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Energetics and mechanics of human breath-hold diving

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Riassunto

Le attività subacquee in apnea sono da sempre praticate dagli esseri umani, inizialmente per sostentamento, poi per diletto e per competizione sportiva. Nonostante l'esplosione di conoscenze di fisiologia in altre attività sportive, l'apnea rimane di difficile indagine, sia per la necessità di strumentazione impermeabile all'acqua, sia perché l'assenza del respiro impedisce di poter analizzare le più comuni variabili cardiorespiratorie. La possibilità di prevenire e ridurre incidenti in quest'ambito passa anche attraverso una migliore percezione delle proprie possibilità, a tutt'oggi limitata dalle insufficienti conoscenze riguardanti il costo energetico (C) durante il nuoto in apnea, la velocità con la quale le scorte di ossigeno si riducono, l'efficacia dei meccanismi di risparmio dell'ossigeno, nonché la meccanica del movimento.

Per prima cosa abbiamo svolto una revisione sistematica della letteratura per avere una panoramica delle soluzioni tecnologiche adottate per poter misurare parametri fisiologici durante le immersion in apnea mediante sensori indossabili. Da questa è emerso che è attualmente possibile in tal modo misurare e monitorare: elettrocardiogramma, temperatura corporea centrale e periferica, pressione arteriosa, saturazione periferica dell'ossigeno, concentrazione di glucosio interstiziale, cardiografia a impedenza, inerzia e orientamento dei segmenti corporei. Di particolare rilevanza sono i pulsossimetri trasmissivi per la loro capacità di stimare le riserve energetiche (di ossigeno), anche se l'incremento della pressione parziale di ossigeno indotta dall'immersione profonda li rende efficaci solo in apnee dinamiche. Infine, i sensori inerziali (IMU) hanno il grande potenziale di poter fornire informazioni riguardo l'efficienza biomeccanica del movimento, un parametro di grande interesse per migliorare C, e quindi, anche a parità di riserve energetiche, le massime prestazioni.

Per meglio comprendere le attività subacquee, abbiamo misurato il lavoro interno della pedalata in immersione in 12 soggetti sani. Esso è stato calcolato attraverso l'estrapolazione a 0 W della relazione lineare tra potenza meccanica del cicloergometro e la corrispondente spesa energetica durante esercizi incrementali a gradini. Abbiamo trovato che la potenza metabolica interna aumenta col cubo della frequenza di pedalata, e tramite il confronto con la pedalata all'asciutto abbiamo elaborato una formula per ottenere potenze metaboliche equivalenti dentro e fuori dall'acqua.

Abbiamo poi progettato e attuato una serie di esperimenti volti a computare il bilancio energetico globale, nonché la spesa energetica totale per unità di distanza, durante apnee dinamiche di durata sottomassimale. Siccome la capacità polmonare totale è un fattore chiave nel computo delle scorte aerobiche dell'organismo, abbiamo inoltre ideato e validato in 20 soggetti sani un nuovo metodo rapido e portatile per misurarla a partire dalla diluizione di una miscela iperossica mediante singolo respiro. Dopodiché tale tecnica, unitamente alla diluzione del monossido di carbonio per la stima della massa emoglobinica e del volume ematico, è stata applicata su 12 apneisti agonisti. Essi hanno coperto una distanza di almeno 50 m con l'uso di bi-pinne o monopinna, al termine dei quali hanno espirato completamente, e poi respirato normalmente, all'interno di un metabolimetro portatile a circuito aperto per la misura del debito di ossigeno. Il metabolismo anaerobico lattacido è stato calcolato mediante un microprelievo di sangue capillare. Il metabolismo anaerobico alattacido mediante la differenza tra debito di ossigeno all'emersione e consumo delle scorte di ossigeno. Mediante diversi IMU posizionati sui

segmenti corporei sono state misurate le principali variabili biomeccaniche. C è risultato 0.63 ± 0.13 kJ m^{-1} per le bi-pinne e 0.42 ± 0.08 kJ m^{-1} per la monopinna ($p<0.05$). La componente aerobica di C è stata di circa il 40%, quella anaerobica lattacida del 15% e quella anaerobica alattacida del 45%. La componente aerobica di C si è rivelata inversamente proporzionale al record personale dei partecipanti. Nonostante le limitazioni dovute al basso numero di partecipanti, che non ha potuto far emergere relazioni tra pattern biomeccanici e prestazioni, questo studio ha posto l'accento sull'importanza della componente anaerobica alattacida, spesso trascurata, nel bilancio energetico del nuoto in apnea.

In conclusione, i presenti risultati rappresentano un passo avanti nelle conoscenze delle attività subacquee, nello sviluppo di nuove tecniche di indagine e più in generale nella conoscenza della fisiologia umana. Essi hanno profonde implicazioni per l'allenamento nell'apnea dinamica e sono da stimolo per ulteriori studi riguardanti le apnee profonde, nonché possono fornire nuove soluzioni per problemi tecnici e sperimentali anche di altri campi di indagine della fisiologia e della medicina.

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Contents

1 Introduction

1.1 Historical background

There are evidences that humans have always been able not only to swim, but also to perform breath-hold (BH) diving, that is the combination of voluntary apnoea and immersion. This activity probably arose in pre-historic times as an adaptation of the hunting-gathering behaviour to the underwater environment and transmitted as a cultural trait across generations. Archaeological finds of mother-of-pearl inlaid works from 4500 BC from the ruins of Bismaya, Babylonia, suggest that BH diving was already performed at that time (Heberlein 1972). Findings from several centuries before Christ in various regions of the world suggest that the skill necessary for BH diving developed independently (Rahn 1987), involving the harvesting of sponges (Greece), pearls (Persian Gulf and India), and seafood (Japan and South Korea). The technique consisted in a head-first immersion and, in case of deep dives, the assistance of a weight and a rope during the descent and the ascent phase, respectively.

The oldest evidence referring to whole populations specialized in BH diving for a living, the *Ama* in Japan and the *Hae-nyo* in South Korea, are from 268 BC and 434 AD, respectively (Hong 1965; Nukada 1965). Also the Sea Nomads (*Bajau*) from Indonesia and the Philippines have conducted a marine-dependent existence for at least 1000 years collecting food through BH diving and now show evidence of genetic adaptations (Ilardo et al. 2018). In the ancient era, naval warfare and salvage became additional applications of BH diving. Divers were employed to destroy the boom defences in harbours by Greeks during the siege of Syracuse, Sicily (415 BC) and by Alexander the Great during the siege of Tyre, Lebanon (333 BC) (Davis 1934). Levantine BH divers, in particular Rhodian, recovered valuables from sunken wrecks in exchange for a proportion of the value of the salved goods, depending on the risk occurred, or – more practically – the depth at which they worked (Davis 1934). With similar rules, in the late Roman Republic and Empire, the corporation of *urinatores*, was involved both in salvage and sabotage tasks (Oleson 1976). Despite the introduction of the very first diving appliances, such as diving bells, for many centuries BH diving was still performed in military operations, as to cut ship's cables in the sieges of Byzantium (196 AD), Andelys (1203), Malta (1565) and Mayence (1793) (Davis 1934). At the beginning of the XIX century, Spanish warships still carried men whose business was to perform BH diving as a requisite for the service of the fleet The introduction of goggles in the second half of XIX century and full-face masks at the beginning of XX century represented a the first major technological advance in BH diving (Teruoka 1932).

