscle activation and recruitment strategies) factors among others. Although the best training methods to improve RSA are widely debated (3,6), the additional use of hypoxic stress in sport-specific test settings has recently been introduced to the discussion (2,39) with the emergence of new promising training strategies including repeated sprints in hypoxia (RSH) (20,22,40,47). Beside the wide range of exercise prescription and denomination beyond the scope of high-intensity intermittent training (55) and according to the recently updated nomenclature for the different hypoxic methods available for team sports (40), we would preferably use the terms related to RSA to define the training prescribed in this study.

Repeated sprints in hypoxia are based on the repetition of "all-out" efforts of short (<30 seconds) duration interspersed with short incomplete recoveries under hypoxic conditions (40). It differs from intermittent hypoxic training because the intensity of the training stimulus is maximal and presumably allows for maintaining high fast-twitch recruitment so that positive results when adding hypoxia to training can be expected. A RSH training stimulus is particularly interesting because under hypoxic conditions (up to 3,800 m) single-sprint performance of short duration (<10 seconds) is generally preserved, whereas fatigue resistance during RSA tests is compromised with earlier and larger decrements in mechanical work (4). For example, Bowtell et al. (4) has recently demonstrated that

Table 2. Training content during a typical training week.*†

Day	1	2	3	4	5	6	7
Morning							
Activity	Football (skills + tactics)	Football (skills + tactics)	Football (skills + tactics)	Football (tactics)	Recovery/ skills	Football (agility + skills)	Off
Duration, min	60	60	60	30	45	30	
Intensity, % HR_{max}	~60	~60	~70	~50	~50	~70	
Afternoon							
Activity	**Conditioning**	Football (skills + tactics)	**Conditioning**	Friendly match (or training)	Football (skills + tactics)	Domestic match	Off
Duration, min	**60**	75	**60**	90	75	90	
Intensity, % HR_{max}	**~90–100**	~70	**~90–100**	~80	~70	~85–90	

*% HRmax = % of maximal heart rate.
†Cells in bold refer to activities performed in hypoxic environment.

during nonmotorized treadmill repeated-sprint runs (10 × 6-s sprints with 30-second rest), the peak running speed was relative resilient to hypoxic exposure (inspired oxygen fraction [F_IO_2] ranging from 12 to 15%).

Compared with similar training at sea level, RSH seems to induce larger benefits on glycolytic pathways, skeletal muscle adaptation, and ventilatory responses, which suggests putative stronger benefits for team sport physical performance (19). For instance, RSH was shown to delay fatigue during an RSA cycling test (i.e., 10-s sprints interspersed with 20-s recoveries) to exhaustion by 40% compared with the same training performed (repeated sprints) in normoxia (RSN) (20). An additional benefit of RSH vs. RSN was also found by Galvin et al. (22) with well-trained academy rugby union and rugby league players' intermittent running performance (i.e., Yo-Yo Intermittent Recovery Test level 1) being improved by 33% after 4 weeks of training including 120 running sprints of 6 seconds. However, with only 1 study including team sport athletes (22), further studies are still needed to endorse the efficacy of RSH in football players, notably by testing more ecological game situations including agility tests and overground (repeated) sprints (19).

Using a randomized, double-blinded placebo-controlled design, this study aimed at comparing the effects of run-based RSH vs. RSN, combined with sea level football-specific training on several physical fitness parameters in highly trained youth male football players. We hypothesized that RSH would improve neuromuscular factors (i.e., lower-limb explosive power and sprinting abilities) and specific repeated-sprint abilities performance to a greater extent than the equivalent training in normoxia (RSN).

Methods

Experimental Approach to the Problem

The study was designed as a randomized, balanced, double-blinded placebo-controlled trial, in which the experimental intervention consisted of an additional RSH ($F_IO_2 = 14.3\%$) vs. RSN ($F_IO_2 = 21.0\%$) training to the usual in-season sea-level football training routine for 5 weeks (~60 minutes/training, 2 $d \cdot wk^{-1}$) at spring 2008.

After baseline, including 2 familiarization training sessions performed once during the week preceding the supervised training period and premeasurements (Pre-), all participants were randomly assigned to 1 of the 2 training groups based on their age and physical performance results. Training content included high-intensity intermittent and RSA exercises and explosive strength/agility tasks either in hypoxia (RSH, $n = 8$) or normoxia (placebo group, RSN = 8) in addition to their usual football-specific training at sea level. Posttesting (Post-) session was repeated the week after the 5-week supervised specific training period (10 training sessions). Both Pre- and Post- tests were performed in an invariant sequence (on 2 separate occasions; day 1: lower-limb explosive power, linear sprint, RSA and repeated-agility [RA] tests; and day 2: incremental field running test to estimate maximal aerobic speed [MAS] with no more than 4 days between each test session) in normoxia on an indoor track at constant temperature of 22.0 ± 0.5° C and 55 ± 10% of relative humidity. Players were told not to perform any intense exercise on the day before the test and to consume their last meal at least 3 hours before the scheduled test time. All players were familiar with all testing procedures and exercises, as part of their usual battery of team fitness testing and training routines. None of the subjects had any previous experience of altitude exposure. All of them were told that training was to be performed in hypoxia.

Subjects

The sample size was estimated using acceptable precision or confidence intervals (CI) a priori using the approach developed for magnitude-based inferences (27). Based on the assumption that a between-group difference in mean RSA time of 1.2 ± 1.1% is meaningful (11,13) and considering a within-subject *SD* (typical error) of 0.8% (29), a sample size of >7 participants per group would provide maximal chances of 0.5 and 25% of type I and type II errors, respectively. Thus, 16 highly trained under-18 male football players participated in this study. This research project was approved by the local research ethics committee, and conformed to the recommendations of the Declaration of Helsinki for use of Human Subjects as per the *Journal of Strength and Conditioning Research* author guidelines. The players and their parents were provided with the procedures and risks associated with participation in the study. Written informed consent was obtained from the players and their parents (for minors). Only outfield players were included in the present study. Subjects' anthropometric characteristics are shown in Table 1.

All the players had more than 4 years training history in a high-performance football academy and participated on average in 12 hours of combined football training and competitive games per week (6–8 football training sessions, 2 conditioning sessions, 1 domestic match each week and 1–2 friendly international club matches every 3 weeks). Football training sessions lasted 60–90 minutes and were dedicated to improve technical skills and tactical attributes. Such work can be qualified as moderate-intensity intermittent aerobic exercise (55–85% of maximal heart rate, HRmax) with regular occurrence of short sprints (12). Matches can be regarded as high-intensity intermittent exercise, with young players likely to spend about half of their playing time at intensity >85% of HRmax (25). Two training sessions (morning and afternoon training sessions or morning training + afternoon match) were programmed on most days. Recovery sessions were planned the day after a match. A typical weekly training content during the length of the study is presented in Table 2.

Procedures

Training Intervention. All players followed their usual football-specific training sessions during the protocol, though excluding sprinting and explosive exercises. Additionally, all groups performed specific run-based training sessions in

Table 3. Outline of the training intervention for the high-intensity training in normoxia (RSN) and hypoxia (RSH) groups.*

High-intensity intermittent treadmill runs							
Week	Interval, s	Sets × reps	Intensity (% MAS)	Rest between sets, min	Rest type	SpO_2, %	% HRmax
1	15:15	2 × 5	90	5 (except for the last set recovery period: 10 min)	Light jogging	RSN, 98 ± 2 RSH, 91 ± 2	95 ± 3 97 ± 2
2	15:15	2 × 6	100	5	Technical skills	RSN, 97 ± 1 RSH, 91 ± 3	97 ± 2 98 ± 1
3	15:15	3 × 7	100	5	Light jogging	RSN, 97 ± 1 RSH, 92 ± 1	96 ± 2 98 ± 1
4	15:15	3 × 8	110	5	Technical skills	RSN, 97 ± 2 RSH, 91 ± 2	98 ± 1 99 ± 1
5	15:15	3 × 9	110	5	Technical skills	RSN, 97 ± 2 RSH, 92 ± 3	98 ± 1 99 ± 1

Repeated-sprint exercises†							
Week	Plyometric jumps (CMJs to box) Sets × reps (height, cm)	Agility drills (ladders) Sets × reps	Plyometric jumps (hurdles) Sets × reps	Acceleration (standing starts) Set × reps (d in m)	Repeated shuttle sprints (<5 s COD) Set × reps (d in m; t in s)	SpO_2, %	% HRmax
1	4 × 4 (30)	4 × 4	4 × 4	4 × 4 (5)	4 × 4 (10; 15)	RSN, 97 ± 1 RSH, 93 ± 1	84 ± 3 90 ± 3
2	5 × 3 (30)	5 × 3	5 × 3	5 × 3 (5)	5 × 3 (10; 15)	RSN, 97 ± 1 RSH, 94 ± 1	83 ± 3 88 ± 3
3	5 × 4 (45)	5 × 4	5 × 4	5 × 4 (10)	5 × 4 (5; 15)	RSN, 97 ± 1 RSH, 93 ± 1	83 ± 2 87 ± 3
4	6 × 3 (30)	6 × 3	6 × 3	6 × 3 (5)	6 × 3 (10; 15)	RSN, 98 ± 1 RSH, 94 ± 1	82 ± 4 88 ± 1
5	6 × 4 (45)	6 × 4	6 × 4	6 × 4 (10)	6 × 4 (5; 15)	RSN, 98 ± 1 RSH, 94 ± 1	81 ± 4 87 ± 2

*RSN = repeated-sprint in normoxia; RSH = repeated-sprint in hypoxia; MAS = maximal aerobic speed; SpO_2 = arterial oxyhemoglobin saturation; % HRmax = % of maximal heart rate; CMJs = countermovement jumps; COD = change of direction; d = distance of run; t = duration of passive recovery.
†All performed at maximal speed/sense of effort.

a normobaric hypoxic room (30 × 30 m; Colorado altitude training, Louisville, CO, USA), where temperature and relative humidity were kept constant (22.0 ± 0.5° C and 55 ± 10%, respectively). The inspired oxygen fraction (F_IO_2) was 14.3% to simulate an altitude of 2,900 m. To blind subjects to altitude, the system was also run for RSN with a normoxic airflow into the chamber. During all training sessions, fingertip pulse oximeters (Wristox 3100; Nonin, Plymouth, MN, USA) were used to estimate arterial oxyhemoglobin saturation (SpO_2), which was recorded at rest and immediately after every sprint (5 seconds) for each experimental trial, whereas only session averaged values are considered for further analysis. Participants were unable to view any heart rate and SpO_2 readings. Furthermore, all investigators, except for the main investigator (G.P.M.), were blinded toward the group assignment.

After a standardized warm-up (~15 minutes) in the normobaric hypoxic room, each RSH or RSN training session (~45 minutes) included:

- high-intensity (ranging from 90 to 110% of MAS or 16 $km \cdot h^{-1}$ < MAS < 20 $km \cdot h^{-1}$) intermittent exercises consisting of 2–3 sets of 5–6 running bouts of durations ~15 seconds performed on a motorized treadmill (Woodway PPS Med; Woodway, Waukesha, WI, USA) interspersed with 15 seconds of passive recovery as adapted from previous studies (12,53). Recovery between the sets (5 and 10 minutes) involved either light running or combined with easy technical exercises inside the room; and
- repeated-sprint exercise including explosive strength/COD/sprint drills consisting of 4–6 series of 3–4 repetitions performed in the training room, aiming exclusively at improving speed, acceleration, and agility

Table 4. Mean changes in lower-limb explosive power, sprinting times, MSS, and MAS after repeated-sprint training performed either in normoxia (RSN) or hypoxia (RSH).*†‡

		Pre-	Post-	Post- Pre- (Cohen's *d*)	Post- Pre- qualitative inference	*p*
CMJ, cm	RSN	41.5 ± 4.6	43.8 ± 3.8	0.54 ± 1.57	Possibly beneficial	<0.001
	RSH	40.3 ± 3.2	42.9 ± 3.1	0.70 ± 0.18	Most likely beneficial	<0.001
	RSH-RSN (Cohen's *d*)	−0.29 ± 1.15				NS
	Qualitative inference	Possibly beneficial				
10 m, s	RSN	1.78 ± 0.06	1.70 ± 0.03	−1.01 ± 2.36	Likely beneficial	<0.05
	RSH	1.84 ± 0.05	1.73 ± 0.05	−1.97 ± 0.67	Most likely beneficial	<0.01
	RSH-RSN (Cohen's *d*)	0.68 ± 1.17				NS
	Qualitative inference	Likely beneficial				
20 m, s	RSN	3.04 ± 0.08	2.95 ± 0.06	−0.46 ± 2.30	Possibly beneficial	<0.01
	RSH	3.08 ± 0.07	2.98 ± 0.08	−1.11 ± 0.60	Most likely beneficial	<0.01
	RSH-RSN (Cohen's *d*)	0.15 ± 1.17				NS
	Qualitative inference	Possibly beneficial				
30 m, s	RSN	4.22 ± 0.11	4.15 ± 0.10	−0.02 ± 2.46	Possibly beneficial	<0.01
	RSH	4.25 ± 0.11	4.15 ± 0.11	−0.80 ± 0.57	Very likely beneficial	<0.01
	RSH-RSN (Cohen's *d*)	0.33 ± 1.17				NS
	Qualitative inference	Possibly beneficial				
40 m, s	RSN	5.39 ± 0.13	5.32 ± 0.14	0.15 ± 2.37	Possibly beneficial	NS
	RSH	5.40 ± 0.13	5.31 ± 0.15	−0.58 ± 0.55	Likely beneficial	<0.05
	RSH-RSN (Cohen's *d*)	0.19 ± 1.17				NS
	Qualitative inference	Possibly beneficial				
MSS, $km \cdot h^{-1}$	RSN	31.0 ± 1.0	30.7 ± 1.1	−0.73 ± 2.25	Possibly trivial	NS
	RSH	31.2 ± 0.8	31.1 ± 0.9	−0.15 ± 0.48	Possibly trivial	NS
	RSH-RSN (Cohen's *d*)	−0.16 ± 1.19				NS
	Qualitative inference	Possibly trivial				
MAS, $km \cdot h^{-1}$	RSN	17.8 ± 1.3	17.8 ± 1.56	−0.77 ± 2.60	Very unlikely beneficial	NS
	RSH	17.3 ± 0.7	17.4 ± 0.6	0.22 ± 0.51	Possibly beneficial	NS
	RSH-RSN (Cohen's *d*)	0.55 ± 1.32				NS
	Qualitative inference	Possibly beneficial				

*Pre- = before training; Post- = after 5-week experimental period; CMJ = countermovement jump; RSN = repeated-sprint in normoxia; RSH = repeated-sprint in hypoxia; NS = not significant; MSS = maximal sprint speed; MAS = maximal aerobic speed; CL = confidence limits.

†Mean ± *SD*, within-condition changes (Cohen's *d* ± 95% CL) and between-condition differences in the change (Cohen's *d* ± 95% CL) in for sprinting times at 10, 20, 30, 40 m and MSS Pre- and Post- test.

‡Qualitative inference: % chances for changes in RSH to be greater/similar/smaller than those observed in RSN.

(e.g., plyometric, agility drills, standing start and very short shuttle sprints, all performed at maximal speed/sense of effort, <5 seconds in duration or ≤10 m distances) as previously described (12); repetitions and series were interspersed with at least 45 seconds and 3 minutes of passive recovery, respectively.

These specific training sessions were completed at the same time of day (±2 hours) and were practiced 2 times per week (i.e., once for each session) for a total of 10 sessions during the experiment. Table 3 shows the details of the training contents/intensities during the experimental period.

Lower-Limb Explosive Power Test. Countermovement jump (CMJ, cm) height was determined as the center of mass vertical displacement calculated from body mass-corrected force development (Bioware software version 4.0; Kistler Instrument Corp., Winterthur, Switzerland) from a force platform (Kistler 9286AA; Kistler Instrument Corp., Winterthur, Switzerland). Each trial was validated by visual inspection to ensure each landing was without any leg flexion and participants were instructed to keep their hands on their hips during all jumps. The CMJ was performed 3 times, separated by 45 seconds of passive recovery, and the highest jump was recorded. Among all popular jumping tests, CMJ was previously demonstrated as the most reliable and valid jumping test (coefficient of variation [CV] = 2.8%) for the estimation of explosive power of the lower limbs in physically active collegial men (19.6 ± 2.1 years) (34).

Single sprints. Players were asked to run 2 maximal, straight-line 40-m sprints during which 10-m split times were recorded. Sprinting time was measured to the nearest 0.01 seconds using dual-beam electronic timing gates (Swift

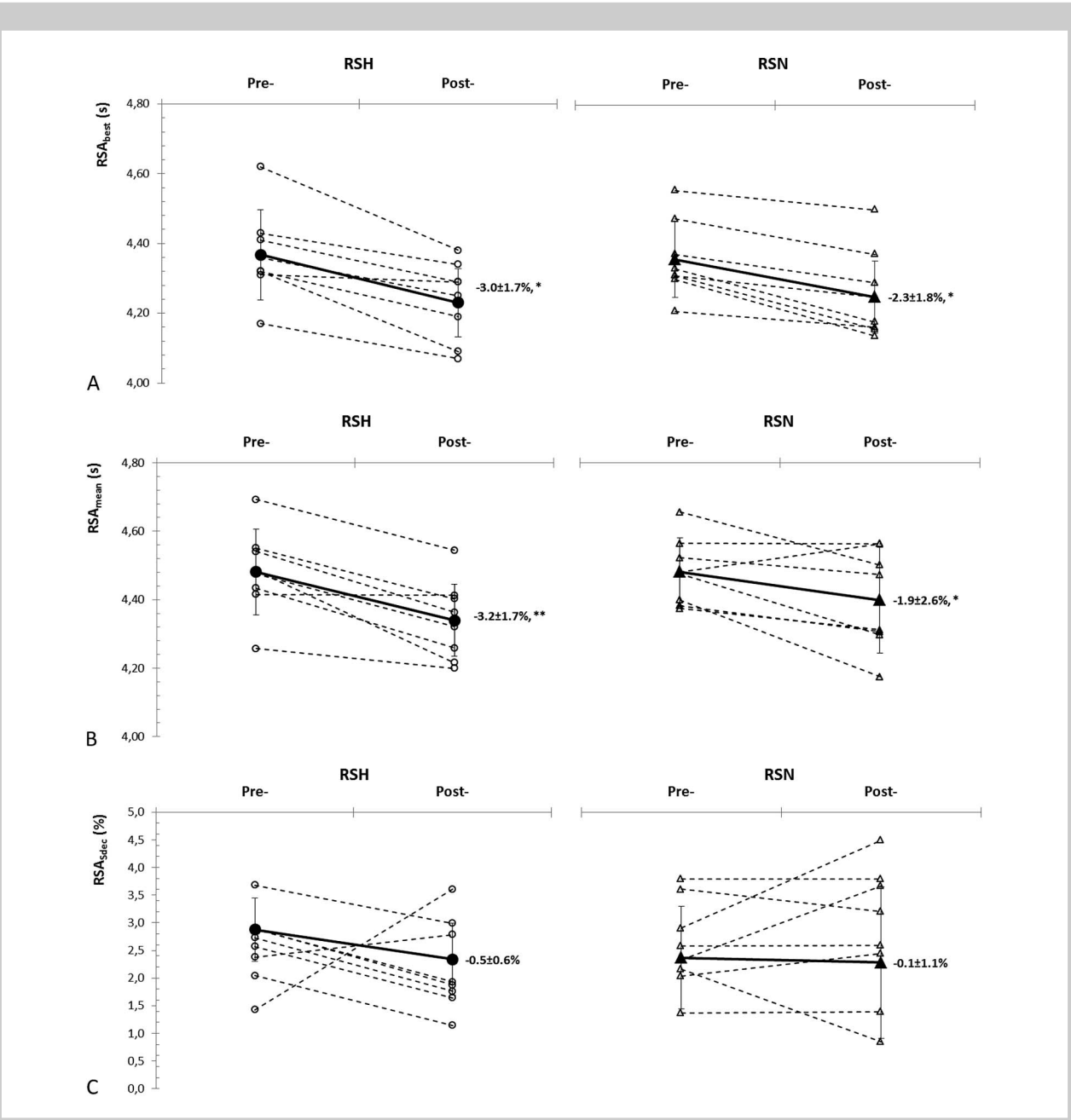

Figure 1. Individual (small symbols, dotted thin lines) and average (large symbols, dark line) best (RSA_{best}; A), mean (RSA_{mean}; B) RSA and sprint decrement (RSA_{Sdec}; C) before (Pre-) and after (Post-) repeated-sprint training in hypoxia (RSH; ○) or normoxia (RSN; Δ). Values are mean ± *SD*. Significant differences from pretest, $*p \leq 0.05$ and $**p < 0.01$, respectively.

Performance Equipment, Lismore, Australia). The height of the photocell was adjusted according to the height of the participant's hip. Players started sprint when ready (thus eliminating reaction time) from a standing static position with their front foot 0.5 m behind the first timing gate and were instructed to sprint as fast as possible over the sprint distance and the best performance was kept for analysis. Players' maximal sprint speed ($km \cdot h^{-1}$) was defined as the average running speed attained during the fastest 10-m split. The reliability of maximal sprinting speed was assessed in a group of young soccer players: the typical error, expressed as CV was 1.4% (7).

Repeated-Sprint Ability and Repeated-Agility Tests. The RSA test involved 10 repetitions of straight-line maximal 30 m in alternating directions interspersed by 30 seconds of passive recovery. A relatively similar RSA field test (i.e., 7 × 30 m with 25 seconds of active recovery) was found reliable

Table 5. Mean changes in RSAs after repeated-sprint training performed either in normoxia (RSN) or hypoxia (RSH).*†‡

		Pre-	Post-	Post- Pre- (Cohen's *d*)	Post- Pre- qualitative inference	*p*
RSA_{best}, s	RSN	4.37 ± 0.11	4.26 ± 0.10	−1.48 ± 1.61	Very likely beneficial	<0.05
	RSH	4.37 ± 0.13	4.23 ± 0.10	−1.04 ± 0.55	Very likely beneficial	<0.05
	RSH-RSN (Cohen's *d*)	0.38 ± 1.24				NS
	Qualitative inference	Possibly beneficial				
RSA_{mean}, s	RSN	4.49 ± 0.10	4.42 ± 0.15	−1.07 ± 1.12	Very likely beneficial	<0.05
	RSH	4.48 ± 0.13	4.35 ± 0.11	−1.10 ± 0.55	Most likely beneficial	<0.05
	RSH-RSN (Cohen's *d*)	0.55 ± 1.32				NS
	Qualitative inference	Possibly beneficial				
RSA_{Sdec}, %	RSN	2.46 ± 0.89	2.38 ± 1.32	−0.08 ± 1.06	Possibly beneficial	NS
	RSH	2.88 ± 0.57	2.34 ± 0.65	−0.54 ± 0.64	Likely beneficial	NS
	RSH-RSN (Cohen's *d*)	−0.44 ± 1.27				NS
	Qualitative inference	Likely beneficial				
RA_{best}, s	RSN	5.87 ± 0.10	5.76 ± 0.11	−0.99 ± 0.72	Very likely beneficial	<0.05
	RSH	6.03 ± 0.19	5.78 ± 0.11	−1.51 ± 0.63	Most likely beneficial	<0.001
	RSH-RSN (Cohen's *d*)	1.24 ± 1.07				<0.05
	Qualitative inference	Very likely beneficial				
RA_{mean}, s	RSN	6.00 ± 0.10	5.85 ± 0.14	−1.08 ± 0.65	Very likely beneficial	<0.05
	RSH	6.14 ± 0.16	5.90 ± 0.14	−1.52 ± 0.23	Most likely beneficial	<0.001
	RSH-RSN (Cohen's *d*)	1.34 ± 1.05				<0.05
	Qualitative inference	Very likely beneficial				
RA_{Sdec}, %	RSN	2.31 ± 0.52	1.61 ± 1.17	−0.69 ± 0.89	Likely beneficial	NS
	RSH	2.14 ± 1.23	2.06 ± 1.01	−0.08 ± 1.92	Possibly beneficial	NS
	RSH-RSN (Cohen's *d*)	0.35 ± 1.12				NS
	Qualitative inference	Possibly beneficial				

*Pre- = before training; Post- = after 5-week experimental period; RSA = repeated-sprint ability; RSN = repeated-sprint in normoxia; RSH = repeated-sprint in hypoxia; NS = not significant; RA = repeated agility; CL = confidence limits.
†Mean ± *SD*, within-condition changes (Cohen's *d* ± 95% CL) and between-condition differences in the change (Cohen's *d* ± 95% CL) in for RSA and RA-related parameters at Pre- and Post- test.
‡Qualitative inference: % chances for changes in RSH to be greater/similar/smaller than those observed in RSN.

(CV <2.7% for sprinting times) in young team sports athletes (45). The RA test consisted of 6 × 20 m maximal sprints with COD departing every 30 seconds (adapted from a previous running agility test that has been shown to be reliable and valid; intraclass correlation coefficient [ICC] = 0.90%, technical error of measurement [TEM] = 2.8%) in assessing agility (21). Three cones, ~1 m in height, were placed 5 m apart in the shape of an "L." Players ran forward 5 m, turned 90° to their left, ran forward 5 m, turned 180° on their left, and followed the same course to return to the finish line.

Both RSA and RA abilities were assessed using the aforementioned dual-beam electronic timing gates (see Single sprints section). After deceleration, participants walked back to the starting line and assumed a standing static position with their front foot 0.5 m behind the first timing gate. Five seconds before starting each sprint, the subjects were asked to assume the ready position as detailed for the sprint tests and await the start signal (i.e., announcement of a countdown before each sprint: "5 seconds, 3-2-1, Go"). Strong verbal encouragement was provided to each subject during all sprints. The best sprint time (RSA_{best} and RA_{best}; seconds), usually the first repetition, was retained, whereas mean repeated-sprint times (RSA_{mean} and RA_{mean}; seconds) and the sprint decrement score (RSA_{Sdec} and RA_{Sdec}; %) (100 − [{best sprint time × number of sprints}/total sprint times] × 100) were calculated (23).

Incremental Field Test. To estimate MAS, an incremental field test, a modified version of the University of Montreal Track Test (i.e., the VAMEVAL maximal incremental running test (31)) was performed. The VAMEVAL test began with an initial running speed of 8.5 $km \cdot hour^{-1}$ with a consecutive speed increase of 0.5 $km \cdot hour^{-1}$ each minute until exhaustion. The players adjusted their running speed according to auditory signals timed to match 20-m intervals delineated by cones around a 200-m-long indoor athletic track. The test ended when participants failed on 3 occasions to reach the next cone in the required time. The average velocity of the last 1-minute stage completed was retained as the player's MAS ($km \cdot h^{-1}$). If the last stage was not fully completed, the MAS was calculated using the formula of Kuipers et al. (30). Whereas the University of Montreal Track Test, which is very similar to the VAMEVAL, was found reliable (r =

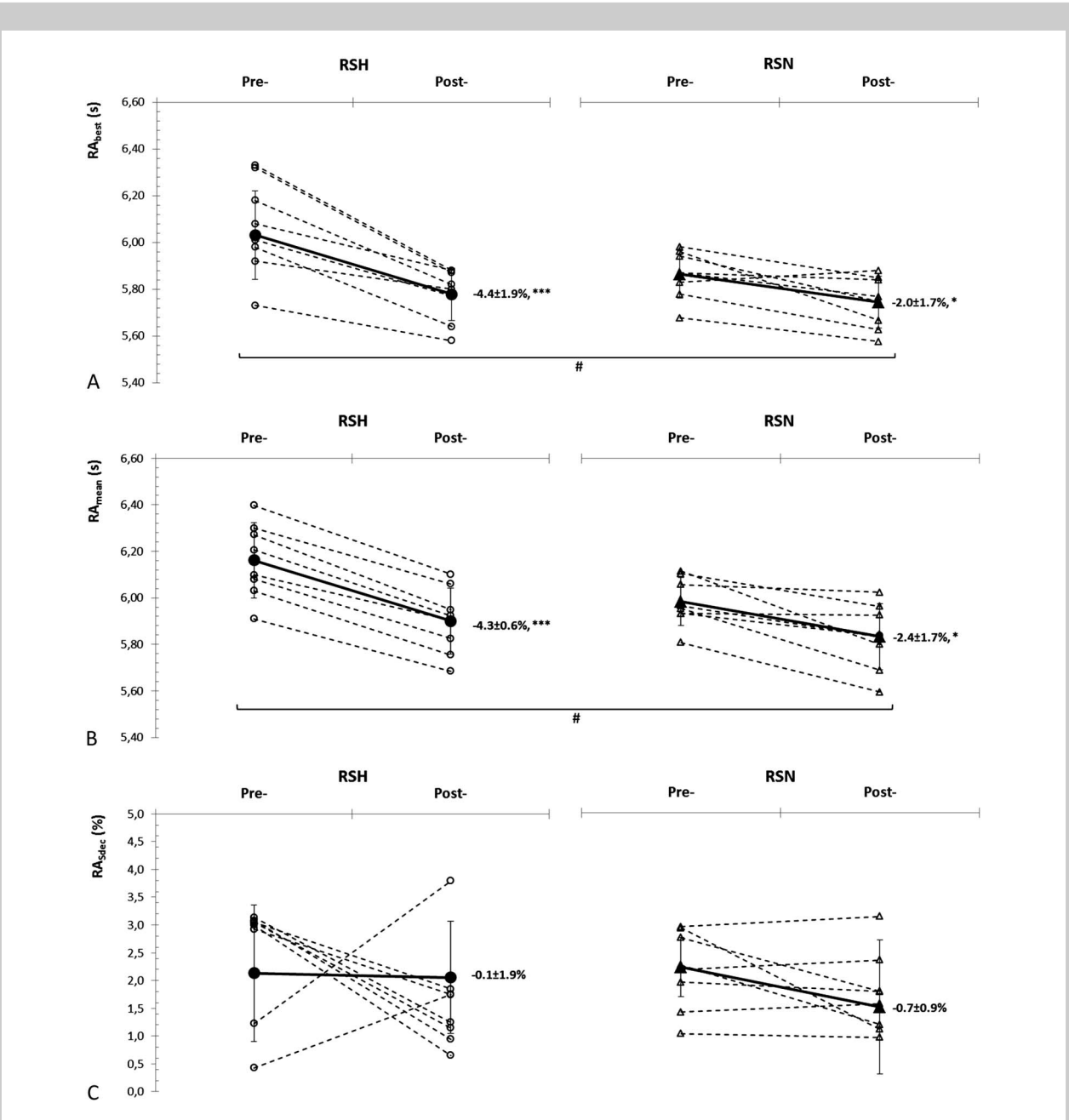

Figure 2. Individual (small symbols, dotted thin lines) and average (large symbols, dark line) in best (RA_{best}; A), mean (RA_{mean}; B) repeated-agility abilities and sprint decrement (RA_{Sdec}; C) before (Pre-) and after (Post-) repeated-sprint training in hypoxia (RSH; ○) or normoxia (RSN; Δ). Values are mean ± *SD*. Significant differences from Pre- test, $*p \leq 0.05$; and $***p < 0.001$; significant differences between groups, $\#p \leq 0.05$.

0.97) (31), the reliability of VAMEVAL to assess MAS was previously reported in a cohort of high-level academy players. The typical error, expressed as a CV, was 3.5% (14).

Statistical Analyses

Data are presented as mean or relative changes (%) with *SD* (±*SD*) unless otherwise stated. Normal distribution of the data was tested using the Shapiro-Wilk test. Data were first analyzed using a 2-factor repeated-measure analysis of variance with 1 between factor (condition; RSH vs. RSN) and 1 within factor (time; Pre- vs. Post- test). Multiple comparisons were made with the Tukey's honestly significant difference (HSD) post hoc test when the Greenhouse-Geisser epsilon correction factor was >0.50, or

with the Bonferroni post hoc test when the epsilon was <0.05. All analyses were made using Sigmaplot 11.0 software (Systat Software, Inc., San Jose, CA, USA). Significance level was set at $p \leq 0.05$.

In addition, an approach based on the magnitudes of differences (27) was used to assess practical significance. Time as well as condition differences were expressed as standardized difference or Cohen's *d* (±95% confidence limits) using pooled *SD*. Threshold values for Cohen's *d* statistics were >0.2–0.5 (small), >0.5–0.8 (moderate), and >0.8 (large). To compare between conditions changes (i.e., period and training effect factors), the chance that the true (unknown) changes for Post- or RSH were higher (i.e., higher than the smallest practically important difference, or the smallest worthwhile [difference] change [0.2 multiplied by the between-subject *SD*, based on Cohen's *d* principle]), similar or lower were calculated. Quantitative chances of greater or lower values were assessed qualitatively as follows: <1%, almost certainly not; 1–5%, very unlikely; 5–25%, unlikely; 25–75%, possible; 75–95%, likely; 95–99, very likely; >99%, almost certain. If the chances of having higher or lower values were both >5%, the true difference was assessed as unclear (27).