For centuries the scientific community considered populations dedicated to BH diving no more than an ethnographic curiosity, while in the early modern period air-breathing animals became the subject of experimental studies on the mechanisms of asphyxia: Robert Boyle (1670) first investigated the increased tolerance to submersion of ducks with respect to hens, while diving bradycardia was first reported in toads and lizards by Edmund Goodwyn (1788) and later in ducks and rats by Paul Bert (1870). In 1833 the observation that pearl fishers can increase BH time thanks to hyperventilation was brought to the attention of Michael Faraday, who applied this practice to rescue operations in toxic atmospheres and endorsed it (Faraday 1833). The earliest allusion to BH diving bradycardia in humans is found the diary of the military exploration of Algerian Sahara

desert by Victor Colonieu (1863). He counted the pulse rate of a member of the guild of well-divers before and immediately after the descent to the bottom of a 40-m deep artesian well, where the hole communicating with the aquifer had to be cleared in case of sand accumulation. Bleeding from ear, nose and mouth was common, and deaths by asphyxia were also reported. This was in agreement with the first publication on accidents suffered by Greek sponge divers (Le Roy de Méricourt 1869) that included also shark attack, paralysis, hearing loss and premature aging.

In the late modern period, apnoeas in dry conditions became a model to address research questions in human respiratory physiology such as the effects of high altitude exposure (Mosso 1898) and the sensitivity to different alveolar gas compositions (Hill and Flack 1908). A remarkable performance was accomplished in 1913 by the exceptional Greek sponge diver Georghios Stotti (or Georgios Haggi Statti according to other sources), accustomed to this work almost from infancy, who recovered the anchor and chain belonging to the Italian battleship *Regina Margherita* which were lost at a depth of 70 m in the Bay of Pigadia, ashore the Dodecanese island of Karpathos (Davis 1934).

In the 1920's Dr. Gito Teruoka, Director of the Institute for Science of Labour in Japan, concerned about the occupational health of the strenuous activity performed by the *Ama* (Nukada 1965), began a systematic investigation on human BH diving physiology. His meticulous observations of their diving times, patterns, depth, and alveolar gas concentration at emersion (Teruoka 1932) were seminal for the development of BH diving science. In the 1930's general interest in the diving human was limited to hard-hat diving and his work was temporarily forgotten, while there was already fervent experimental activity on the physiology of diving animals (Irving 1939; Scholander 1940). During World War II, BH swimming was still performed by frogmen of the United States Navy's Underwater Demolition Teams, who continued to train in this discipline even after the war (Rahn 1987). This military corps was responsible also for the invention of the neoprene wetsuit (Rainey 1998).

In the post-war period, the greater accessibility of the self-contained underwater breathing apparatus (SCUBA) and the mass-production of masks, snorkels, arouse public interest in undersea exploration and adventure sports. Spearfishing and diving clubs were established, and challenges for BH depth and time records naturally arose. In 1950, Raimondo Bucher set the first recognized depth record of 30 m. Therefore, groups from all over the world became interested in the physiological consequences of BH diving, such as temperature regulation, alveolar and tissue gas exchange, chest mechanics, cardiovascular responses as well as energetics (Rahn 1987). Always in the post-war, the publication of previously classified works performed by Wallace Fenn, Hermann Rahn, and Arthur Otis for the US Air Force on alveolar gas exchange and the CO_2-O_2 diagram (Farhi 1990) were fundamental for the subsequent scientific developments. Studies on BH diving were comprehensively reviewed for the first time in 1965 the "Symposium on the Physiology of Breath-Hold Diving and the Ama of Japan" held in Tokyo in conjunction with the XXIII International Congress of Physiological Sciences, sponsored by the International Union of Physiological Sciences and several institutions of the United States, such as the Office of Naval Research and the National Academy of Sciences – National Research Council (Rahn and Yokoyama 1965).

In the contemporary period populations routinely practising BH diving for a living are decreasing, being engaged in more profitable and less physically demanding occupations as a

result of urbanization and the growing tourist industry (Hong 1965; Nukada 1965). In spite of this, Hong et al. (1991) reported that 13,000 divers, mostly men, in Japan, and 16,000 women in South Korea, were still active in professional sea diving for food harvesting in 1990, to be compared with a total population of about 30,000 divers that were censed 30 years earlier. In general, those populations do not dive below depths of 10-20 m, while far greater depths have been reached during competitive BH diving or record attempts. After a slow record evolution in the 1950's and 1960's, a faster evolution occurred, which was interrupted in the mid 1970's for safety concerns, when the threshold of 90 m of depth was approached by Enzo Maiorca (Ferretti 2001). Even though scientists did not endorse the activity of deep BH diving, they kept trying to understand the mechanisms that determine apnoea breaking points, depth limits and the associated risks, with positive repercussions also on the knowledge of the pathophysiology of "involuntary" BH diving, i.e. drowning. For this reasons, another important workshop on "The Physiology of Breath-hold Diving" was held in 1985 at the State University of New York at Buffalo, sponsored by the New York Sea Grant Institute (Lundgren and Ferrigno 1987).

The more recent years have been characterised by dramatic record improvements (AIDA 2020), which were facilitated by the technical developments and the loose regulations. Performances and safety-related issues are still a major concern for physicians and scientists, and technological advances in the BH diving equipment and monitoring are now crucial for future developments. Over the last decades, most advancements in underwater physiology involved surface, eupnoeic swimming. Conversely, analogous studies involving breath-hold swimming are rarer due to the inherent technical challenges. This discrepancy is exemplified in the most important performance parameter of underwater locomotion: the energy cost.