Results

Normoxic and Hypoxic Training Exposure

All players were able to combine their usual football-specific training sessions with the experimental-specific training sessions throughout the duration of the study. Number of training sessions, volume, and intensity were identically matched among the 2 groups. During training sessions over the 5-week training period, averaged SpO_2 values of the RSH group decreased at 91–93%, whereas it remained at 97–98% for the RSN group.

Performance

From Pre- to Post- training, the lower-limb explosive power (CMJ) improved ($p < 0.001$) to a similar extent in both groups (+6.5 ± 1.9% vs. +5.0 ± 7.6% for RSH and RSN, respectively). However, benefit appeared most likely for RSH, whereas it was only possible for RSN with a possibly beneficial effect for RSH compared with RSN (Table 4). Although sprinting performances increased significantly after 5 weeks of training compared with baseline (Pre-) with no significant difference between groups (Table 4), greater magnitudes were observed for RSH for all sprinting distances (likely to most likely beneficial) than that for RSN (possibly to likely beneficial). Qualitative inferences between groups appeared possibly beneficial for all sprint distances except for 10-m sprint (likely beneficial) for RSH compared with RSN. Despite MAS being unchanged throughout the protocol (+0.9 ± 2.3% and −0.2 ± 4.6% for RSH and RSN, respectively), there was, however, a greater magnitude perceived for RSH (possibly beneficial) than that for RSN (unlikely beneficial) with a possible beneficial effect for RSH compared with RSN (Table 4).

Both RSH and RSN groups improved RSA_{best} (−3.0 ± 1.7% and −2.3 ± 1.8%, respectively; both $p \leq 0.05$) and RSA_{mean} (−3.2 ± 1.7%, $p < 0.01$ and −1.9 ± 2.6%, $p \leq 0.05$ for RSH and RSN, respectively; Figures 1A, B), whereas RSA_{Sdec} did not change (−0.5 ± 0.6% and −0.1 ± 1.1% for RSH and RSN, respectively; Figure 1C). There was no significant interaction between time and condition for any RSA-related parameter. Although training impact appeared of similar magnitude on RSA_{best} and RSA_{mean} (ranging from very likely to most likely) for both groups, RSH seemed possibly beneficial compared with RSN (Table 5). The RA_{Sdec} remained unchanged between Pre- and Post- in both groups (−0.1 ± 1.9% and −0.7 ± 0.9% for RSH and RSN, respectively; Figure 2C). Significant condition × time interactions effects were found for RA_{best} ($p \leq 0.05$) and RA_{mean} ($p \leq 0.05$) with larger decrease for RSH (−4.4 ± 1.9% and −4.3 ± 0.6% for RA_{best} and RA_{mean}, respectively; $p < 0.001$) than that for RSN (−2.0 ± 1.7% and −2.4 ± 1.7%, respectively; $p \leq 0.05$) group (Figures 2A, B). Repeated-agility performance-related parameters improved in both condition with a very likely advantage for RSH compared with RSN (Table 5).

Discussion

In the present study, we investigated the effect of hypoxia exposure when 2 weekly high-intensity intermittent running-based training sessions were added over a 5-week in-season period to the usual football training routine of highly-trained under-18 male players. The major findings of this investigation were that (a) the addition of 10 specific run-based training sessions to their regular football practice substantially improved several neuromuscular fitness components related to on-field football physical performance, and (b) high-intensity training in normobaric hypoxia in a team sport applied setting (i.e., high-intensity intermittent and repeated-sprint runs, agility, and conditioning exercises) appeared more efficient than the same training in normoxia at enhancing RA ability, with the inclusion of direction changes.

The addition of high-intensity intermittent running, sprints, and all-out efforts into normoxic training programs has been shown to be effective for team sport players (9,10). It is therefore believed that these types of training (e.g., repeated-sprint training or sprint interval training) challenge at different respective levels relative to the training content, both the metabolic and the neuromuscular/musculoskeletal systems (8). However, despite our limited understanding of these dose-response relationships between the training load and training-induced changes in physical capacities and performance, the primarily expected benefits of such exercises are to maximize cardiorespiratory fitness (10). The results of our study did not show any significant improvement in MAS for either RSH or RSN groups, although RSH was found possibly more effective on MAS than RSN. This was expected because the total duration of hypoxic exposure (e.g., hypoxic

dose) was too low for inducing any positive hematological adaptations. Moreover, intensities and exercise-rest ratios probably did not elicit $\dot{V}O_2$ responses near $\dot{V}O_2$max (38,41) and normoxic $\dot{V}O_2$max or peak power output are not further enhanced by hypoxic than normoxic training (52). Rather, other central (i.e., ventilatory, hemodynamics, or neural adaptations) or peripheral (i.e., muscle-buffering capacity, economy, mitochondrial biogenesis, lactate transport, pH regulation) factors outside hematological adaptations have also the potential to improve match-related performance in team sports (19). Because team sport training implicates a myriad of metabolic and neuromuscular systems simultaneously (26), anaerobic glycolytic energy contribution and neuromuscular load/musculoskeletal strain are logically likely the more important variables to consider (9).

During high-intensity intermittent exercise, exercise capacity not only depends on energy supply and energy depletion but also on the function of the neuromechanical system (9). Resistance exercise training is well known to elicit morphological and functional adaptations in the skeletal muscle (42). Degrees of muscular hypertrophy and strength gains after resistance training are thought to be dependent on the intensity of exercise, in such a way that an intensity of approximately 70–85% of 1 repetition maximum (1RM) is required to achieve a substantial effect (36). However, recent research suggests that similar muscle mass and strength gains can be accommodated with low or high-intensity resistance exercise training performed under intermittent hypoxic conditions (33,44). Nishimura et al. (44) reported accelerated increases in muscle cross-sectional area of the elbow flexors and extensors in untrained male student after 6 weeks of hypoxic resistance training (4 sets of 10 repetitions at 70% 1RM, 2 times per week, $F_IO_2 = 16\%$) compared with normoxia. In the present study, the improvement in lower-limb explosive strength (6.5%) and sprinting performances (6.0% at 10 m) following RSH indicate greater magnitude (possibly and likely higher benefits) compared with RSN improvement (5.0 and 4.3%, respectively). These greater hypoxic-induced strength gains might at least partially explain the larger improvements for agility factors (~4% for RSH vs. ~2% for RSN), which arguably request higher muscular solicitation (e.g., eccentric load during the braking phase of COD). Such improvements could not be observed during our RSA test, as the strength component is less decisive on this test where sprints are performed in the same direction, presumably resulting in an absence of difference between conditions. It is possible that this improved RA performance (i.e., as a result of a better production/force application technique) is because of neural adaptations. Although speculative, this may include increased motor unit synchronization or agonist muscle activation, as evidenced from greater electromyography signal amplitudes during posthypoxic intervention maximal voluntary contractions (33).

Because football efforts are characterized by high-intensity running bouts repeated throughout the game, improving RSA is important to delay premature or excessive fatigue, eventually improving players' match-related physical performance (48). Our results, showing substantial improvements of both RSA (Figure 1) and RA (Figure 2), therefore confirm the practical relevance of the present training intervention. However, the question of the transfer of such training-induced individual physical performance improvements (as inferred from specific performance tests) to match performance enhancement (i.e., high-intensity running or involvement with the ball) and ultimately team's game results is highly debated and unknown (16,17). It is particularly interesting that RA was further improved after RSH as, in addition to the ability to repeat high-intensity efforts, the ability to COD is an important fitness component for successful participation in football (51). Introducing COD into repeated-sprint sequences increases the mechanical demands of the repeated accelerations inherent to consecutive COD (46). In fact, such running modality is likely to increase peripheral (particularly biarticular locomotor muscles) demands, resulting in a greater taxing of the anaerobic energy system (1). Consecutive training adaptation would be partly related to an upregulation of the glycolytic potential and to an increased anaerobic capacity (33,35), which might be helpful for team sport athletes. Besides, by challenging the functional reserve in muscle oxygen diffusing capacity likely used in hypoxia (15), RSH has the potential to stimulate beneficial adaptations in terms of phosphocreatine resynthesis and oxygen utilization mediated by hypoxia-inducible factors at the muscular level. Based on the suggestion that (a) the ability to resynthesize phosphocreatine is probably the major determinant of RSA (37), and (b) RSH efficiency is likely to be fiber-type dependent (19), adding a hypoxic stimulus to training can maximize fatigue resistance during high-intensity intermittent exercises.

One potential limitation of this study is the omission of resting skeletal muscle microbiopsy samples to confirm putative training-induced adaptive mechanisms at the muscular level (20). The main reason not to perform invasive measurement belongs to ethical considerations as this study was conducted within a realistic applied setting on developing athletes. Besides, the failure of previous researches to observe any significant change in total hemoglobin mass, red cell volume, or any other red cell indices after intermittent hypoxic training compared with sea level (2,24) convinced us not to investigate erythropoiesis response here because the low "altitude dose" associated with is unlikely to stimulate "the erythropoeitic pathway to the point that it enhance postaltitude sea-level endurance performance" (32,56). Another point to mention is that performance changes should not only be monitored shortly (i.e., few days) after the intervention but also few weeks after the last day of exposure to distinguish the short (or immediate) from mid/long-term (or delayed) effects. Indeed, after return to sea level, performance might depend from some or all of the individual time course of

the changes in red blood cell mass, ventilatory acclimatization, biomechanical, and neuromuscular factors (18). Last, it is worth noting that whereas statistical significance and standardized magnitude of differences indicate greater increase for RSH than for RSN, caution is needed in interpreting our results given the large CI reported. Moreover, despite the double-blinded design used in this study, the addition of a "control" group (i.e., without the 2 weekly high-intensity sessions) to assess the fitness improvement of such training is lacking.

In conclusion, the use of the so-called RSH, a new hypoxic training method developed to overcome some of the inherent limitations of intermittent hypoxic training was shown to be as efficient as RSN for lower-limb explosive power and maximal sprinting performance improvement and to improve RSA performance of highly trained under-18 male footballers. A key feature of this study that used a carefully controlled double-blind design in a team sport–applied setting over a 5-week period was to demonstrate that the addition of 10 RSH sessions to players' regular football training induced twice larger enhancements of RA ability than the same training in normoxia. Although RSH is a promising training strategy to induce a synergetic effect on performance over sea-level training in young high-level football players, further applied research bringing together physiologists and conditioning coaches is still needed to improve our understanding of how optimally design exercise and recovery parameters (intensities or exercise-rest ratios (39)) when implementing an RSH training regimen with team sports players.

Practical Applications

This study reported that football-specific high-intensity and repeated-sprint exercises, be it performed in normoxic or hypoxic condition, is efficient to improve physical fitness performance factors in young football players. Such training intervention should therefore be recommended during preparation or in-season to boost players' performance and delay premature fatigue. Moreover, performing repeated-sprint and RA sequences at maximal intensity in hypoxia may provide additional activation of anaerobic and neuromuscular pathways beyond that observed in normoxia. Therefore, the higher practical effect of RSH shown in this study suggests that the prescription of high-intensity hypoxic exercises is sufficient to have a synergetic effect on physical performance over sea-level training in football players. As also supported by recent evidence (22), RSH may also lead to putative benefits for other team sports like, rugby union or field hockey, where the ability to repeat high speed runs during an entire game is essential for overall performance.

Given the relevance of explosive power for footballers and the prevalence of neuromuscular qualities determining RSA (5), the present results suggest that adding hypoxic explosive exercises (i.e., heavy resistance or plyometric/agility [i.e., COD]/sprint drills) improve power-related factors and fatigue resistance. However, it is worth noting that no uniform gains resulting from an hypoxic training intervention should be expected for all age, level, and gender, because of (a) possible individual responsiveness (adherence, compliance, and variability) to hypoxia and (b) position and tactical dependence to match running performance (40). Besides, in an applied setting, the benefits-to-costs ratio and the use of this method have to be weighted with factors such as practicalities, financial, and logistics of this type of approach and the magnitude of the transfer of the RSA and RA improvements to match performance (e.g., high-intensity running and involvements with the ball during competitions) (2).

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Chapter 11

Article 6 – ‘Live High-Train Low and High’ Hypoxic Training Improves Team-Sport Performance

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"Live High–Train Low and High" Hypoxic Training Improves Team-Sport Performance

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[1]ISSUL, Institute of Sports Sciences, University of Lausanne, Lausanne, SWITZERLAND; [2]Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, SWITZERLAND; [3]Section for Elite Sport, Swiss Federal Institute of Sport, Magglingen, SWITZERLAND; [4]Faculty of Motor Sciences, Université Libre de Bruxelles, Brussels, BELGIUM; and [5]Athlete Health and Performance Research Center, ASPETAR, Qatar Orthopedic and Sports Medicine Hospital, Doha, QATAR.

ABSTRACT

BROCHERIE, F., G. P. MILLET, A. HAUSER, T. STEINER, J. RYSMAN, J. P. WEHRLIN, and O. GIRARD. "Live High–Train Low and High" Hypoxic Training Improves Team-Sport Performance. *Med. Sci. Sports Exerc.*, Vol. 47, No. 10, pp. 2140–2149, 2015. **Purpose**: This study aims to investigate physical performance and hematological changes in 32 elite male team-sport players after 14 d of "live high–train low" (LHTL) training in normobaric hypoxia (≥14 $h \cdot d^{-1}$ at 2800–3000 m) combined with repeated-sprint training (six sessions of four sets of 5 × 5-s sprints with 25 s of passive recovery) either in normobaric hypoxia at 3000 m (LHTL + RSH, namely, LHTLH; $n = 11$) or in normoxia (LHTL + RSN, namely, LHTL; $n = 12$) compared with controlled "live low–train low" (LLTL; $n = 9$) training. **Methods**: Before (Pre), immediately after (Post-1), and 3 wk after (Post-2) the intervention, hemoglobin mass (Hb_{mass}) was measured in duplicate [optimized carbon monoxide (CO) rebreathing method], and vertical jump, repeated-sprint (8 × 20 m–20 s recovery), and Yo-Yo Intermittent Recovery level 2 (YYIR2) performances were tested. **Results**: Both hypoxic groups similarly increased their Hb_{mass} at Post-1 and Post-2 in reference to Pre (LHTLH: +4.0%, $P < 0.001$ and +2.7%, $P < 0.01$; LHTL: +3.0% and +3.0%, both $P < 0.001$), whereas no change occurred in LLTL. Compared with Pre, YYIR2 performance increased by ~21% at Post-1 ($P < 0.01$) and by ~45% at Post-2 ($P < 0.001$), with no difference between the two intervention groups (vs no change in LLTL). From Pre to Post-1, cumulated sprint time decreased in LHTLH (−3.6%, $P < 0.001$) and LHTL (−1.9%, $P < 0.01$), but not in LLTL (−0.7%), and remained significantly reduced at Post-2 (−3.5%, $P < 0.001$) in LHTLH only. Vertical jump performance did not change. **Conclusions**: "Live high–train low and high" hypoxic training interspersed with repeated sprints in hypoxia for 14 d (in season) increases the Hb_{mass}, YYIR2 performance, and repeated-sprint ability of elite field team-sport players, with benefits lasting for at least 3 wk postintervention. **Key Words:** HEMOGLOBIN MASS, HYPOXIA, REPEATED-SPRINT ABILITY, TEAM SPORTS

To date, altitude training has mainly been used by individual "endurance" athletes with the primary goal of further improving exercise performance upon return to sea level. Several paradigms—"live high–train high" (residing and training at altitude) and, more recently, "live high–train low" (LHTL) (sleeping at altitude but training near sea level) approaches (30,31)—have subsequently been introduced to reach this goal. To date and despite ongoing debate on its efficiency in elite endurance athletes or on the nature of its underlying mechanisms (17,24), LHTL is widely recognized as the "gold-standard" altitude training method (23) for athletic performance enhancement. Its success belongs to the erythropoietic effect of chronic hypoxia initiated by continuously residing at natural/terrestrial (hypobaric hypoxia) or simulated (normobaric hypoxia) altitude, with the possibility of maintaining high training intensity and rates of oxygen flux at sea level (43).

An emerging concept is that positive gains associated with the LHTL method may rely on the magnitude of hypoxia-induced increase in hemoglobin mass (Hb_{mass}) (24). Reportedly, LHTL was found to be less efficient in (endurance) athletes with high preintervention Hb_{mass} (16). Because team-sport (28,41) athletes are generally characterized by moderate Hb_{mass} (24) and/or maximal oxygen uptake ($\dot{V}O_{2max}$), one may speculate on substantial gains in Hb_{mass} after LHTL. Because aerobic metabolism dominates energy delivery in most team sports (e.g., soccer, rugby, or field hockey), it is likely that LHTL would benefit some team-sport athletes (13). Although positive short-term benefits on Hb_{mass} have been reported after

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only 13–19 d of exposure in Australian Football League (AFL) (28), soccer (41), or water-polo (12) players, the level of evidence of the usefulness of LHTL in improving sport-specific performance in team-sport athletes is still limited (2).

Team sports share a common feature—high-intensity intermittent exercise patterns, where the ability to repeatedly perform maximal or near-maximal bouts with incomplete recoveries [i.e., repeated-sprint ability (RSA)] for sustained periods is important for match outcome. In field hockey, for instance, although the distances covered by high-intensity running (~8%) and sprinting (~1%) only represent a small percentage of the total match duration, both decrease from the first half to the second half, which may increase chances to evade an opponent and create scoring opportunities (26). In order to better resist fatigue in the most intense periods of a game or toward match end, innovative hypoxic training methods have recently been tailored for team-sport use (2,13,29). In this vein, we have recently updated the panorama of the different hypoxic methods currently available to add repeated-sprint training in hypoxia (RSH) (30) as a new form of "live low–train high" regimen. Briefly, RSH, which includes maximal-intensity efforts under moderately hypoxic conditions, has proved superior to repeated-sprint training in normoxia (RSN) in enhancing peripheral adaptations (9,33,38) and thereby to RSA (8,9,11). With physical performance only acutely (within days) assessed after the RSH intervention, however, the long-term (few weeks) deacclimatization effects (if any) of this hypoxic method are currently unknown. Although all available RSH studies have so far been conducted in a laboratory environment, only two have adopted a running mode (i.e., nonmotorized treadmill [5,11]), yet none of them have used overground sprints, which would considerably increase the ecological validity of literature findings.

Evaluating the combination of altitude training methods and its effects on the magnitude and time course of several aspects of match-related performance and adaptive physiological response is an integral part of the role of research scientists. The capacity of team-sport athletes to repeatedly perform high-intensity actions depends not only on their Hb_{mass} but also on skeletal muscle tissue adaptations and the efficiency of their neuromuscular system. Theoretically, for team-sport athletes and coaches looking to elicit concurrent aerobic and anaerobic adaptations to improve sea-level performance, "live high–train low and high" [LHTLH; i.e., 2–3 wk of sleeping at 2500–3000 m with training at sea level, except for a few (two to three) hypoxic training sessions per week], suggested as early as 2010 (30,31), is an attractive combination. However, it is currently unknown whether combining LHTL and RSH in a cohort of team-sport athletes would produce larger performance gains than would concurrent LHTL and RSN.

Using a randomized, double-blind, controlled design, this study aimed to investigate the immediate (few days) and prolonged (3 wk) effects of the "traditional" LHTL approach, combined with either RSH or RSN (both compared with controls), on team-sport-specific sea-level performance and Hb_{mass} in elite field hockey players. We hypothesized that, combined with traditional "LHTL" exposure, RSH (LHTL + RSH, namely, LHTLH) *versus* RSN (LHTL + RSN, namely, LHTL) provides similar hematological adaptations but larger sport-specific physical performance gains, persisting at least 3 wk postintervention.

METHODS

Subjects

Thirty-six lowland elite male field hockey players (mean ± SD: age, 25.3 ± 4.6 yr; height, 178.4 ± 6.0 cm; body weight, 75.8 ± 7.9 kg; estimated $\dot{V}O_{2max}$, 52.1 ± 1.9 $mL \cdot min^{-1} \cdot kg^{-1}$ [22]) were recruited from among Belgium, Spanish, and Dutch first division clubs (nine of the participants were national team members of their respective countries) to participate in this study. The experiment was approved by the Anti-Doping Lab Qatar institutional review board (agreement SCH-ADL-070) and conformed to current Declaration of Helsinki guidelines. Subjects gave their written informed consent after having been informed in detail of all experimental procedures and possible risks [e.g., severe intensity nature of the proposed exercise, acute mountain sickness (AMS), including headache, dizziness, tiredness, shortness of breath, and nausea, in isolation or in combination], associated with the experiments. Exclusion criteria for participation were acclimatization or exposure to hypoxia of more than 2000 m for more than 48 h during a period of 6 months before the study and any history of altitude-related sickness and health risk that could compromise the subject's safety during training and/or hypoxia exposure. During the study, one subject from the control group (see "Study Design") was excluded after the lead-in period due to insufficient fitness level (i.e., incapacity to satisfy the criterion score for physical performance tests; see "Pre, Post-1, and Post-2 Testing Sessions"), whereas three others (control group, n = 1; experimental groups, n = 2) were excluded due to illness or injury.

Study Design

The experimental design (Fig. 1) consisted of the following: a 2-wk lead-in period (from middle of December to beginning of January) at sea level where training sessions were supervised and load quantified; a 1-wk preintervention period at sea level where baseline testing (Pre) was performed; a 14-d hypoxic intervention period; and, finally, a 3-wk postintervention period at sea level with training sessions supervised and load quantified, where Post-1 (2–3 d) and Post-2 (22–23 d) test sessions were performed.

Each of the three test sessions (Pre, Post-1, and Post-2) was 48 h in duration and involved the 32 players on the same sea-level testing site (Belgium). At this occasion, physical performance and Hb_{mass} were evaluated in invariant order under similar temperate conditions (±2 h). After the completion of Pre, subjects were randomly assigned to one of the three following groups according to their initial fitness

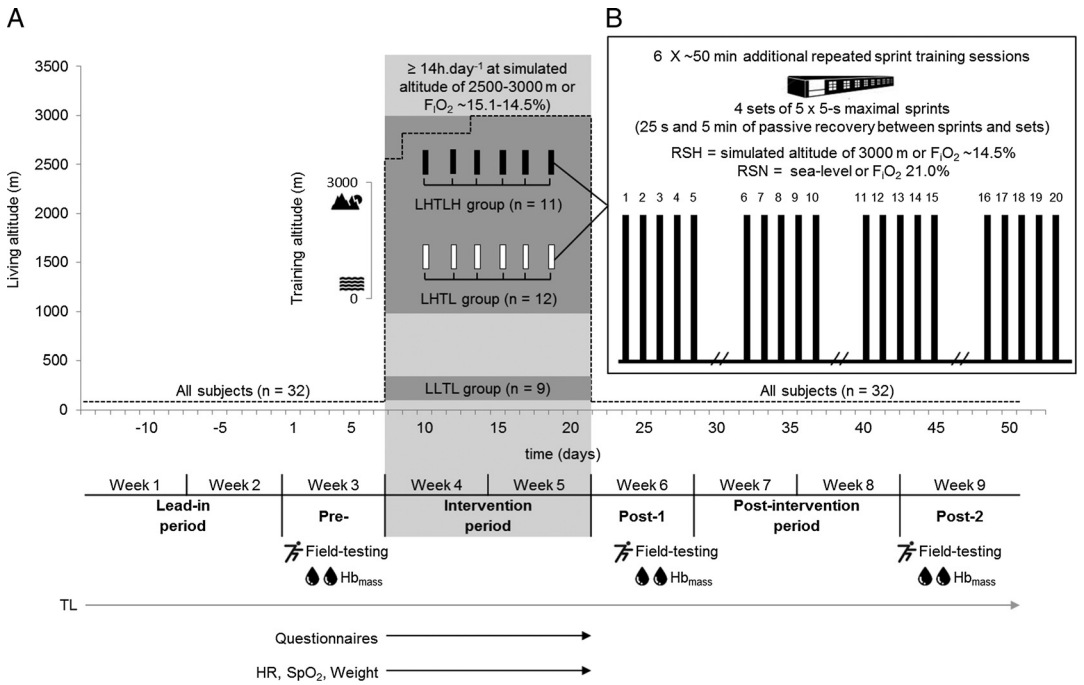

FIGURE 1—Protocol overview. General procedure (A) and description of a typical repeated-sprint training session (B). Field testing, testing including vertical jumps, 30-m sprint (with intervals at 5, 10, and 20 m), RSA, and YYIR2; Hb_{mass}, total hemoglobin mass measured via CO rebreathing and performed in duplicate; Post-1, 1 wk after termination of the intervention period; Post-2, 3 wk after termination of the intervention period; Pre (or baseline), 2-wk after lead-in period; TL, training load; wellness, morning wellness monitoring, including the Lake Louise score questionnaire, DALDA questionnaire, and GSQS.

level and playing position (Table 1): 14 d of "LHTL" altitude training (>14 $h \cdot d^{-1}$ and simulated altitude of 2500–3000 m) during which players trained (i.e., regular field hockey practice) at sea level with the addition of six repeated-sprint training sessions either in normobaric hypoxia simulating an altitude of 3000 m (LHTL + RSH, namely, LHTLH; $n = 11$) or in normoxia (LHTL + RSN, namely, LHTL; $n = 12$) and "live low–train low" (LLTL) training ($n = 9$). LLTL players resided at sea level yet under comfort conditions similar to those in the two experimental groups. Although LLTL players were not enrolled in training camp (Qatar, January 2014, normal environmental conditions), they followed the same training/competition routine (i.e., without the completion of any additional repeated-sprint training session) as LHTLH or LHTL players. All subjects were familiar with the testing procedures as part of their regular physical performance assessment implemented in their clubs. Although not recorded, particular attention was paid to food intake, hydration, and sleep habits during the experiment, such that players from all three groups were provided with similar diets and bedtime schedules, which were based on club guidelines and experience gained from previous training camps.

To evaluate physical performance, subjects performed a test battery at sea level on a well-ventilated indoor synthetic ground (Taraflex®) gymnasium at a constant temperature of ~22°C. Pre, Post-1, and Post-2 testing sessions were performed in the exact same sequence as follows: (i) jump tests; (ii) after 10 min of rest, repeated sprints; and (iii) after an additional 15 min of recovery, and Yo-Yo Intermittent Recovery Level 2 (YYIR2). Due to the extreme intensity of the tests, subjects were asked to arrive at the testing sessions in a rested and hydrated state (at least 3 h after a meal and having avoided strenuous training in the preceding 24 h). In all cases, subjects were asked to reproduce their last meals, avoiding alcohol and caffeine intakes during the 24 h before each test scheduled in the same time slot. Tap water was provided *ad libitum*. For all tests, subjects were vigorously encouraged during all efforts. Before the test battery, a standardized 15-min warm-up, including athletic and acceleration drills, was supervised by two investigators.

TABLE 1. Subject characteristics.

	LHTLH ($n = 11$)	LHTL ($n = 12$)	LLTL ($n = 9$)
Age (yr)	27.6 ± 4.8*	25.3 ± 4.2	22.3 ± 4.6
Height (cm)	179.1 ± 8.6	178.9 ± 4.7	178.1 ± 5.2
Weight (kg)	76.5 ± 7.9	76.1 ± 8.6	74.3 ± 8.9
MAV ($km \cdot h^{-1}$)	17.5 ± 0.4	17.6 ± 0.3	17.3 ± 0.2
RSA_{TT} (s)	27.23 ± 1.15	27.05 ± 0.81	26.98 ± 1.03
Position	1GK/4DF/3MF/3FW	1GK/5DF/2MF/4FW	1GK/4DF/3MF/1FW

Values are presented as mean ± SD.
*$P < 0.05$ for LHTLH players older than those in the other groups.
DF, defender; FW, forward; GK, goalkeeper; MAV, maximal aerobic velocity; MF, midfield; RSA_{TT}, repeated-sprint cumulated sprint time.

Living hypoxic exposure. The sleeping and recreational hypoxic facilities were fully furnished normobaric hypoxic rooms with O_2 filtration (CAT system; Colorado Altitude Training, Louisville, Colorado, USA). Three days before the start of the study, all rooms were controlled and calibrated by qualified engineers. Furthermore, all investigators, except for the main investigator, were blinded to group assignment. In all rooms, air pumps were constantly turned on. O_2 fraction in each room was continuously monitored from independent O_2 probes connected to a control panel located in a room where access was restricted to the main investigator only. The two intervention groups were exposed to normobaric hypoxia equivalent to 2500 m [fraction of inspired oxygen (F_iO_2), 15.1%; blood pressure, 768.0 mm Hg; partial pressure of inspired oxygen (P_iO_2), 108.3 mm Hg] for the first 24 h of the intervention period (day 1). Thereafter, O_2 fraction was further decreased to the equivalent of 2800 m (F_iO_2, 14.5% ± 0.1%; blood pressure, 766.8 ± 1.1 mm Hg; P_iO_2, 104.5 ± 0.6 mm Hg; days 2–5) and 3000 m (F_iO_2, 14.2% ± 0.1%; blood pressure, 765.3 ± 1.5 mm Hg; P_iO_2, 101.7 ± 0.8 mm Hg; days 6–14). Subjects were strictly confined (as verified by the main investigator) to their rooms from 2200 to 0700, from 0800 to 1000, and again from 1300 to 1600 during these 2 wk. However, they were encouraged to spend more time in their rooms, if desired. Concentrations of ferritin (143.6 ± 68.9 $\mu g \cdot L^{-1}$; range, 45–279 $\mu g \cdot L^{-1}$) and soluble transferrin receptor (254.7 ± 33.3 $mg \cdot dL^{-1}$; range, 202–330 $mg \cdot dL^{-1}$) measured during the lead-in period indicated that none of our subjects were iron deficient at the time of study entry.

Daily physiological measures and questionnaires. During the intervention period, arterial oxyhemoglobin saturation (SPO_2) and HR were recorded in a blind manner, using fingertip pulse oximeters (GO_2™ Achieve 9570-A; Nonin, Plymouth, MN, USA), every morning upon waking up. Afterward, participants had to fill three different questionnaires: (1) the Lake Louise score questionnaire, which included five simple questions (scale from 0 to 3) that were sensitive to quantifying AMS severity (34). Overall Lake Louise score was determined by summing all scores; (2) the Daily Analysis of Life Demands for Athletes (DALDA) questionnaire (36), which was used to monitor psychological status (i.e., mood state); parts A and B of the DALDA questionnaire represent the sources and manifestations of stress (general fatigue and feelings) in the form of signs and symptoms, respectively. For both parts, the number of items marked as "worse than normal" (i.e., *a* scores) was tallied and reported; and (3) the 15-item Groningen Sleep Quality Scale (GSQS), which was used to evaluate high-altitude sleep disturbance (42). Finally, subjects (in minimal clothing) were weighted with a digital balance (±0.1 kg; Seca, Hamburg, Germany) before breakfast. All aforementioned variables were averaged over the 14-d training camp for subsequent analysis.

Field hockey training sessions. During the entire study (from the start of the lead-in period to Post-2), each field hockey training session and match was monitored. Players' training loads [arbitrary units (a.u.)] were calculated as total training/competition duration (min) × session RPE (Borg's scale from 6 to 20), collected within 10 min of completing each training session. On days with two training sessions, daily training load was taken as the sum of the sessions performed. On average, tested players practiced ~7.0–9.0 $h \cdot wk^{-1}$ (three to four field hockey sessions + two to three fitness sessions + one to two matches) during the season (i.e., within the 3 months preceding the lead-in period).

Supervised training protocol. In addition to their usual field hockey practice, players of the two intervention groups completed six specific repeated-sprint training sessions during the 14-d intervention period with at least 36 h recovery in between. Sessions were completed on synthetic grass inside a mobile inflatable simulated hypoxic equipment (Altitude Technology Solutions Pty Ltd, Brisbane, Queensland, Australia), as recently described (14). Briefly, it comprised a polyvinyl chloride inflatable running lane tunnel (length, 45 m; width, 1.8 m; height, 2.5 m) and a state-of-the-art hypoxic trailer (a 55-kW screw compressor), generating more than 3000 L of hypoxic air per minute, with F_iO_2 between 21% and 10% (a simulated altitude of up to 5100 m). F_iO_2 was continuously measured (every 5 s) by two sensors located at 15 and 30 m in the tunnel and displayed on the control panel, which was managed only by the main investigator. Air input flow was sufficient for safe, comfortable, and stable training conditions, with temperature and humidity maintained at ~25°C and ~55% relative humidity, respectively. For RSH, ambient air was mixed with nitrogen (from pressurized tanks) to reduce F_iO_2 to ~14.5% in order to simulate an altitude of 3000 m. In order to blind subjects to altitude, the system was also run for RSN with normoxic airflow (F_iO_2, 21.0%) into the tunnel. For motivational reasons and reinforcement of the subjects' blinding to group classification, all players were assigned to different teammates during each of the six training sessions.