1.2 The energy cost of locomotion in water

In aquatic locomotion, thanks to the Archimedes lift, gravitational work is much reduced compared to land and the metabolic energy (E) is mainly expended to generate work to overcome hydrodynamic resistance (W_D), with a specific "drag" efficiency of the transformation (η_D):

$$E = \frac{W_D}{\eta_D} \tag{1.1}$$

Mechanical work and efficiency

W_D is the product of resistance exerted by water to the body in motion (active drag, D) times the distance (d) of progression. However, W_D is only a partition of the total mechanical work (W_{tot}) performed by the swimmer (Zamparo et al. 2020), therefore η_D is lower than the efficiency of muscle contraction. W_{tot} includes also the work that, despite giving water kinetic energy, does not contribute to thrust (wasted external work) as well as that needed to accelerate/decelerate the limbs with respect to the centre of mass (internal work, negligible at low speeds and low stroke frequencies). The ratio W_D/W_{tot} is called the propelling efficiency (η_p), while the overall muscular efficiency (η_{tot}) is the ratio W_{tot}/E. The product $\eta_p \cdot \eta_{tot}$ is equal to η_D, implying that the drag efficiency is determined both by true muscular efficiency and propelling efficiency. Therefore, Equation 1.1 can be rearranged in the form:

$$\frac{E}{d} = \frac{D}{\eta_{tot} \cdot \eta_p} = C \quad (1.2)$$

The ratio E/d represents the energy cost (C) of swimming, that is therefore mostly influenced by D and η_p. Those parameters can be independently influenced by swimming (speed, style, and technique), anthropometric (regional body density, length/thickness ratio, body surface area), and water (depth, temperature, density) characteristics. D increases is a power function of swimming speed (*v*), through a proportionality constant (*k*) reflecting the characteristics of the swimmer and the hydrodynamic characteristics (di Prampero et al. 1974).

$$D = k \cdot v^n \quad (1.3)$$

D is the sum of pressure drag, friction drag, and wave drag. Pressure drag is created when water is separated to allow the passage of the body. When we deal with pressure drag only, n = 2 and

$$k = ½ \cdot \rho \cdot A \cdot C_d \quad (1.4)$$

where ρ is water density, *v* is the swimming speed, A the projected frontal area in the direction of movement of the body in motion and C_d the drag coefficient accounting for its shape. Due to lower body density in the thorax and higher in the legs, the body is subject to a "leg sinking" that varies with anthropometric characteristics. This phenomenon affects C by increasing both A (due to body inclination) and the wasted external mechanical work (part of the leg kick thrust is employed to counteract further body inclination) (Pendergast et al. 1977). The leg-sinking torque is prevailing at slow speeds and progressively decreases as speed is increased.

Friction drag depends on the surface characteristics of the body and on the features of the layer of stationary fluid immediately surrounding the body in motion. It independent of speed and is low in human swimming, while it is critical for boats (Pendergast et al. 2005).

Wave drag is the result of accelerating the water away from the body, which forms waves at the water surface. It increase as the fourth power of swimming speed and decreases in proportion to the level of immersion of the body (Pendergast et al. 2005; Zamparo et al. 2020).

Metabolic energy

Most aquatic locomotion studies report data of submaximal energy expenditure after the metabolic steady state is attained, a condition for which it is not necessary to estimate the contribution of the anaerobic energy sources (Ferretti et al. 2017), the determination of which is based on several assumptions. Steady-state oxygen consumption ($\dot{V}O_2$) can be measured at the mouth with the aid of snorkels to collect expired air, or estimated by backward extrapolation of the breath-by-breath $\dot{V}O2$-off kinetics during the first 30-60 s immediately after exercise (Zamparo et al. 2020). In the case of substantial contribution of the anaerobic energy sources, as in short-duration, high-intensity exercises, anaerobic energy source must be computed to calculate the overall energy balance in the form (di Prampero 1981; Ferretti 2015):

$$E = a \cdot PCr + b \cdot La + c \cdot VO_2 \quad (1.5)$$

where E is total metabolic energy, PCr is the amount of phosphocreatine hydrolysis, La the net increase of blood lactate concentration, and VO_2 the amount of oxygen consumed for the combustion of nutrients. The constants *a*, *b*, and *c* are the energetic equivalents of the three metabolic pathways. While La and VO_2 can be easily measured, the assessment of PCr needs to be estimated from pure theoretical assumptions (the initial PCr concentration, the time constant, the complete PCr depletion at steady state) (Capelli et al. 1998), or by measuring either the $\dot{V}O_2$-on (accumulated oxygen deficit) (Reis et al. 2010) or the $\dot{V}O_2$-off-kinetics (alactic oxygen debt) (Sousa et al. 2013).

1.3 The energy cost of breath-hold diving

With respect to surface swimming, BH diving is characterized by 1) the absence of breathing and therefore of a metabolic steady state 2) being completely underwater 3) the common use of fins and wetsuits. Concerning the first point, it is clear that only the $\dot{V}O_2$ of recovery and peak blood lactate can be directly measured. The second two points affect biomechanics and energetics of movement. Complete submersion reduces wave drag (Zamparo et al. 2020), while cold water exposure reduces η_p (Pendergast 1987). The use of fins reduces both wasted external work and internal work (the latter due to a reduction in stroke rate), resulting in an increase in η_p (Zamparo et al. 2002). These advantages are greater in monofin with respect to bi-fins, at least at surface swimming (Zamparo et al. 2006). The wetsuit reduces friction drag and help to prevent the cold-associated decline in η_p (Pendergast 1987; Zamparo et al. 2020).

Deep apnoeas

Deep diving implies three phases: descent, bottom activity, and ascent. During early descent, a force of positive buoyancy acts on the diver and adds to D, due to the large lung volume. Then, at a depth depending upon initial lung volume and body composition, the buoyancy become negative, aiding the descent (Pendergast 1987). During bottom activity, only D must be overcome for horizontal propulsion, however during competitive BH dives bottom activity is negligible. The opposite phenomena described in the descent occur during ascent.

Existing studies focused on occupational BH diving and estimated the total energy and thermal cost of a work shift from the $\dot{V}O_2$ measured during resting periods after repetitive dives, when a sort of "steady-state" $\dot{V}O_2$ was attained (Kang et al. 1965, 1983; Craig and Medd 1968; Shiraki et al. 1986). However, the variability in diving pattern, equipment and environmental conditions makes a quantitative analysis of the data from these studies impossible (Pendergast 1987). Of note, Craig and Medd (1968) found that in addition to energy derived from aerobic metabolism, lactic acid built up in the blood after the first dive and decreased thereafter, indicating that part of the E for the dive came from anaerobic glycolysis and, as indicated by the large $\dot{V}O_2$ during recovery, from high energy phosphate depletion. Another approach was attempted by Ferretti et al. (Ferretti et al. 1991) by measuring change in lung O_2 stores and assuming a fixed blood oxygen store depletion, along with assessment of the anaerobic lactic contribution to energy balance, estimating a metabolic power equal to 20-30% of the subject's maximal oxygen consumption ($\dot{V}O_{2max}$). However, it was impossible to quantify the anaerobic alactic energy contribution.