Specific training sessions. Each session lasted ~50 min, including a 15-min warm-up, repeated-sprint training routine, and a 10-min recovery phase (i.e., a total of 300 min for the six sessions during the 14-d training camp). Specifically, the repeated-sprint training routine included four sets of 5 × 5-s maximal sprints interspersed with 25 s of recovery with 5 min of passive recovery between sets, finally ending with a 10-min cooling-down period (Fig. 1B). Subjects were constantly reminded to exert "all-out" effort in trying to reach peak acceleration and to maintain the highest possible running speed for every 5-s sprint bout. Up to six subjects trained simultaneously in the inflatable marquee. Commercially available energy drinks and bottled water were provided *ad libitum* during training to ensure appropriate hydration.

Blinding. This research was run in a double-blind controlled manner. With the exception of the control group, subjects in both the LHTLH group and the LHTL group were told (based on the head coach's request to increase team motivation) that they were both residing in and training under hypoxic conditions but had no accurate information

about the simulated altitude levels inside the rooms and inflatable marquee. The efficacy of the blinding process was evaluated upon experiment termination (i.e., immediately after Post-2) by administering Likert scales (100 m marks from 0 to 4000 m), where each participant had to indicate (separately) which simulated altitude he believed he had been living at and training in for the first and second weeks of the altitude camp (i.e., scores for weeks 1 and 2 were then averaged to report only one value for the total duration of the camp).

Pre, Post-1, and Post-2 Testing Sessions

Vertical jumps. Players performed the following vertical jump tests with their hands kept on the hips to eliminate any influence of arm swing: (i) squat jump (SJ) starting from a static semisquatting position (~90° of flexion) maintained for ~1 s and without any preliminary movement; (ii) countermovement jump (CMJ) starting from a standing position, squatting down to ~90° angle, and extending the knee in one continuous movement; and (iii) one set of multirebound jumps (MRJ) with rebounds to the highest possible point six times. For SJ and CMJ, subjects were asked to perform two maximal trials, and the highest jump was recorded. During MRJ, they were instructed to keep their knees as stiff as possible ("ankle jumps") and to have as brief a contact time as possible. Jump heights were calculated by recording flight times (SJ, CMJ, and MRJ) and ground contact (MRJ) with an optical measuring apparatus (Optojump; Microgate, Bolzano, Italy).

Repeated sprints. The subjects underwent an RSA test consisting of eight 20-m sprints departing every 20 s. The sprints were performed back and forth to allow for passive recovery during the short rest period. Players had to complete the distance in a straight line as fast as possible. Three seconds before the start of each sprint, they were asked to assume the ready position and to await the start signal with a 3-s countdown (3, 2, 1, "go"). Each sprint was initiated from a standing position, 50 cm behind the photocell gate, which started a digital timer. Sprint times were measured to the nearest 0.01 s using photocells connected to an electronic timer (Polifemo Radio Light; Microgate), whose height was adjusted according to the height of the subject's hip. The two photocells were placed at 0- and 20-m distance intervals. During the first sprint, subjects were required to achieve at least 95% of their criterion score (i.e., defined as the best of three single 20-m sprints interspersed with 2 min of recovery; data not presented) as a check on pacing. All of the subjects satisfied this criterion score. Two scores were calculated during the RSA test: cumulated sprint time and percentage of sprint decrement calculated as follows: [(cumulated sprint time)/(best sprint time $\times$ 8) $-$ 1] $\times$ 100 (15). A similar RSA test (i.e., 6 $\times$ 30 m departing on 25 s) in highly trained field hockey players was found to be very reliable, as evidenced by a typical error of 0.7% for the total sprint time (39).

Specific aerobic capacity. To assess high-intensity intermittent running performance, subjects performed an incremental running test up to exhaustion (YYIR2) (1). Briefly, the test consisted in repeated 20-m shuttle runs at increasing speeds (starting at 13 $km \cdot h^{-1}$) controlled by audio beeps interspersed by 10 s of active recovery. When the subject failed to reach the finish line in time twice, the distance covered was then recorded and represented the test result. HR (Polar Electro, Kempele, Finland; 5 s on average) was measured, with the highest value retained as HR_{max}. During the YYIR2 test, none of the subjects reported an HR_{max} <95% of their age-predicted HR_{max} (i.e., traditional 220 $-$ age formula), indicating maximal exhaustion. This test is reproducible and is a sensitive tool for assessing aerobic capacity in team-sport players (20).

Hemoglobin mass. Hb_{mass} was measured in duplicate at each time point by using a slightly modified version (40) of the optimized CO rebreathing method described by Schmidt and Prommer (37). Briefly, subjects spent 5 min in sitting position before three capillary blood samples (35 μL) were taken from the earlobe and analyzed immediately for baseline carboxyhemoglobin values (ABL 800flex; Radiometer A/S, Copenhagen, Denmark). Subjects then rebreathed for 2 min a gas mixture of 100 mL of pure CO (Multigas SA, Domdidier, Switzerland) and 3.5 L of oxygen in a closed-circuit system (glass spirometer; Blood Tec GbR, Bayreuth, Germany). During the rebreathing period, a CO gas analyzer (Dräger PAC 7000; Dräger Safety, Lübeck, Germany) was used to check for possible CO leakage at the nose, mouthpiece, and spirometer system. On minutes 6 and 8 after the start of CO rebreathing, two final capillary blood samples were taken from the earlobe and averaged as a 7-min postcarboxyhemoglobin value. Directly before and 2 min after rebreathing, the same CO gas detector as described above was used to quantify end-tidal CO concentration (in parts permillion). Hb_{mass} was calculated from the mean change in carboxyhemoglobin before and after CO rebreathing, as described previously by Steiner and Wehrlin (40). Both measurements were performed on two consecutive days (12-h to 24-h time lag between measures), and the results were averaged. In our mobile laboratory, the typical error was 1.6% for CO rebreathing method and 1.1% for mean duplicate Hb_{mass} measurement over all measurement time points.

Statistical Analysis

All data in the text and figures are presented as mean ± SD. Relative changes (%) in performance are expressed with 95% confidence intervals (95% CI). LHTLH and LHTL room and inflatable marquee exposures were compared with paired *t*-test. One-way ANOVA was used to test differences in training load, questionnaires, and physiological measures between groups. Two-way ANOVA with repeated measures [Time (Pre vs Post-1 vs Post-2) $\times$ Condition (LHTLH vs LHTL vs LLTL)] was used to compare physical performance and Hb_{mass} data. ANOVA assumptions were verified before all statistical analyses. Pairwise differences were identified using the Holm–Sidak *post hoc* analysis procedure

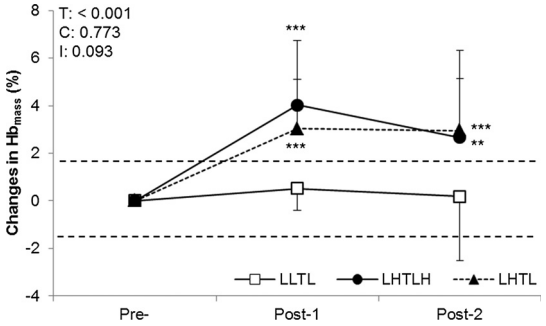

FIGURE 2—Mean changes in Hb_{mass} from baseline (Pre) to the end of the intervention (Post-1) and 3 wk after the intervention (Post-2) in the LHTLH, LHTL, and LLTL groups. Dashed lines represent the typical error of the CO rebreathing procedure in the present study (1.6%). C, condition effects; I, interaction effects; T, time effects. **$P < 0.01$, significantly different from Pre. ***$P < 0.001$, significantly different from Pre.

adjusted for multiple comparisons. Pearson's product–moment correlation analysis was employed to determine correlations between Pre and Post-1 and/or Post-2 changes between Hb_{mass} and physical performance tests. The null hypothesis was rejected at $P < 0.05$. All statistical calculations were performed using Sigmaplot 11.0 software (Systat Software, San Jose, CA).

RESULTS

Hypoxic dose and efficacy of the blinding procedure. Mean daily room confinement (14.5 ± 0.8 and 14.4 ± 0.7 $h \cdot d^{-1}$; $P = 0.52$), total RSH/RSN exposure (3.9 ± 0.7 and 3.7 ± 1.1 h; $P = 0.51$), and total hypoxic dose (202.5 ± 4.5 and 201.2 ± 3.0 h; $P = 0.75$) were similar between LHTLH and LHTL. Participants from both experimental groups were not able to correctly identify the simulated altitude they were residing at [2591 ± 767 m (range, 200–3700 m) and 2491 ± 658 m (range, 1500–3500 m) for LHTLH and LHTL, respectively] and training in [2445 ± 771 m (range, 1000–4000 m) and 2648 ± 761 m (range, 1000–3750 m) for LHTLH and LHTL, respectively]. Overall, this indicates that the blinding process was successful and that subjects were unaware of the hypoxic group classification.

Morning HR, SPO_2 and questionnaires. No difference ($P = 0.74$) in the mean values of wake-up HR was found between groups during the experimental period (60 ± 8, 60 ± 3, and 59 ± 7 bpm for LHTLH, LHTL, and LLTL, respectively). Mean SPO_2 for LLTL (97.2 ± 0.7%) was higher ($P < 0.001$) than those for both intervention groups, and LHTLH showed lower SPO_2 than LHTL during hypoxic exposition (92.3 ± 0.9% and 93.2 ± 0.9%, respectively; $P < 0.05$). No change in mean body weight was observed between groups ($P = 0.87$) during the study.

The mean Lake Louise score was 1.0 ± 0.9 for LHTLH, 1.2 ± 0.8 for LHTL, and 1.4 ± 0.7 for LLTL, and no difference ($P = 0.54$) was found between groups. Part A (0.6 ± 0.5, 0.7 ± 0.8, and 0.9 ± 0.7 for LHTLH, LHTL, and LLTL, respectively; $P = 0.41$) and part B (1.2 ± 1.3, 2.1 ± 1.7, and 2.6 ± 2.1 for LHTLH, LHTL, and LLTL, respectively; $P = 0.14$) of the DALDA questionnaire were not different between groups. The mean GSQS value for sleep quality during the intervention period was comparable for all groups (2.0 ± 0.9, 2.0 ± 1.1, and 2.1 ± 1.0 for LHTLH, LHTL, and LLTL, respectively; $P = 0.93$), indicating no disturbed sleep. Similarly, no difference in GSQS waking state (1.2 ± 0.6, 1.2 ± 0.6, and 1.1 ± 0.5 for LHTLH, LHTL, and LLTL, respectively; $P = 0.90$) was observable.

Training load. Overall training load was closely matched among the three groups during the study (3534 ± 412, 3702 ± 570, and 3179 ± 309 a.u. for LHTLH, LHTL, and LLTL, respectively; $P = 0.35$). No difference in mean field hockey training load occurred during the lead-in period (981 ± 142, 1054 ± 196, and 1070 ± 55 a.u.; $P = 0.57$), 2-wk intervention period (976 ± 112, 985 ± 143, and 1016 ± 99 a.u.; $P = 0.86$), and 3-wk postintervention period (987 ± 142, 981 ± 142, and 1094 ± 254 a.u.; $P = 0.59$) among the LHTLH, LHTL, and LLTL groups. Note that no significant difference ($P = 0.90$) in mean training load monitored during both specific RSH (590 ± 76 a.u.) and RSN (594 ± 35 a.u.) sessions was observable.

Hemoglobin mass. Compared with Pre, both hypoxic groups similarly increased their Hb_{mass} at Post-1, with no further change at Post-2 [LHTLH: 888 ± 107, 924 ± 114 ($P < 0.001$), and 912 ± 127 g ($P < 0.01$); LHTL: 931 ± 131, 957 ± 140 ($P < 0.001$), and 956 ± 137 g ($P < 0.001$)] (Fig. 2). Note that the increase in Hb_{mass} at Post-1 and Post-2, which exceeded the typical error of 1.6% for the CO rebreathing procedure, occurred in 18 and 15 of the 23 subjects composing the two intervention groups, respectively. Hb_{mass} remained unchanged for LLTL at Pre, Post-1, and Post-2 (929 ± 171, 934 ± 170, and 930 ± 163 g, respectively). Finally, no significant correlation among Pre, Post-1, and/or Post-2 changes in Hb_{mass} and any physical performance data could be evidenced.

Physical performance. With the exception of CMJ height (time effect, $P < 0.05$), none of the vertical jump test data displayed a main effect of condition or any significant interaction between time and condition (Table 2).

TABLE 2. Vertical jump performance results before (Pre) and after (Post-1 and Post-2) 14 d of "LHTL" exposure and additional RSH (LHTL + RSH, namely, LHTLH), additional RSN (LHTL + RSN, namely, LHTL), or in the control group (LLTL).

	LHTLH			LHTL			LLTL			*P* Value		
	Pre	Post-1	Post-2	Pre	Post-1	Post-2	Pre	Post-1	Post-2	Time	Condition	Interaction (Time–Condition)
SJ (cm)	36.2 ± 3.2	37.3 ± 3.0	36.1 ± 3.7	36.2 ± 5.4	36.8 ± 4.8	36.3 ± 4.8	37.3 ± 5.3	38.6 ± 5.6	37.5 ± 4.5	0.080	0.757	0.903
CMJ (cm)	39.2 ± 3.5	38.7 ± 3.6	38.2 ± 3.9	38.9 ± 5.6	39.0 ± 5.5	37.3 ± 5.8	41.5 ± 5.6	41.1 ± 5.8	39.8 ± 4.5	0.013	0.506	0.924
MRJ (cm)	28.9 ± 4.4	30.9 ± 4.2	30.7 ± 4.0	30.0 ± 6.5	30.6 ± 5.6	29.8 ± 6.2	30.6 ± 5.5	32.0 ± 5.9	31.4 ± 5.7	0.128	0.847	0.895

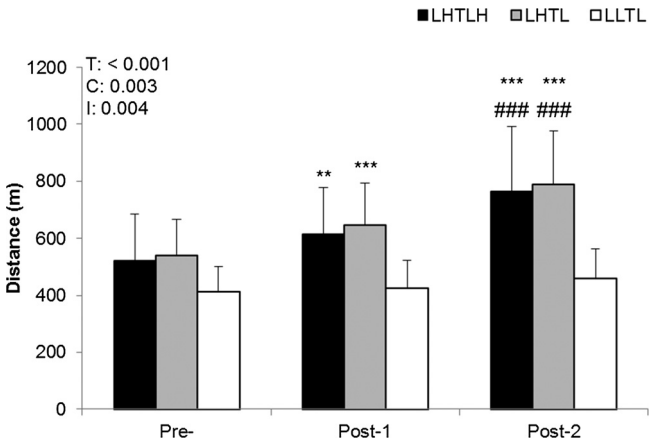

FIGURE 3—Distance (m) covered during YYIR2. Measurements were taken before (Pre), immediately after the intervention (Post-1), and 3 wk after the intervention (Post-2). C, condition effects; I, interaction effects; T, time effects. **$P < 0.01$, significantly different from Pre. ***$P < 0.001$, significantly different from Pre. ###$P < 0.001$, significantly different from Post-1.

From Pre to Post-1, the intervention resulted in similar increases in YYIR2 performance in LHTLH [from 520 ± 165 to 615 ± 162 m; +21% (95% CI, 7% to 36%); $P < 0.01$] and LHTL [from 540 ± 126 to 647 ± 147 m; +22% (95% CI, 10% to 34%); $P < 0.001$], whereas no significant change occurred in LLTL [from 413 ± 89 to 427 ± 96 m; +4% (95% CI, −19% to 26%)] (Fig. 3). At Post-2, both hypoxic groups further increased their YYIR2 performance by a mean of +45% (95% CI, 21% to 74%) (764 ± 227 m) and +19% (95% CI, 21% to 75%) (789 ± 187 m) in reference to Pre and Post-1 (both $P < 0.001$), respectively.

During the RSA test (Fig. 4), cumulated sprint time decreased from Pre to Post-1 in both LHTLH [from 27.23 ± 1.15 to 23.23 ± 1.02 s; −3.6% (95% CI, −5% to −2%); $P < 0.001$] and LHTL [from 27.05 ± 0.81 to 26.54 ± 0.77 s; −1.9% (95% CI, −3% to −1%); $P < 0.01$], with no significant change in LLTL [from 26.98 ± 1.03 to 26.81 ± 1.47 s; −0.7% (95% CI, −3% to 1%)]. Compared with Pre, cumulated sprint time at Post-2 remained significantly shorter ($P < 0.001$) for LHTLH [26.21 ± 1.09 s; −3.5% (95% CI, −5% to −2%)], whereas no difference was observed for LHTL [26.63 ± 0.90 s; −1.5% (95% CI, −3% to 0%)] and LLTL [26.86 ± 1.31 s; −0.8% (95% CI, −3% to 1%)]. Sprint decrement score [averaged for all conditions, 4.0% (95% CI, −1% to 9%)] did not change throughout the protocol ($P = 0.14$).