Dynamic apnoeas

Dynamic apnoea involves covering a horizontal distance in breath-hold while submerged. Swimming underwater prevents the over-water arm recovery that is typical of surface swimming where propulsion is primarily provided by the arms. Therefore, propulsion is generally provided totally from a flutter type leg kick, which *per se* has a lower η_p and a higher C with respect to front crawl at the surface, where the non-propulsive action of the arms takes place out of the water (Holmér 1974). Besides fins, another countermeasure for dynamic apnoea to reduce D and increase η_p is the use of a counterweight at the base of the neck, that abolishes the leg-sinking torque and helps maintaining neutral buoyancy (Schagatay 2010). Moreover, selecting the correct swimming speed and technique is critical for dynamic apnoea: it appears that the increased C at high speeds is not recovered during a "glide" due to the high D (Pendergast 1987)

The only known bioenergetic feature about dynamic apnoea is the increase in blood lactate concentration with respect to eupnoeic exercise (Olsen et al. 1962; Andersson et al. 2004; Tagliabue et al. 2005; Rodríguez-Zamora et al. 2018), with a positive correlation swimming speed (Breskovic et al. 2011). No data are present in literature on the C of dynamic apnoea. However, the C of non-apnoeic underwater swimming has been calculated in few studies. With the SCUBA asset and fins, C depended primarily on speed and secondarily on anthropometric characteristics, ranging from 35 to 55 ml of O_2 m^{-1} (Goff et al. 1957; Pendergast et al. 1996, 2003a, b). More relevant to dynamic apnoea, C of swimming with fins with legs only was ~65% higher underwater than at surface due to a greater D, being approximately 43 ml of O_2 m^{-1} (0.89 kJ m^{-1}) at 1 m s^{-1} (Pendergast et al. 2005).

1.4 Aim of the thesis

Despite the high development of swimming science, the energy cost of breath-hold swimming and its biomechanical determinants is technically challenging and there are no studies in this direction. First, we must frame the problem of technological limitations in the monitoring of the subject underwater, which is relevant for the development of sensors-equipped suits (smart suits). Then, an investigation on the bioenergetics and biomechanics of BH diving is attempted using dynamic apnoea as a preliminary model. However, since total lung capacity is the key parameter affecting BH performance and oxygen stores calculations (Ferretti 2001), a portable technique for its measurement was developed and validated. Therefore, the aim of this thesis was:

- To review current technical developments in assessing physiological parameters during complete water immersion
- To study the effect of water resistance on underwater movement
- To develop a new portable technique for measuring total lung capacity
- To calculate energy balance and biomechanical variables during dynamic apnoeas

1.5 Thesis outline

The thesis is composed by several works organized as paper manuscript. Some of them have been already published on peer-reviewed journals.

- Chapter 2 is a systematic review on wearable sensors for breath-hold diving

- Chapter 3 in an experimental study on the internal work of water cycling
- Chapter 4 describes and validate a novel technique to assess total lung capacity in field conditions
- Chapter 5 shows the results of an experimental expedition aimed to investigate the energy sources and cost of dynamic apnoea, as well as to collect biomechanical data via inertial sensor technology
- Chapter 6 is a general conclusion discussing the expected impact of this work is to enhance safety and performance of BH diving as well as increasing general physiological knowledge on human underwater activities 6

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2 The current use of wearable sensors to enhance safety and performance in breath-hold diving: A systematic review

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2.1 Abstract

Introduction. Measuring physiological parameters at depth is an emergent challenge for athletic training, diver's safety and biomedical research. Recent advances in wearable sensor technology made this challenge affordable; however, its impact on breath-hold diving has never been comprehensively discussed.

Methods. We performed a systematic review of the literature in order to assess what types of sensors are available or suitable for human breath-hold diving, within the two-fold perspective of safety and athletic performance.

Results. In the 52 studies identified, sensed physiological variables were: electrocardiogram, body temperature, blood pressure, peripheral oxygen saturation, interstitial glucose concentration, impedance cardiography, heart rate, body segment inertia and orientation.

Conclusions. Limits and potential of each technology are separately reviewed. Inertial sensor technology and transmission pulse oximetry could produce the greatest impact on breath-hold diving performances in the future.

2.2 Introduction

Underwater human activities are commonly performed for recreational, occupational or competitive purposes (Buzzacott 2017; Fitz-Clarke 2018). The most common approaches include either a self-contained underwater breathing apparatus (SCUBA) or the breath-holding. These activities carry an intrinsic health risk due the physiological stresses related to hypoxemia, hyper- or hypocapnia, hydrostatic pressure and cold water (Fitz-Clarke 2018), potentially resulting in loss of consciousness and drowning. Given that the majority of reported adverse events are related to a delay in recognizing a life-threatening problem (Buzzacott 2017), the risk can be minimized through primary and secondary prevention strategies. In these contexts, field measurement of relevant physiological parameters is an emergent challenge, as the improvement of divers' safety requires a better understanding of diving physiology. This challenge is being met thanks to technological advances in wearable sensors – i.e., water and pressure proofing, miniaturization

and underwater communication (Sieber et al. 2010; Cibis et al. 2017). In breath-hold (BH) diving, remarkable increase in the number of active competitors and dramatic improvement in diving performance have been occurred in the last 20 years (AIDA 2020). As BH divers rely only on their own physiological capabilities, sensor technology provides potential for training feedback and enhancement of human performance and safety.

This work aimed to systematically review wearable technologies usable during BH diving, with the twofold perspective of inferring its potential applications to safety and performance. Specifically, this review aimed at addressing the following questions:

- What type of wearable sensors can be used in human BH diving?
- What wearable sensors used for SCUBA diving are potentially applicable also to BH diving?
- What water- and pressure-proofing strategies have been adopted to adapt monitoring technology to the underwater environment?
- At which depth have the various approaches been reported to work?

Although some of the physiological changes discussed in this review may apply to all types of diving – see e.g. the blood pressure increase (Almeling et al. 2005) – the conclusions arrived at are specific to BH diving. Analogous reviews on SCUBA diving were previously published (Sieber et al. 2010; Cibis et al. 2017) and the interested reader may refer to them.

2.3 Methods

Article selection was based on a systematic search following the PRISMA guidelines (Moher et al. 2009) of the scientific databases PubMed and Scopus. To avoid outdated technology, only items published after January 2000 were included in the search. The keyword string was: (sensor OR ECG OR electrocardiogram OR “heart rate” OR “blood pressure” OR hemodynamics OR “oxygen saturation” OR EEG OR IMU OR “inertial measurement unit” OR accelerometer OR gyroscope OR “body temperature” OR “blood glucose”) AND (diver OR diving).

Title and abstract of each result were reviewed and evaluated based on the relevance to the aims of this study. When appropriate, full-text was obtained for a more detailed analysis. Article references were examined for further pertinent publications.

Inclusion criteria were that the publication: appeared in a peer-reviewed academic source; was related to the utilization or the development of wearable sensors explicitly or potentially applicable to human BH diving; included experiments carried out completely below the water level, at a depth (either real or simulated with a hyperbaric chamber) of more than 2 m. Exclusion criteria were: studies performed in shallow water (less than 2 m deep); sensors applicable only to SCUBA diving; invasive or non-portable sensors.

The following information was extracted from articles meeting the inclusion criteria: sensed physiological variable, sensor technology, sensor sealing precautions, studied diving mode, test environment, maximal tested depth.

2.4 Results and discussion

The initial search yielded 1,565 titles on Scopus and 760 on PubMed updated to 28 February 2019. Duplicates were removed, 248 abstracts were further analysed and subsequently 106 full-text papers were downloaded. After full-text assessment, we finally selected 52 publications for inclusion in this review (Figure 1). The parameters extracted from each publication are specifically reported in Tables 1-6, which will be discussed in detail below.

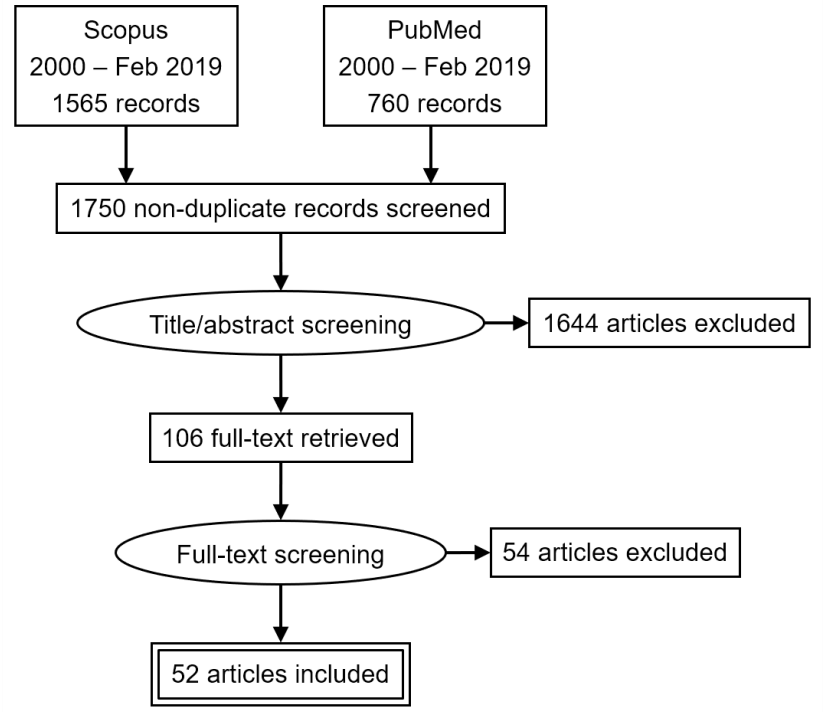

Figure 1. PRISMA flow diagram for the systematic review.

Thirteen studies involved BH diving, 38 SCUBA diving and one saturation diving. Tested depths ranged from 2 to 160 metres' sea or fresh water. Sensed physiological variables were: electrocardiogram (ECG, 19 studies), body temperature (six studies), arterial blood pressure (ABP, five studies), peripheral oxygen saturation (SpO_2, five studies), interstitial glucose concentration (five studies), impedance cardiography (four studies), heart rate (13 studies), body segments inertia and orientation (three studies), electroencephalogram (one study). Six studies involved simultaneous measurements of multiple parameters, such as ECG and impedance cardiography (one study), ECG and SpO_2 (one study), ECG, ABP and SpO_2 (two studies), heart rate and core and skin temperature (one study), ECG and body temperature (one study). In all studies, an appropriate casing was used for the water- and pressure-proofing of electronic components.

Electrocardiogram and heart rate

The ECG has previously been applied to BH diving (Wyss 1956) and unsurprisingly was the most common physiological variable recorded. As reported in Table 1, depth ranged between 2–70 m. In ECG monitoring there are two different elements that must be waterproofed: the electrodes and the electronics. Performing differential measurements between devices, such as

ECG, the front-end electronics typically requires amplifiers which present high input impedance, high level of gain a and large common-mode rejection ratio (CMRR). These must provide a large amount of gain for very low-level signals, often in the presence of high noise levels. Immersion in salt water introduces a parallel resistance between electrodes, increasing the load and decreasing the signal by an amount that depends on water conductivity (i.e. salinity) and electrodes properties. Therefore, the optimal and most widespread solution was to place electrodes under a dry suit (González Olea et al. 2001; Istepanian and Woodward 2003; Cibis et al. 2015; Olędzki et al. 2017; Noh et al. 2018). Alternatively, electrodes insulation was achieved via direct coverage with biocompatible adhesive patches (Schipke and Pelzer 2001; Ehrmann et al. 2004; Lemaître et al. 2013; Bosco et al. 2014; Berry et al. 2017; Schuster et al. 2017) or with hydrophobic dental impression material (Sieber et al. 2008a; Kuch et al. 2009; Breskovic et al. 2011; Kiviniemi et al. 2012). All the reported solutions avoided modifying the original manufactured skin-electrode interface while maintaining the correct inter-electrode insulation. Finally, a novel solution based on intrinsically waterproof electrodes has been recently developed (Noh et al. 2016).

The ECG signal analysis can be restricted to heart rate only, as in commercial cardiotachometers and diving computers, which were studied at 3–65 m depth (Koehle et al. 2006; Flouris and Scott 2009; Chouchou et al. 2009; Ljubkovic et al. 2010; Møllerløkken et al. 2011; Bilopavlovic et al. 2013; Zanchi et al. 2014; Madden et al. 2014, 2016; Castagna et al. 2015; Lee et al. 2016; Steinberg and Doppelmayr 2017; Susilovic-Grabovac et al. 2018) or in shallow water during static apnoea competitions (Lemaître et al. 2005; Lindholm et al. 2006). Cardiac arrhythmias are common during BH diving (Ferrigno et al. 1991; Gentile and La Scala 2001) and real-time ECG analysis can be used to trigger alert signals based on pre-determined criteria (Cibis et al. 2015). Moreover, heart rate response to exercise is only partially suppressed by the diving reflex and still remains influenced by the metabolic rate (Bergman et al. 1972; Smeland et al. 1984; Manley 1990; Wein et al. 2007; Lemaître et al. 2013): it could be therefore monitored by an experienced diver as a real-time surrogate of the energy cost of underwater swimming.

Table 1. Studies reporting measurement of electrocardiogram and heart rate. BH: breath-hold; BT: Bluetooth; HVP: hydrophilic vinyl polysiloxane; NS: not specified; PC: personal computer; S: SCUBA; trans.: transmission; WHC: wet hyperbaric chamber; *probably not monitored in real-time due to Bluetooth underwater constraints.