DISCUSSION

To the best of our knowledge, the present study is the first randomized, double-blind, controlled investigation verifying the usefulness of combining hypoxic training methods when attempting to improve sea-level performance in elite team-sport athletes. We have administered "traditional" LHTL with RSH (namely, "LHTLH" hypoxic training) and compared its immediate (few days) and prolonged (few weeks) effects with a combination of LHTL and RSN. Our results are clear and compelling: first, similar increases in Hb_{mass} and specific aerobic fitness (YYIR2 performance) in the two intervention groups despite a low altitude (≥200 h); second, YYIR2 performance was further enlarged at Post-2 in reference to Post-1 in the two intervention groups, whereas Hb_{mass} was maintained; and third, RSA was improved in the two intervention groups yet with twice-larger gains measured at Post-1 in LHTLH compared with LHTL. Three weeks after the intervention, RSA performance improvements were only maintained in the LHTLH group. Overall, this short-term LHTLH method (i.e., 14 d of LHTL exposure + 6 RSH sessions, as performed "in season") demonstrated greater effects on Hb_{mass} and sport-specific physical performance (YYIR2 and RSA) than did LHTL and LLTL training in elite field hockey players, with the benefits lasting for at least 3 wk postintervention.

LHTL(H) as stimulus for increasing Hb_{mass}. In the present study, we have demonstrated that 14 d of LHTL in normobaric hypoxia at ~2800–3000 m were sufficient to immediately increase Hb_{mass} by ~3%–4%. This increase is greater than the magnitude observed in previous studies with similar hypoxic doses in normobaric hypoxia or is in line with longer exposure (i.e., 1% for every 100 h) to hypobaric hypoxia (18). More specifically, our results are very similar

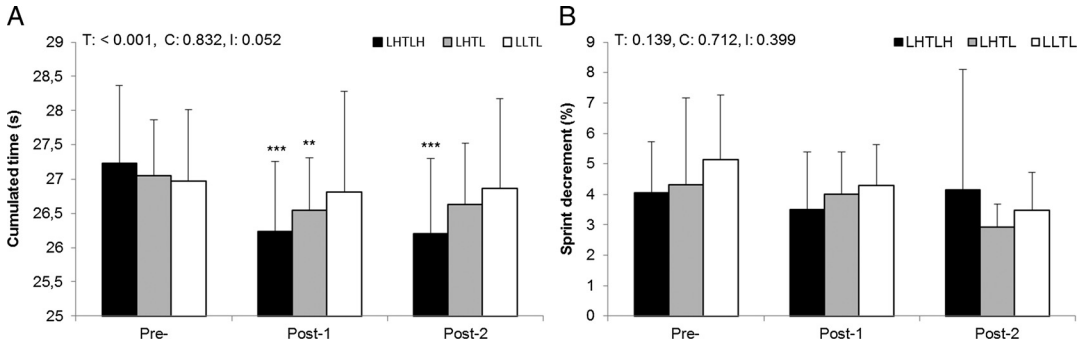

FIGURE 4—Cumulated sprint times (A) and sprint decrement score (B) during RSA test (8 × 20 m–20 s passive recovery). Measurements were taken before the intervention (Pre), immediately after the intervention (Post-1), and 3 wk after the intervention (Post-2). C, condition effects; I, interaction effects; T, time effects. **$P < 0.01$, significantly different from Pre. ***$P < 0.001$, significantly different from Pre.

to those involving team-sport populations. Reportedly, Hb_{mass} increased by ~3%–4% after (i) 18–19 d of preseason moderate-altitude (~2100 m) training camp in elite AFL players (28); (ii) 10 d of simulated LHTL in international-level water-polo players (12); and (iii) 13 d spent at 3600 m altitude in U17 soccer players (41). Direct comparisons of Hb_{mass} gains between the aforementioned studies are difficult because the magnitude of hypoxia-induced hematological changes would differ according to various "dose–response" relationships (25), training content (31), and individual responsiveness (6). Of importance, with no significant difference in training load between our three groups, the lack of change in Hb_{mass} in the LLTL group would indicate that any specific hockey practice/training-induced increase in Hb_{mass} is unlikely for this short period. Despite ongoing debate surrounding the importance of hematological factors in driving adaptations induced by chronic exposure to hypoxia (18), it has been acknowledged that, in elite endurance (16) and team-sport (28) athletes, hypoxia-induced Hb_{mass} response is inversely related to its initial level (27). In our study, preintervention Hb_{mass} values (mean of the three groups, 916 ± 133 g) were in agreement with those measured in other team-sport athletes (926 ± 118 g, ranging from 721 to 1023 g in AFL, soccer, field hockey, and water-polo players) (12,19,28,41).

With similar room confinement time for our two intervention groups, the addition of six RSH sessions (~5 h at a simulated altitude of 3000 m) for LHTLH had no measurable impact on Hb_{mass} increase. This demonstrates that hypoxic dose is the main factor for Hb_{mass} increase and that RSH *per se* has no "erythropoietic" effect. It is also known that, at moderate altitude, the occurrence of negative side effects (e.g., AMS symptoms) is very low (35). This was confirmed with the present athletes who displayed low perceptual scores to questionnaires. Taken as a whole, this fully supports the notion that a relatively short period of normobaric hypoxic LHTL or LHTLH exposure (~2 wk and low hypoxic dose of ~200 h at simulated altitudes of ~2800–3000 m) may be sufficient to increase Hb_{mass} in team-sport athletes.

Immediate effect on sea-level physical performance. The aforementioned increase in Hb_{mass} at Post-1 was also transposed into an immediate improved specific aerobic performance, as evidenced by the large (~21%) and similar increase in YYIR2 distance in the two intervention groups. Note that the absence of an increase in jumping performance after LHTL, combined with repeated sprinting (under hypoxic or normoxic conditions), would suggest that leg power was not modified in response to such training and was therefore probably not directly involved in marked aerobic performance gains. Our substantial in-season increments in YYIR2 performance are in line with the findings of Galvin et al. (11), who reported that a 4-wk RSH treadmill sprint intervention induced +33% improvements in well-trained academy rugby players' intermittent running performance (i.e., Yo-Yo Intermittent Recovery level 1). Therefore, the present data confirm that within no more than 14 d of residing in simulated altitude (irrespective of additional RSH training) there were substantial ergogenic benefits of LHTL for elite team-sport athletes when tested at sea level.

Along with YYIR2 performance, improvement in RSA also occurred in our two experimental groups, with a two-fold superior benefit seen in LHTLH compared with LHTL (cumulated sprint time, −3.6% vs −1.9%, respectively). In addition to this shorter cumulated sprint time, the unchanged percentage of sprint decrement observed at Post-1 strengthens this result. By using sport-specific ecological training and testing setting (i.e., repeated sprinting on synthetic grass with players wearing their field hockey shoes inside a mobile inflatable hypoxic marquee under normobaric hypoxic conditions), the present results therefore strengthen the validity of previous studies (5,9,11,33). In particular, it appears of practical relevance to solve some of the problems related to the congested calendars of the majority of professional team-sport athletes, which do not allow players to afford time for the usually recommended 3- to 4-wk blocks of altitude training. Furthermore, it suggests that the proposed mechanisms (see later) were not blunted by the Hb_{mass} increase. The rationale for this study was based on the assumption that, if the LHTL paradigm works to improve sea-level "endurance" performance and if additional RSH works to improve sea-level-specific performance (i.e., higher tolerance for repeated-sprint exercises) (5,9,30), then the physiological benefits of the LHTLH method must derive from the combination of these two hypoxic methods. Whereas the mechanisms behind coupling different hypoxic methods are currently unknown, our findings provide evidence that both "aerobic" and "anaerobic" benefits acutely improve sea-level team-sport physical performance.

Although the main mechanism for improved sea-level performance after "traditional" LHTL exposure relies on an increase in red cell mass (23), other hypoxia-induced physiological adaptations are possible and may include improved muscle buffer capacity (17). Similarly, exercise capacity during high-intensity intermittent tasks depends not only on Hb_{mass} but also on molecular adaptations at the skeletal muscle level and on the efficiency of the neuromuscular system (8,9,11). When used in isolation (8), RSH was superior to RSN in enhancing peripheral adaptations (i.e., oxidative capacity, capillary density, and muscle glycolytic potential, as well as increased expression of hypoxia inducible factor 1α and downstream genes for oxygen and transport) (9,38). Pending confirmatory research, this would suggest a hypoxia-induced increase in anaerobic glycolytic activity in muscle and a more efficient use of fast-twitch muscle fibers (8,9). With comparable Post-1 Hb_{mass} gain between LHTLH and LHTL, the twice-larger improvement in RSA in the former group, compared with the latter group, is likely linked to the aforementioned RSH-specific adaptations. Nevertheless, one limitation relates to our inability to examine the independent effects of repeated-sprint training and residing in hypoxia. Although the addition of a group of

players living near sea level with additional RSN would have improved our test design, increasing our sample number was unrealistic in our cohort of elite players.

Delayed effects on sea-level performance. When players were retested 3 wk after completion of the hypoxic intervention, the LHTLH and LHTL groups displayed maintenance of Hb_{mass} but enhancement of YYIR2 performance at Post-2 in reference to Post-1, whereas only LHTLH players were able to preserve their hypoxia-induced RSA gains. Within competitive field hockey matches, research has reported a reduction in total distances achieved by players at high intensity (26). Bearing in mind that decisive events during competitive games are often reliant on transient RSA (10,32), the larger improvement in RSA performance in LHTLH players compared with LHTL players is likely to give them further competitive edge in the most intense periods of a game or toward match end. Three main components have been suggested to primarily influence rate of deacclimatization or change in performance (i.e., training responsiveness and exercise capacity) after an altitude training stimulus (7): timing in decay in Hb_{mass}, consequences of ventilatory acclimatization, and alterations in biomechanical and neuromuscular factors associated with force production. In the present study, the increase (~3%) in Hb_{mass} at Post-2 appears consistent with the model estimated by Gore et al. (18), which was susceptible to be maintained for up to 20 d after LHTL. Considering the paucity of data describing the decay and/or normalization of hematological response after return to sea level, it is unclear whether a period of reacclimatization to sea level is necessary to obtain the full effects of additional high-intensity altitude training, whereas this effect may well relate to ventilatory factors (i.e., time course of the decay of ventilatory acclimatization with return to sea level [44]). In our study, however, there was no difference in YYIR2 improvement during the postaltitude periods between LHTLH and LHTL, making it unlikely that the postintervention improvement represents a generic "delayed" response to altitude. Conversely, it could be hypothesized that, due to the positive acclimatization response to hypoxia, the ability to train at a higher level after return to sea level may allow achievement of higher fitness levels (i.e., improved training responsiveness). Although postintervention training load was strictly controlled and monitored for 3 wk (i.e., similar between groups), it cannot be completely ruled out that easier subjective ratings to produce the same "external physical output" may have resulted from the LHTLH intervention, which deserves further research attention. Finally, although speculative, hypoxia-induced improvement in the active musculature neural drive (4) may have up-regulated musculoskeletal stiffness, leading to faster stride frequencies and thereby better sea-level RSA (3). Reportedly, after 28 d of LHTL—where, out of as many as 40 total training sessions, seven training sessions classified as higher intensity were performed at lower altitudes (365–1150 m), no alteration in stride length, stride frequency, ground contact, or aerial time was observed in a group of six elite distance runners tested at common racing speeds (18–25 $km \cdot h^{-1}$) (21). However, as this later study neither recruited team-sport participants nor employed sprinting speeds, with measurements restricted to the period immediately after intervention, more research is required.

CONCLUSIONS

This study is the first to combine traditional LHTL with RSH, compared with similar training in normoxia, in team-sport players and to determine its short-term (few days) and long-term (3 wk) effects on hematological parameters and sport-specific physical performance. With only a low hypoxic dose (≥200 h), "LHTLH" conducted for 2 wk during the in-season period of elite field hockey players is an attractive intervention to elicit "aerobic" and "anaerobic" benefits for improving sea-level performance. Our results displayed similar immediate up-regulated Hb_{mass} and increase in specific aerobic performance in the two experimental groups. However, the superiority of LHTLH to LHTL was demonstrated in the RSA test with twice-larger acute performance gains, with those being well maintained at least for 3 wk after the LHTLH intervention only. This advocates that nonhematological factors outside the role played by oxygen-carrying capacity are probably more robust to explain performance enhancement and/or maintenance after LHTLH. Determination of optimal characteristics for combining hypoxic methods and identification of the hematological, ventilatory, and biomechanical mechanisms of adaptation and individual rates of decay in deacclimatization of the newly proposed LHTLH method also require future research.

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Chapter 12

Article 7 – Maximal-intensity hypoxic exercise superimposed to hypoxic residence boost HIF-1α and related genes transcription in human skeletal muscle

12. Article 7 - Repeated maximal-intensity hypoxic exercise superimposed to hypoxic residence boosts HIF-1α and related genes transcription in human skeletal muscle

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Abstract

Aim. To determine the nature and magnitude of human skeletal muscle molecular adaptations occurring immediately (Post-1) and three weeks (Post-2) after 14 days of passive normobaric hypoxic exposure (≥14 $h.day^{-1}$ at FiO_2 14.5-14.2%) with the addition of 6 maximal-intensity exercise sessions either in normobaric hypoxia (FiO_2 ~14.2%) (LHTLH; n = 9) or in normoxia (FiO_2 20.9%) (LHTL; n = 11) both compared to a control group living in normoxia with no additional maximal-intensity exercise (LLTL; n = 10).

Methods. Muscle biopsies were obtained from the *vastus lateralis*.

Results. Hypoxia inducible factor-1α subunit, vascular endothelial growth factor, myoglobin, peroxisome proliferator-activated receptor-gamma coactivator 1 alpha and mitochondrial transcription factor A mRNA levels increased at Post-1 (all $P \leq 0.05$) in LHTLH, but not in LHTL or LLTL, and returned near baseline levels at Post-2. The protein expression of citrate synthase increased in LHTLH ($P<0.001$ and $P<0.01$ at Post-1 and Post-2, respectively) and LLTL ($P<0.01$ and $P<0.05$ at Post-1 and Post-2, respectively), whereas it decreased in LHTL at Post-1 and Post-2 (both $P<0.001$).

Conclusion. Combined with residence in normobaric hypoxia, repeated maximal-intensity hypoxic exercise induces short-term beneficial changes in muscle transcriptional factors that are of larger magnitude or not observed with similar normoxic exercise. The decay of molecular adaptations was relatively fast, with most of benefits already absent 3 weeks post-intervention.

Introduction

Human skeletal muscle is a greatly specialized and a highly adaptive tissue. Increased oxygen (O_2) consumption and/or a lowered tissue O_2 tension (hypoxia) are known to initiate a cascade of systemic, local and cellular adaptations, all aiming to restore O_2 homeostasis (Semenza, 1999, Semenza, 1998). Whatever the origin of the hypoxic stimulus [*i.e.*, exercise-induced (Ameln et al., 2005) or environmentally- (O_2-deprived environments) (Hoppeler and Vogt, 2001)], the hypoxia inducible factor-1α subunit (HIF-1α), an O_2 sensitive transcriptional activator that stabilizes in the nucleus under hypoxic conditions, is the main factor mediating these responses (Wang and Semenza, 1995). HIF-1α is a key regulator responsible for the induction of hypoxia-induced genes (Ke and Costa, 2006) in turn involved in erythropoiesis/iron metabolism, angiogenesis, glucose metabolism as well as cell proliferation/survival and apoptosis (Lundby et al., 2009, Semenza, 1998, Semenza, 1999).

The current scientific literature indicates that prolonged hypoxic residence (≥10-12 $h.day^{-1}$ for a minimum of 10 consecutive days) would only induce minimal adaptations in human skeletal muscle tissue. Specifically, down-regulation of HIF-1α (-49%) and its target genes [*e.g.*, vascular endothelial growth factor (VEGF) (-66%)] (D'Hulst et al., 2016), absence of muscle angiogenesis as well as marginal changes in oxidative enzymes [*e.g.*, citrate synthase (CS)] (Lundby et al., 2009) have been reported. Contrastingly, exercising in hypoxia appears appealing to promote structural and functional adaptations in skeletal muscle (Lundby et al., 2009, Hoppeler and Vogt, 2001). While these changes have been reported to occur immediately (within few days) after the aforementioned hypoxic interventions, the extent to which delayed (after several weeks) positive muscle phenotype adaptations also occur is unknown.