Sensor	References	Diving mode	Setting	Tested depth (m)	Manufacturer	Real-time display	Data storage or display	Data trans.	Water- and pressure-proofing of the wearable sensor	Max. depth (m)
Electrodes	(Gentile and La Scala 2001)	BH	WHC	55	NS	Yes	ECG recorder	NS	NS	-
Electrodes + ECG transmitter	(González Olea et al. 2001)	S	WHC	27	Fukuda Denshi	Yes	ECG recorder (Dynascope DS-1040)	Wireless	All inside diver's dry suit	-
	(Istepanian and Woodward 2003)	S	Pool	2	Prototype	Yes	Data logger	Wireless (acoustic)	Electrodes: under diver's dry suit; ECG transmitter: inside a cylindrical housing attached to an aqualung	-
	(Togawa et al. 2006)	S	Sea	20	Nihon Kohden	No	ECG transmitter	Cable	Electrodes: NS; ECG transmitter: water- and pressure-proof case.	-
Electrodes + ECG recorder	(Lemaître et al. 2013)	BH	Sea	70	Sorin Group	Yes	ECG recorder (storage); PC (display)	Cable	Electrodes: covered with transparent adhesive (Tegaderm, 3M, St. Paul, Minn., USA); ECG Holter: plastic tube (Comex SA, Marseille, France)	190
	(Schipke and Pelzer 2001)	S	Pool	4	Reynolds Medical	No	ECG recorder	Cable	Electrodes: waterproof tape; ECG recorder:	-

									professional diving pouch (TMT, Ewa-Marine)	
	(Winkler et al. 2011)	S	Lake	8	PicoMed	No	ECG recorder	Cable	Electrodes: special clips; ECG recorder: NS	-
	(Bosco et al. 2014)	S	Sea	25	Mortara	No	ECG recorder	Cable	Electrodes: first layer (Visulin, Hartmann) + second layer (Steri-Drapes, 3M); ECG Holter recorder: pressure-proof anticorodal aluminium housing, with a plexiglass cover (Metralab Srl)	50
	(Olędzki et al. 2017; Noh et al. 2018)	S	Sea	30 (Olędzki et al. 2017) 61 (Noh et al. 2018)	Rozinn Electronics (Noh et al. 2018) NS (Olędzki et al. 2017)	No	ECG recorder	Cable	All inside diver's dry suit	-
Electrodes patch embedded in an ECG recorder	(Ehrmann et al. 2004)	BH	WHC	20	NS	No	ECG recorder	Embedded	Electrodes: special adhesive patch; ECG recorder: water- and pressure-proof case	-
Electrodes + data logger	(Sieber et al. 2008a; Kuch et al. 2009; Breskovic et al. 2011; Kiviniemi et al. 2012)	BH	Pool	2 (Breskovic et al. 2011; Kiviniemi et al. 2012) 10.5 (Sieber et al. 2008a; Kuch et al. 2009)	Prototype	Yes	Data logger	Cable	Electrodes: HVP dental impression material (Elite H-D+, Zhermack); Data logger: lexan tube	200
	(Berry et al. 2017)	S	Pool	4.6	UFI	Yes	Data logger	Cable	Electrodes: benzoin + waterproof tape + moleskin; Data logger: not shown	-
Electrodes + ECG sensor + Smartphone	(Cibis et al. 2015)	S	Pool	2.7	Shimmer Research Ltd	Only vibratory alerts	Smartphone	Wireless (BT)	All inside diver's dry suit; Smartphone: professional diving pouch	-
Electrodes + Monitoring board	(Schuster et al. 2017)	S	Sea	30	Prototype	Yes	PC	Cable to a BT buoy	Electrodes: hot glue + self-adhesive waterproof film (Tegaderm, 3M); ECG Monitor: Case (DryCase 2000, OtterBox)	-
Electrodes chest strap + ECG transmitter	(Noh et al. 2016)	S	Pool	4.5	Prototype	Yes*	PC	Wireless (BT)	Electrodes: intrinsically waterproof (hydrophobic, Carbon Black/Polydimethylsiloxane electrodes, meshed with embedded copper mesh); ECG transmitter: NS	-
Electrodes chest strap + wrist monitor	(Flouris and Scott 2009; Castagna et al. 2015; Lee et al. 2016)	BH (Lee et al. 2016) S (Flouris and Scott 2009; Castagna et al. 2015)	Sea	3 (Castagna et al. 2015) 5 (Flouris and Scott 2009) 20 (Chouchou et al. 2009; Lee et al. 2016)	Polar	Yes	Wrist monitor	Wireless	Electrodes: built-in water insulation (textile electrodes); Monitor: built-int waterproof case	50
	(Koehle et al. 2006)	S	Pool	4.5	Timex	Yes	Wrist monitor	Wireless	NS	-
	(Ljubkovic et al. 2010; Møllerløkken et al. 2011; Bilopavlovic et al. 2013; Madden et al. 2014, 2016; Zanchi et al. 2014; Steinberg and Doppelmayr 2017; Susilovic-Grabovac et al. 2018)	S	Sea	18 (Bilopavlovic et al. 2013; Madden et al. 2014, 2016; Zanchi et al. 2014; Susilovic-Grabovac et al. 2018) 20 (Steinberg and Doppelmayr 2017) 54 (Møllerløkken et al. 2011) 65 (Ljubkovic et al. 2010)	Scubapro-Uwatec	Yes	Wrist monitor	Wireless	Electrodes: built-in water insulation; Monitor: built-int waterproof case	120

Arterial blood pressure

Underwater ABP measurement was successfully carried out at depths between 2–10.5 m (Table 2). In designing the pressure transducer, electrical components waterproofing without preventing (or excessively delaying) barometric equalization in the reference chamber is critical to allow correct measurement in the aquatic environment, especially in dynamic conditions (ascent and descent) (Sieber et al. 2009). We found only two different approaches to achieving this. The first solution was putting a commercial ABP device inside a downwardly-open plexiglass housing (Almeling et al. 2005), whose resulting water-air interface was set at the level of the middle of the blood pressure cuff. Subsequent studies improved the portability of the sensor, with the ABP device encapsulated into a Lexan tube directly located over the cuff, inflated with the gas coming from SCUBA tank (Sieber et al. 2008b, 2009; Breskovic et al. 2011; Kiviniemi et al. 2012). BH was reported to increase ABP either modestly (Sieber et al. 2009) or dramatically (Ferrigno et al. 1997). Therefore, it would be useful to monitor individual ABP responses to BH diving for research or screening purposes.

Table 2. Studies reporting measurement of arterial blood pressure. BH: breath-hold; NS: not specified; S: SCUBA; trans.: transmission.

Sensor	References	Diving mode	Setting	Tested depth (m)	Manufacturer	Real-time display	Data storage or display	Data trans.	Water- and pressure-proofing of the wearable sensor	Max. depth (m)
Cuff + Differential pressure sensor + microcontroller (based on Korotkoff sounds)	(Almeling et al. 2005)	S	Pool	3	Bosch + Sohn (BoSo)	Yes	On screen via a video-camera	Cable	Plexiglass housing for inflator/display (downwardly open for hydrostatic pressure equalization); silicone sheath for cuff microphones	-
Cuff + differential pressure sensor + microcontroller (based on the oscillometric method)	(Sieber et al. 2008b, 2009; Breskovic et al. 2011; Kiviniemi et al. 2012)	BH (Sieber et al. 2009; Breskovic et al. 2011; Kiviniemi et al. 2012) S (Sieber et al. 2008b)	Pool	10.5	Prototype	Yes	Micro-controller	Cable	Lexan tube, inflation air coming from a SCUBA tank	200

Impedance cardiography

Impedance cardiography allows for non-invasive monitoring of the electrical impedance changes in the thorax thus providing estimation of the cardiac stroke volume and, together with the ECG measurement, of several derived cardiovascular parameters. These systems usually rely on the use of a set of electrodes (at least four) placed on the thorax. An alternating high frequency and small amplitude current is applied through two electrodes, whereas the electrical potential difference is measured by using the other pair. Secured in a pressure chamber (Gentile and La Scala 2001) or into an underwater torch case in open sea (Tocco et al. 2012, 2013; Marongiu et al. 2014), the device allowed to take measurements up to 55 m depth (Table 3). While it represents an index of myocardial performance, it adds limited benefits for BH diving safety and performance compared to ECG alone.

Table 3. Studies reporting measurement of impedance cardiography. BH: breath-hold; trans: transmission; WHC: wet hyperbaric chamber.

Sensor	References	Diving mode	Setting	Tested depth (m)	Manufacturer	Real-time display	Data storage or display	Data trans.	Water- and pressure-proofing of the wearable sensor	Max. depth (m)
Electrodes	(Gentile and La Scala 2001)	BH	WHC	55	Bomed	Yes	Recorder	NS	NS	-
Electrodes + recorder	(Tocco et al. 2012, 2013; Marongiu et al. 2014)	BH	Pool (Tocco et al. 2012, 2013) Sea (Marongiu et al. 2014)	3 (Tocco et al. 2012, 2013) 30 (Marongiu et al. 2014)	2C Technologies Inc	No	Recorder	Cable	Recorder: underwater torch case; Electrodes: surgical 15x10 cm patches (Plastod, Bologna, Italy)	90

Peripheral oxygen saturation

Arterial haemoglobin saturation is a key performance parameter for BH and reflects the partial pressure of O_2 in the arterial blood. It can be measured non-invasively in the peripheral circulation (SpO_2), although motion artefacts and reflex peripheral vasoconstriction prevent the utilization of classical transmission pulse oximeters at fingertip or earlobe. Accordingly, only reflectance pulse oximeters at the forehead were used underwater (Kuch et al. 2009, 2010; Breskovic et al. 2011; Kiviniemi et al. 2012), at a depth of 2–10 m (Table 4). In the design of the device, waterproofing was specifically obtained by soaking it in a highly sealing and electrically insulating polymeric material. Battery change was facilitated by introducing a separate waterproof compartment.

The descent phase of the BH dive cannot be guided by pulse oximetry, because the consumption of oxygen stores is counterbalanced by transmission of the surrounding hydrostatic pressure to the alveolar gas, thus increasing arterial partial pressure of oxygen and resulting in a fairly stable SpO_2 at 100%. Only during a prolonged period at depth and/or during the ascent (when there is reversal of the above process) would O_2 depletion manifest as a decrease in SpO_2. As a consequence of circulation time between lungs and forehead, the nadir of SpO_2 at forehead occurs 4–8 s after surfacing (Choi et al. 2010), or even later if cardiac output is reduced by a marked diving response (Tocco et al. 2013).

Table 4. Studies reporting measurement of peripheral oxygen saturation. BH: breath-hold; trans: transmission.

Sensor	References	Diving mode	Setting	Tested depth (m)	Manufacturer	Real-time display	Data storage or display	Data trans.	Water- and pressure-proofing of the wearable sensor	Max. depth (m)
Reflectance sensor (8000R) + module (OEM III) + data logger	(Kuch et al. 2009, 2010; Breskovic et al. 2011; Kiviniemi et al. 2012)	BH	Pool	2 (Breskovic et al. 2011; Kiviniemi et al. 2012), 10.5 (Kuch et al. 2009, 2010)	Nonin	Yes	Data logger	Cable	Data logger: either (i) inside a lexan tube or (ii) filled with silicone gel (SilGel 612, Wacker Chemie AG) with a water- and pressure-proof compartment for battery.	200

Body segments inertia and orientation

Classical movement analysis systems (optical motion capture, force and pressure measurement sensors, global positioning system) are not suitable to deep underwater environment. Inertia measurement units (IMU) incorporate accelerometers, gyroscopes and magnetometers in a small space and can be easily waterproofed. For these reasons, IMU arose as a powerful tool for

the investigation of competitive swimmers' biomechanics (Mooney et al. 2016) and the energetics of air-breathing diving animals (Halsey et al. 2011; Elliott 2016). IMUs have been used in experimental studies on human divers only in three occasions (Table 5), two conducted at a depth of 2 m (Kuch et al. 2011; Groh et al. 2015) and one at 10 m (Goodfellow et al. 2015). Electrical insulation was achieved by means of either external cases (Kuch et al. 2011; Groh et al. 2015) which can be easily acquired and applied, or by embedding the electronics in a polymeric potting compound (Goodfellow et al. 2015).

The main outcomes of Kuch et al. (2011) and Goodfellow et al.(2015) were, respectively, the reconstruction of diver's posture (to detect anomalous behaviours) and path (to build an inertial based underwater navigation system). However, potential applications of IMU to BH diving extend to investigating the energy cost of underwater swimming, a major determinant of BH distance or depth (Ferretti 2001)- Feedback on swimming economy would be crucial for improving performances of both dynamic and deep apnoeas, especially if given real-time. Groh et al.(2015) moved in that direction, trying to establish a biomechanical model to describe leg and upper body orientation during fin kicking. Their proposed algorithm has the potential to be implemented into a wider training system for competitive or recreational divers. However, additional parameters still need to be measured in order to obtain a complete biomechanical model.

Table 5. Studies reporting measurement of body segments inertia and orientation. PC: personal computer; S: SCUBA; trans: transmission; *personal communication.

Sensor	References	Diving mode	Setting	Tested depth (m)	Manufacturer	Real-time display	Data storage or display	Data trans.	Water- and pressure-proofing of the wearable sensor
Accelerometer + magnetometer + gyroscope (IMU)	(Kuch et al. 2011)	S	Pool	2	ST Microelectronics + InvenSense	Yes	PC at surface	Cable	Lexan tube
	(Goodfellow et al. 2015)	S	Pool	10	Pololu	Yes	PC at surface	Cable	Spokes: 3D printed housing, filled with polyurethane potting compound; Hub: 5083 grade aluminium alloy housing
	(Groh et al. 2015)	S	Pool	2*	Prototype	No	IMU	Cable	Professional diving case or pouch for cameras or mobile phones

Body temperature

Superficial, rectal and ingestible temperature sensors were easily adapted to hyperbaric environments to investigate heat exchanges in SCUBA (Risberg and Hope 2001; Hope et al. 2005; Vrijdag et al. 2013; Castagna et al. 2015; Schuster et al. 2017) and saturation diving (Mekjavić et al. 2001) at 3–160 m depth (Table 6). Built-in cases are the most common solutions to properly insulate superficial sensors, while rectal and ingestible sensors are designed to be resistant to gastrointestinal fluids and thus are already waterproof. However, no specifications were found concerning maximum ambient pressure in which those sensors may be operated. Electrical insulation of the data loggers was achieved by means of cases or housing designed to allow easy access.