While chronic low-intensity 'aerobic' exercise in hypoxia may evoke cellular adaptations via HIF-1α activation (Vogt et al., 2001, Zoll et al., 2006), the magnitude of these responses likely depends on the hypoxic dose (Lundby et al., 2009), and may not necessarily translate in

substantial physical performance benefits in endurance individuals (Lundby et al., 2012, Roels et al., 2007, Truijens et al., 2003). Interestingly, it has been postulated that exercise intensity in hypoxia per se modulates muscle molecular mechanisms of O_2 homeostasis with '*adaptations that compensate for the reduced availability of O_2 during exercise*' (Hoppeler and Vogt, 2001). Reportedly, maximal-intensity hypoxic exercise, where short 'all-out' efforts (≤ 10 s) with incomplete recoveries (< 30 s) are repeated (namely repeated sprints in hypoxia or RSH), induces several molecular adaptations at the skeletal muscle level compared to similar exercise in normoxia (RSN) (Faiss et al., 2013b). Specifically, the mRNA expression of genes involved in O_2 signaling (HIF-1α), O_2 carrying [myoglobin (Mb)] and pH regulation [carbonic anhydrase-3 (CA-3)] were up-regulated after RSH but not after RSN. Nonetheless, the observation of a concomitant down-regulation of genes involved in mitochondrial biogenesis [mitochondrial transcription factor A (TFAM) and peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α)] after RSH would suggest a shift from aerobic to anaerobic glycolytic activity in the muscle and a more efficient use of fast twitch (FT) muscle fibers (Faiss et al., 2013a, Faiss et al., 2013b, Puype et al., 2013).

Therefore, the purpose of this study was to investigate the acute and delayed skeletal muscle molecular adaptations associated with 14 days of passive normobaric hypoxic exposure [the so-called 'live high-train low' (LHTL) paradigm] combined with RSH (LHTL+RSH, namely LHTLH) or RSN (LHTL+RSN, namely LHTL) [both compared to a control condition, *i.e.*, 'live low-train low' (LLTL)]. Our hypothesis was that, when combined to passive normobaric hypoxic residence, repeated maximal-intensity hypoxic exercise induces acute muscle molecular adaptations – *i.e.*, HIF-1α pathway and its target genes – not observed (or to a lower extent) with similar normoxic exercise. We also expected that the molecular adaptations would be maintained for a longer period when combining passive normobaric hypoxic residence with repeated maximal-intensity hypoxic exercise compared to the other

conditions tested. This is the first study to investigate the effects of prolonged passive normobaric hypoxic exposure with superimposed maximal-intensity exercise sessions in hypoxia (LHTLH) *vs*. normoxia (LHTL) on molecular adaptations in human skeletal muscle.

Materials and methods

Subjects. Thirty lowland elite male field hockey players (age 25.1 ± 4.5 years, height 177.8 ± 5.6 cm, body weight 75.2 ± 7.7 kg and estimated VO_2max 52.0 ± 1.9 $mL.min^{-1}.kg^{-1}$) were recruited among Belgium, Spanish and Dutch first division clubs to participate in this study. The subjects were fully informed of the possible risks involved in the study before providing written consent. The study was approved by the Anti-Doping Lab Qatar institutional review board (Agreement SCH-ADL-070) and was conducted according to the Helsinki Declaration. Exclusion criteria for participation were acclimatization or exposure to hypoxia of more than 2000 m for more than 48 h during a period of 6 months before the study, and any history of altitude-related sickness and health risk that could compromise the subject's safety during exercise and/or hypoxia exposure. During the study, two subjects (control group: n = 1; experimental groups: n = 1) were excluded due to injury.

Procedures. The experimental design and physical performance results have been described in details elsewhere (Brocherie et al., 2015). Briefly, it consisted in three testing sessions before (Pre-), immediately (2-3 days, Post-1) and three weeks (Post-2) after 14 days of intervention. After the completion of Pre-, subjects were randomly assigned to one of the three following groups: 14 days of residence in normobaric hypoxia (≥14 $h.day^{-1}$ at 2800-3000 m, FiO_2 14.5-14.2%) during which subjects exercised (*i.e.*, regular field hockey practices) at sea level with the addition of six maximal-intensity exercise sessions either in normobaric hypoxia simulating an altitude of 3000 m (LHTL+RSH, namely LHTLH; n = 8) or in normoxia (LHTL+RSN, namely LHTL; n = 11) and control (LLTL; n= 9) where

subjects did not performed any additional specific exercise. Importantly, this research was successfully run in a double-blinded, controlled manner (Brocherie et al., 2015).

Living hypoxic exposure. The sleeping and recreational hypoxic facilities were fully furnished normobaric hypoxic rooms with O_2 filtration (CAT system, Colorado Altitude Training, Louisville, Colorado, USA). The two intervention groups (LHTLH and LHTL) were exposed (i.e. from 22:00 to 07:00, from 08:00 to 10:00 and again from 13:00 to 16:00; and were encouraged to spend more time in if desired) to a normobaric hypoxia equivalent to 2500 m (FiO_2 15.1%, BP 768.0 mmHg, PiO_2 108.3 mmHg) for the first 24 h of the intervention period (day 1). Thereafter, the O_2 fraction was further decreased to the equivalent of 2800 m (FiO_2 14.5 ± 0.1%, BP 766.8 ± 1.1 mmHg, PiO_2 104.5 ± 0.6 mmHg; days 2-5) and 3000 m (FiO_2 14.2 ± 0.1%, BP 765.3 ± 1.5 mmHg, PiO_2 101.7 ± 0.8 mmHg; days 6-14). Concentrations of ferritin (155.2 ± 78.7 $\mu g.L^{-1}$, range: 45-279 $\mu g.L^{-1}$) and soluble transferrin receptor (256.6 ± 33.7 $mg.dL^{-1}$, range: 202-330 $mg.dL^{-1}$) measured during the 2-weeks lead-in period at sea level indicated that none of the subjects was iron deficient at the commencement of the study.

Supervised exercise protocol. In addition to their usual field hockey practices and matches [carefully monitored and reported in (Brocherie et al., 2015)], subjects of the two intervention groups (LHTLH and LHTL) completed six specific maximal-intensity exercise sessions on a synthetic grass ground, inside a 45-m long mobile inflatable simulated hypoxic equipment (Altitude Technology Solutions Pty Ltd, Brisbane, Queensland, Australia), as described elsewhere (Girard et al., 2013). For RSH, ambient air was mixed with nitrogen (from pressurized tanks) to reduce FiO_2 to ~14.2% in order to simulate an altitude of 3000 m. In order to blind subjects to altitude, the system was also run for RSN with normoxic airflow (FiO_2 21.0%) into the tunnel.

Each session lasted ~50 min including a 15-min warm-up, the maximal-intensity exercise and a 10-min recovery phase (*i.e.*, a total of 300 min for the 6 sessions among the 14-days intervention). Specifically, the maximal-intensity exercise included 4 sets of 5 × 5-s maximal sprints interspersed with 25 s of passive recovery with 5 min of standing rest between sets.

Muscle Biopsy Samples. Biopsy samples were all taken by the same experienced medical doctor with a 5-mm Bergström type needle (in conjunction with a suction device to create a negative pressure) in the mid portion of the *vastus lateralis* muscle after local anesthesia (1% xylocaine, subcutaneously). Biopsies were preceded by 48 h without any exercise activity and were taken randomly in opposite legs (*i.e.*, left-right-left or vice versa) during subsequent test sessions. For mRNA analysis, the muscle tissue portion was immediately frozen in liquid nitrogen and stored at -80°C until required for analyses.

Western blotting. Frozen muscle tissue (~20 mg) was homogenized 3 × 5 s with a Tissuelyser (Qiagen, Hilden, Germany) in an ice-cold buffer (1:10, w/v) [50 mM Tris-HCl pH 7.0, 270 mM sucrose, 5 mM EGTA, 1 mM EDTA, 1 mM sodium orthovanadate, 50 mM glycerophosphate, 5 mM sodium pyrophosphate, 50 mM sodium fluoride, 1 mM DTT, 1% Triton-X 100 and a protease inhibitor cocktail (Roche Applied Science, Vilvoorde, Belgium)]. After centrifugation of homogenates at 10 000 g for 10 min at 4°C, the supernatants were stored at -80°C. Protein concentration was measured using the DC protein assay kit (Bio-Rad laboratories, Nazareth, Belgium) with bovine serum albumin as a standard. Proteins (30-50 µg) were separated by SDS-PAGE (7.5 – 12.5%) and transferred to PVDF membranes. Subsequently, membranes were blocked with 5% non-fat milk for 1 h and incubated overnight (4°C) with the following antibodies: glucose transporter 4 (GLUT-4, #PA1-1065, Thermo Scientific, Erembodegem, Belgium), phosphofructo kinase (PFK, #166722, Santa Cruz, Huissen, The Netherlands), monocarboxylate transporter-1 (MCT-1,

#AB3538P, Millipore, Overijse, Belgium), monocarboxylate transporter-4 (MCT-4, #AB3316P, Millipore), CA-3 (#135995, Abcam, Cambridge, UK), AMP-activated protein kinase alpha (AMPKα, #2532, Cell Signaling, Leiden, The Netherlands), phospho-AMPKα Thr172 (#2535, Cell Signaling), CS (#14309, Cell Signaling), eukaryotic elongation factor 2 (eEF2, #2332, Cell Signaling). Appropriate horseradish peroxidase-conjugated secondary antibodies (Sigma-Aldrich, Bornem, Belgium) were used for chemiluminescent detection of the proteins of interest. Membranes were scanned and quantified with Genesnap and Genetools softwares (Syngene, Cambridge, UK), respectively. The results are presented as the ratio protein of interest/eEF2 or as the ratio phosphorylated/total form for AMPK.

Real-time quantitative Polymerase Chain Reaction. RNA was extracted using TRIzol (Invitrogen, Vilvoorde, Belgium) from 20–25 mg of frozen muscle tissue. RNA quality and quantity were assessed by spectrophotometry with a Nanodrop (Thermo Scientific, Erembodegem, Belgium). One µg of RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Gent, Belgium) according to manufacturer's instructions. A SybrGreen-based master mix (Applied Biosystems, Erembodegem, Belgium) was used for real-time PCR analyses using the ABIPRISM 7300 (Applied Biosystems). Real-time PCR primers were designed for human HIF-1α, VEGF, Mb, cytochrome c oxidase subunit 4 (COX-4), PGC-1α, TFAM, endothelial nitric oxide synthase (eNOS) and, neuronal nitric oxide synthase (nNOS) (Table 1). Thermal cycling conditions consisted of 40 three-step cycles including denaturation of 30 s at 95°C, annealing of 30 s at 58°C and extension of 30 s at 72°C. All reactions were performed in triplicate. To compensate for variations in input RNA amounts and efficiency of reverse transcription ribosomal protein L19 (RPL19) and beta-2-microglobulin (B2M) mRNA were quantified, and results were normalized to these values. These genes were chosen out of three normalization genes using

the GeNorm applet according to the guidelines and theoretical framework described elsewhere (Vandesompele et al., 2002).

Gene	Forward Primer	Reverse Primer
HIF-1α	GCCCCAGATTCAGGATCAGA	TGGGACTATTAGGCTCAGGTGAAC
VEGF	TTTCTGCTGTCTTGGGTGCATTGG	ACCACTTCGTGATGATTCTGCCCT
Mb	GCCACCAAGCACAAGATC	GGCATCAGCACCAAAGT
COX-4	GAGAGCTTTGCTGAGATGAA	CCGTACACATAGTGCTTCTG
PGC-1α	GGGATGATGGAGACAGCTATGG	CTCTTGGTGGAAGCAGGGTC
TFAM	AGCGTTGGAGGGAACTTCCTGATT	TTCTTTATATACCTGCCACTCCGCCC
eNOS	CAGTTACCAGCTAGCCAAAGT	CTCATTCTCCAGGTGCTTCAT
nNOS	CAGAACTCACACAAGGTCTATC	GTTGACCGACTGGATTTAGG
RPL19	CGCTGTGGCAAGAAGAAGGTC	GGAATGGACCGTCACAGGC
B2M	ATGAGTATGCCTGCCGTGTGA	GGCATCTTCAAACCTCCATG

Table 1. PCR Primers sequences.

Data and statistical analysis. Values are expressed as means ± SD. Percent changes in means (Post-1 and Post-2 *vs*. Pre-) are given for biopsies, with Pre- values being normalized to 1. Two-way ANOVA with repeated measures [Time (Pre- *vs*. Post-1 *vs*. Post-2) × Group (LHTLH *vs*. LHTL *vs*. LLTL)] was used to compare each measured variable. When significant modifications were found, Holm-Sidak post-hoc test was performed to localize the effect. All analyses were made using Sigmaplot 11.0 software (Systat Software, CA, USA). Null hypothesis was rejected at $P < 0.05$.

Results

Skeletal muscle mRNA expression analysis and enzyme activity. Figures 1 and 2 display changes from Pre- to Post-1 and Post-2 in mRNA and protein expression levels in the three groups.

O_2 signaling. Compared to Pre-, HIF-1α mRNA levels increased at Post-1 ($P < 0.01$) in LHTLH only, before returning to near Pre- values at Post-2 (Fig. 1). At Post-1, higher HIF-1α mRNA levels were observed in LHTLH compared to LHTL and LLTL (both $P < 0.05$).

O_2 carrier. Compared to Pre-, higher mRNA levels of VEGF ($P = 0.05$) and Mb ($P < 0.05$) occurred at Post-1 in LHTLH (Fig. 1). At Post-1, VEGF and Mb values were also higher in LHTLH than in LLTL (both $P < 0.05$).

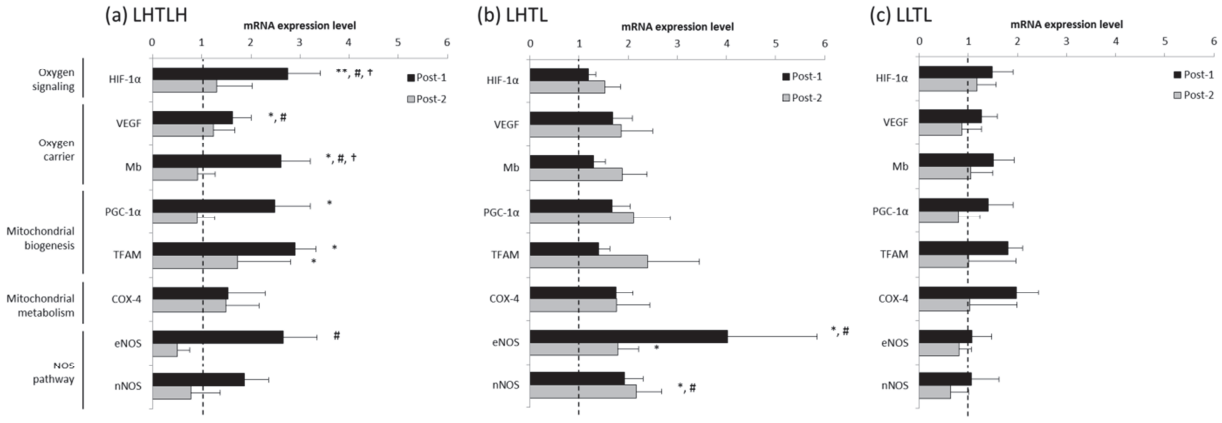

Figure 1. Relative changes in selected mRNA expression markers from before (Pre-) to immediately (Post-1) and 3 weeks (Post-2) after the intervention. The intervention consisted in 14-days of passive normobaric hypoxic exposure combined with (a) repeated maximal-intensity hypoxic exercise in hypoxia (LHTLH) or (b) normoxia (LHTL). (c) A control group followed a 'live low-train low' (LLTL) protocol. Black and grey bars represent Post-1 and Post-2 values of mRNA concentrations in *vastus lateralis* muscle, respectively. These values were normalized to baseline values (Pre-), which were set to 1 (dashed line). Values are means ± SD. * $P < 0.05$, ** $P < 0.01$ *vs*. Pre-intervention; # $P < 0.05$ *vs*. LLTL and † $P < 0.05$ *vs*. LHTL. HIF-1α, hypoxia inducible factor-1a; VEGF, vascular endothelial growth factor; Mb, myoglobin; COX-4, cytochrome oxidase 4; PGC1-α, proliferator-activated receptor gamma coactivator-1α; TFAM, mitochondrial transcription factor A; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase.

Mitochondrial biogenesis and metabolism. Transcript levels of regulators of mitochondrial biogenesis PGC-1α ($P < 0.05$ at Post-1) and TFAM ($P < 0.05$ at Post-1 and Post-2) were increased in LHTLH only. No significant changes were detected in COX-4 mRNA levels. The protein expression of CS increased in LHTLH ($P < 0.001$ at Post-1and $P < 0.01$ at Post-2) and LLTL ($P < 0.01$ at Post-1, and $P < 0.05$ at Post-2), whereas it decreased in LHTL ($P < 0.001$ at Post-1 and Post-2) (Fig. 2).

Nitric oxide synthase pathway. Compared to Pre-, eNOS and nNOS mRNA levels tended to increase at Post-1 in LHTLH ($P = 0.30$ and $P = 0.09$, respectively) (Fig. 1). In LHTL, only

eNOS mRNA levels increased significantly from Pre- to Post-1 ($P < 0.01$), while values of both eNOS and nNOS were higher at Post-2 *vs*. Pre (both $P < 0.05$). The mRNA levels of eNOS at Post-1 were higher in LHTLH and LHTL compared to LLTL (both $P < 0.05$).

pH regulation. The protein expression of MCT-1, but not of MCT-4 and CA-3, was increased in LHTLH ($P < 0.05$) and LHTL ($P < 0.05$) at Post-1 in reference to Pre- (Fig. 2). Whereas MCT-1 at Post-2 returned to near Pre- values in LHTLH, lower values were observed from Pre- to Post-2 in LHTL ($P < 0.05$).

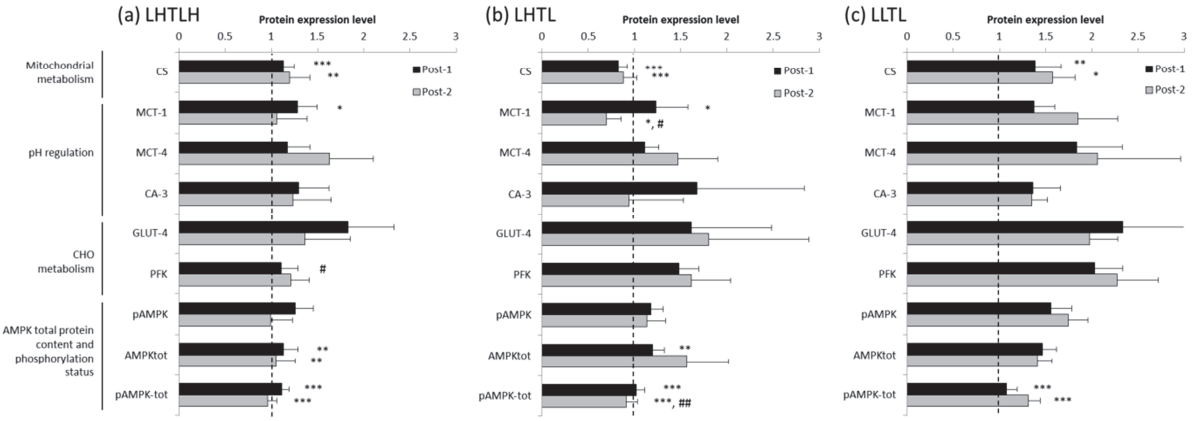

Figure 2. Relative protein expression of selected markers from baseline (Pre-) to the end of the intervention (Post-1) and after 3 weeks (Post-2). The intervention consisted 14-days of passive normobaric hypoxic exposure combined with (a) repeated maximal-intensity hypoxic exercise in hypoxia (LHTLH) or (b) normoxia (LHTL). (c) A control group followed a 'live low-train low' (LLTL) protocol. Black and grey bars represent Post-1 and Post-2 values of protein concentrations in *vastus lateralis* muscle, respectively, and were normalized to baseline values (Pre-), which were set to 1 (dashed line). Values are means ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, *vs*. Pre- and # $P < 0.05$, ## $P < 0.01$ *vs*. LLTL. GLUT-4, glucose transporter 4; PFK, phosphofructokinase; CA-3, carbonic anhydrase III; CS, citrate synthase; MCT-1, monocarboxylate transporter 1; MCT-4, monocarboxylate transporter 4; AMPK, AMP-activated protein kinase.

Glucose metabolism. No changes were measured in the protein expression levels of GLUT-4 (Fig. 2). At Post-1, the protein expression of PFK was lower in LHTLH compared to LLTL ($P < 0.05$).

AMPK total protein content and phosphorylation status. Compared to Pre-, AMPK total protein content ($P < 0.01$ for LHTLH and LHTL) and pAMPK-tot ($P < 0.001$ for LHTLH and LHTL) increased significantly at Post-1, whereas lower pAMPK-tot values were noted at Post-2 ($P < 0.01$ and < 0.001 for LHTLH and LHTL, respectively) (Fig. 2).

Discussion

This is the first study to investigate the effects of prolonged passive exposure to normobaric hypoxia with superimposed maximal-intensity exercise sessions in hypoxia (LHTLH) *vs*. normoxia (LHTL) on molecular regulations in human skeletal muscle. The novel findings are that LHTLH elicits higher short-term (first few days) molecular responses of factors implicated in the regulation of O_2 signaling and carrying, mitochondrial biogenesis, as well as of enzymes implicated in mitochondrial metabolism compared to LHTL [with also no change in control (LLTL)]. We further indicate that the majority of these positive molecular responses disappeared already three weeks post-intervention. We confirm our hypothesis of larger specific transcriptional responses when passive normobaric hypoxic exposure and repeated maximal-intensity hypoxic exercise are combined, yet with normalization of molecular adaptations three weeks after the intervention.

O_2 signaling and gene regulation. In the present study, the level of HIF-1α mRNA increased immediately after the intervention in LHTLH but, unexpectedly, it was not the case for LHTL. First, the absence of HIF-1α increase after LHTL supports the hypothesis that HIF-1α response to hypoxia is time-dependent (Lundby et al., 2009). Accordingly, it has been previously demonstrated that HIF-1α protein levels peak within the first hours of hypoxic exposure then progressively decline toward basal levels (Stroka et al., 2001, Vigano et al., 2008), suggesting a possible local or systemic 'acclimatization' after several days (Lundby et al., 2009). As the protein level of HIF-1α is only briefly increased after a hypoxic stimulus and our intention was to determine stable molecular changes after repeated expositions to hypoxia, we solely quantified the mRNA level of this transcription factor, which reflects the long-term activation of the HIF-1α pathway (Galban and Gorospe, 2009). With this in mind, the enhanced mRNA level of HIF-1α after LHTLH clearly suggests that the addition of repeated maximal-intensity exercise in hypoxia (but not in normoxia) plays an important role

for up-regulating the activation of the HIF-1α pathway and its downstream genes (Zoll et al., 2006, Vogt et al., 2001, Faiss et al., 2013b). This is further supported by the return to near HIF-1α mRNA Pre- values at 3 weeks post-intervention when no additional maximal-intensity exercises and/or hypoxic stimulation were performed.

Activation of HIF-1α is known to lead to cellular adaptations [*i.e.*, O_2 carrying-capacity (Wenger and Gassmann, 1997), neovascularization (Forsythe et al., 1996), glucose oxidation (Wenger and Gassmann, 1997)], which in turn would positively influence exercise capacity in humans (Vogt et al., 2001, Zoll et al., 2006, Faiss et al., 2013b). Accordingly, the mRNA levels for the capillary growth factor VEGF, *i.e.*, an HIF-1-regulated gene (Semenza, 1999, Semenza et al., 1999, Wenger and Gassmann, 1997) and Mb mRNA, significantly increased after LHTLH, whereas no changes were observed in both LHTL and LLTL. This corroborates previous works, which demonstrated Mb mRNA or protein levels enhancement after hypoxic endurance exercise [*i.e.*, 3-5 sessions of intermittent hypobaric (~2300 m) or normobaric hypoxic (~3850 m) training per week for 4-6 weeks (Vogt et al., 2001, Terrados et al., 1990)]. In addition to an exercise intensity effect (Vogt et al., 2001), the shift from type I (oxidative) to type II (glycolytic) fibers in hypoxia (Ishihara et al., 1994, Itoh et al., 1995) could at least in part explain differences in VEGF gene expression between LHTLH and LHTL. Meanwhile, as previously suggested (Millet and Faiss, 2012), RSH (compared to RSN) would enhance the behavior of fast twitch type II fibers via larger blood perfusion levels (*i.e.*, microvascular O_2 delivery) (McDonough et al., 2005) and/or compensatory vasodilation (Casey and Joyner, 2012). In addition to the heterogeneity of the ratio between local muscle blood flow, metabolic rate and O_2 utilization (Koga et al., 2014), it is therefore conceivable that the molecular effects – *i.e.*, up-regulating HIF-1α pathway and its downstream genes – of the superimposed maximal-intensity hypoxic exercise are more manifest in type II muscles, especially given their greater reliance on fractional O_2 extraction compared to type I muscles.

Metabolic Phenotype. After hypoxic endurance exercise, it has been demonstrated that mRNA levels for PGC-1α (Zoll et al., 2006), COX-1, COX-4 and CS (Zoll et al., 2006, Vogt et al., 2001, Terrados et al., 1990) increase to a greater extent compared to similar intervention in normoxia. In the present study, LHTLH induced larger mitochondrial adaptations (*i.e.*, increased mRNA levels for PGC-1α and TFAM) compared to LHTL and LLTL, thereby suggesting a preponderant role of the superimposed RSH for muscle phenotypic adaptations. Whereas the protein expression of CS decreased immediately after LHTL, it increased after both LHTLH and LLTL. Conversely, when used in isolation (*i.e.*, with normoxic residence), Faiss et al. (2013b) indicated that RSH induced a down-regulation in mitochondrial biogenesis (PGC-1α and TFAM), despite unchanged oxidative capacity (CS). Some methodological differences (*i.e.*, exercise mode, frequency and duration) between studies might be responsible for these discrepant findings. Potentially, LHTLH improved skeletal respiratory capacity and muscle function (*i.e.*, CS enzymatic adaptation) to a larger extent than LHTL.

Compensatory vasodilation. Among the HIF-1α target genes, VEGF is a critical signal in vascular remodeling, which maintains vascular integrity and stimulates the production of the vasodilatory mediator nitric oxide. Hence, eNOS plays a key role in blood flow regulation and vascular tone (Gielen et al., 2011). Concomitantly, nNOS expression exerts a functionally significant effect in hypoxic tissue, thereby influencing tissue O_2 delivery (Fish et al., 2007, Tsui et al., 2014), ventilatory regulation and metabolic adaptations to hypoxia (Gardiner et al., 2011). Accordingly, our eNOS and nNOS data for LHTLH support the idea that the activation of the HIF-1α pathway contributes to a larger release of both eNOS and nNOS (Vanhoutte et al., 2009). Although we did not measure the protein expression, an increase in eNOS and nNOS may promote changes in the blood flow and vascular tone (Gielen et al., 2011), participate in angiogenesis (Viboolvorakul and Patumraj, 2014) and enhance O_2 delivery.

Because fiber-type selective peripheral vascular effects of nNOS have been reported during acute high-speed treadmill running, but not at slower speeds (Copp et al., 2013), with enhanced blood flow and vascular control mostly visible in fast twitch II fibers (Ferguson et al., 2013), we could speculate on the superimposed effect of RSH/RSN to prolonged passive normobaric hypoxic exposure. However, to specifically address this question, this would require the inclusion of an additional experimental group residing in hypoxia without any specific exercise.

pH-regulating System. Lactate production during maximal-intensity exercise is markedly enhanced with this effect mainly visible in fast twitch type II fibers, which predominantly contain MCT-1 and MCT-4 transporters to facilitate lactate removal (Juel et al., 2003). In line with previous sprint interval intervention studies (Puype et al., 2013, Burgomaster et al., 2007), LHTLH and LHTL increased muscle MCT-1 protein content, whereas MCT-4 and CA-3 contents did not change. Because MCT-1 protein expression, but not MCT-4, is inversely related to rate of fatigue development during short-duration maximal-intensity exercise (Thomas et al., 2005), MCT-1 probably has a preponderant role in lactate metabolism up-regulation following maximal-intensity exercise (with or without additional hypoxic stress).

Delayed effects on skeletal muscle transcriptional regulation. We recently demonstrated that LHTLH and LHTL resulted in similar short (2-3 days) hematological adaptations (*i.e.*, increase in hemoglobin mass) that were maintained for at least 3 weeks post-intervention (Brocherie et al., 2015). Contrastingly, our novel findings indicate that most of the transcriptional adaptations were back to near Pre- values 3 weeks post-intervention. The present study is the first to show the time course of HIF-1α and related genes transcription in human skeletal muscle adaptations following residence in normobaric hypoxia superimposed

with high-intensity exercise in hypoxia or normoxia. With the post-intervention carefully supervised and controlled (*i.e.*, similar between groups), the fact that this period did not include any environmental hypoxic stress – be it during residence or exercise in normobaric hypoxia – indicate that the lack of external hypoxic 'stimulus' could be responsible for the rapid reversal of beneficial muscle function adjustments, while it apparently did not affect hemoglobin mass (Brocherie et al., 2015). Additionally, as the post-intervention period did not include any maximal-intensity exercise session (as performed six times during the 14-days intervention) as well, the influence of such exercise modality and/or frequency on normalization of molecular responses should not be overlooked. One aspect that deserves more research attention is whether additional hypoxic stress and/or intense exercise when individuals return to sea level after an hypoxic intervention induces a better maintenance of molecular adaptations. The decay of molecular responses over time after return to sea level, that occur more rapidly than the hematological adaptations, would suggest that mechanisms (not measured here) such as those involved in the factor-inhibiting HIF regulation (Lindholm and Rundqvist, 2016) may be at play.

Strengths and limitations. One potential strength of this study is that elite field hockey players volunteered to participate, while mechanistic studies are generally carried out with recreational participants. In these studies, results are often extrapolated to elite athletes while exercise-related adaptations are probably less prominent as the level of practice/performance increases. Here, we found that LHTLH was the most efficient strategy to activate in the short-term the transcription of specific genes involved in key physiological processes for adaptations such as angiogenesis or O_2 transport. It is therefore tempting to associate the molecular regulations we measured at Post-1 and the enhanced performance that we previously reported at both Post-1 and Post-2 in LHTLH (Brocherie et al., 2015).

Methodologically, it is known that most gene expressions generally peak within 2-8 h after a single exercise stimulus (Pilegaard et al., 2003). With muscle biopsies obtained 48 h following the last exercise session in our subjects, we are confident that the augmented expression of several genes actually is the result of the proposed intervention *per se*, and not a side effect of the final exercise session. Despite careful control of biopsy sampling (*i.e.*, no physical activity allowed between the last exercise session, similar sampling timing between 08:00 and 10:00 a.m. during the three test sessions and corresponding samples ran in the same assay) (Fluck et al., 2005), a large inter-subject gene and protein expression variation occurred here. Potentially, this may be explained by: (i) DNA sequence variations resulting from HIF-1α gene polymorphism in the promoter region of HIF-1α gene (Prior et al., 2006), (ii) fiber type differences in the sampling from *vastus lateralis* muscle, which has an unequal typology and metabolic properties (Pette, 1985), (iii) pulsative nature of gene expression in muscular fiber (Newlands et al., 1998), and (iv) involvement of reactive oxygen species in the regulation of HIF-1α mRNA and HIF-1α target genes (Pialoux et al., 2009). That said, it is worth mentioning that biopsies obtained alternatively from the left and right *vastus lateralis* muscle actually give similar mRNA expression profiles when the two legs are compared (Lundby et al., 2005).

In conclusion, combined with normobaric hypoxic residence, repeated maximal-intensity hypoxic exercise elicited higher short-term (first few days) skeletal muscle molecular beneficial adaptations not observed (or at least in smaller proportions) after similar normoxic exercise. The large and specific adaptations in mRNA levels of factors involved in O_2 signaling and transport, mitochondrial biogenesis, as well as in enzymes implicated in mitochondrial metabolism, are seen to highlight the prominence of superimposed maximal-intensity hypoxic exercise, yet with a rapid decay and normalization of molecular adaptations after cessation of the intervention.

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Conflict of interest

The authors report no conflicts of interest.

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