Monitoring body temperature would be useful in repetitive diving, such as spearfishing competitions and professional dives, because it allows a timely diagnosis and prevention of hypothermia (Pendergast and Lundgren 2009). This was reported to be a quite frequent event in Ama (Kang et al. 1965), which could eventually elicit chronic adaptation to cold (Ferretti and

Costa 2003). The usefulness of such monitoring is underscored also by the reduction in maximal BH duration in cold water due to an increased resting metabolic rate (Sterba and Lundgren 1985). Some commercially available diving computers offer skin temperature measurement from the heart rate chest strap (Ljubkovic et al. 2010; Møllerløkken et al. 2011; Bilopavlovic et al. 2013; Madden et al. 2014, 2016; Zanchi et al. 2014; Steinberg and Doppelmayr 2017; Susilovic-Grabovac et al. 2018), but have not been the subject of published scientific studies. In fact, it is noteworthy that the gold standard for a comprehensive characterization of human thermal balance is to measure both skin and core temperature (Ferretti et al. 1989).

Table 6. Studies reporting measurement of body temperature. BT: Bluetooth; NS: not specified; PC: personal computer; S: SCUBA; Sat.: saturation diving; trans: transmission.

Sensor	References	Diving mode	Setting	Tested depth (m)	Manufacturer	Real-time display	Data storage or display	Data trans.	Water- and pressure-proofing of the wearable sensor
Ingestible temperature sensor + data logger	(Vrijdag et al. 2013)	S	Pool	3	HQ Inc.	No	Data logger	Wireless	Temperature sensor: capsule; data logger: inside diver's dry suit
	(Castagna et al. 2015)	S	Pool	3	Philips Respironics	Yes	Data logger	Wireless	Temperature sensor: capsule; data logger: NS
	(Schuster et al. 2017)	S	Sea	30	Philips Respironics	Yes	Data logger + PC on surface	Wireless (to data logger) Cable to BT (to PC)	Temperature sensor: capsule; data logger: case (DryCase 2000, OtterBox)
	(Mekjavić et al. 2001)	Sat.	Sea	160	Biomed d.o.o.	No	Data logger	Wireless	Temperature sensor: capsule; data logger: professional diving pouch
Rectal temperature sensor + data logger	(Risberg and Hope 2001)	S	Sea	10	Grant Instruments	Yes	Data logger	Cable	Temperature sensor: inside divers' dry suit; data logger: above water surface
Skin temperature sensor + data logger	(Risberg and Hope 2001)	S	Sea	10	Grant Instruments	Yes	Data logger	Cable	Temperature sensor: inside divers' dry suit; data logger: above water surface
	(Hope et al. 2005)	S	Sea	8	Grant Instruments	Yes	Data logger	Cable	Temperature sensor: surgical tape (Blenderm, 3M); data logger: above water surface
	(Castagna et al. 2015)	S	Sea	3	Philips Respironics	Yes	Data logger	Wireless	Temperature sensor: built-in waterproof case; Data logger: NS
	(Schuster et al. 2017)	S	Sea	30	Philips Respironics	Yes	Data logger + PC on surface	Wireless (to data logger) Cable to BT buoy (to PC)	Temperature sensor: built-in waterproof case; Data logger: Case (DryCase 2000, OtterBox)
	(Mekjavić et al. 2001)	Sat.	Sea	160	Biomed d.o.o.	No	Data logger	Embedded	Temperature sensor: embedded in the data logger; data logger: professional diving pouch

Interstitial glucose concentration

Subcutaneous sensors for interstitial glucose concentration have been waterproofed with adhesive films and dental impression material (Lormeau et al. 2005; Bonomo et al. 2009) or simply kept under the dry suit (Adolfsson et al. 2008, 2009) or even the wet suit (Pieri et al. 2016). In this case, there is no issue related to the direct contact with water, since the sensor (i.e. a thin needle) is placed within the interstitial fluid. Electrical insulation had to be ensured only to avoid issues related to power supply and data transmission. Devices were studied at depths 22–40 m (Table 7). In insulin-dependent diabetic SCUBA divers these devices may diagnose hypoglycaemia during the dive, although the very short immersion times hamper their usefulness for BH diving.

Table 7. Studies reporting measurement of interstitial glucose concentration. S: SCUBA; Sat.: saturation diving; trans: transmission.

Sensor	References	Diving mode	Setting	Tested depth (m)	Manufacturer	Real-time display	Data storage or display	Data trans.	Water- and pressure-proofing of the wearable sensor
Subcutaneous glucometer + monitor	(Bonomo et al. 2009)	S	Sea	21.5	Medtronic	No	Monitor	Cable	Glucose sensor: hydrophilic vinylpolysiloxane material (Elite H-D, Zhermack) + doubled plastic adhesive dressing + an elastic collodion film between the two dressings; monitor: pressurized aluminium container
	(Lormeau et al. 2005)	S	Sea	20	Medtronic	No	Monitor	Cable	Glucose sensor: taped with an Opsite film; monitor: water- and pressure-proof case
	(Adolfsson et al. 2008, 2009)	S	Sea	22 (Adolfsson et al. 2008) 24 (Adolfsson et al. 2009)	Medtronic	No	Monitor	Cable	All inside diver's dry suit
	(Pieri et al. 2016)	S	Sea	40	Dexcom	Yes	Monitor	Wireless	Glucose sensor: under diver's wet suit; monitor: waterproof case with glass screen

Electroencephalogram

One pilot study obtained electroencephalographic (EEG) recordings 4 m underwater (Schneider et al. 2014) by protecting the electrodes under a full-face latex mask, further covered by a bathing cab. In this case, waterproofing is essential to ensure inter-electrode insulation and prevent surface biopotentials becoming equipotential, as discussed earlier in relation to ECG and impedance cardiography. Signals were transmitted via cable to an amplifier at the surface. Although acute cognitive impairment is an important safety issue in BH diving, real-time applicability of EEG in this field remains unfeasible at this time. Nevertheless, it would be important to develop portable underwater EEG devices, especially to study development of adaptive changes in EEG reported by some authors on trained breath-hold divers (Ratmanova et al. 2016).

2.5 Conclusions

Since the first tests on BH diving populations (Teruoka 1932; Rahn and Yokoyama 1965), the potential for carrying out physiological measurements during actual BH diving has increased dramatically. The wearable sensors implemented so far have contributed significantly to our understanding of BH diving physiology and to the safety of dives. Adequate waterproof characteristics seem to be achievable for systems originally designed for terrestrial use, provided that the issues of both sensor-body interface and electrical insulation are taken into account. However, the intrinsic depth limits of the adopted technology were not reported in several studies.

Another great recent improvement involves the transmission and real-time processing of physiological measurements. On-line medical and physiological information transmission during diving could allow a prompt recognition of an increased risk or a clinical adverse event, leading to timely termination of the dive (for example, significative cardiac arrhythmias or an excessive

rise in ABP). Further advances could be obtained by integrating different sensors into a unique "smart" suit.

In addition to safety, the analysis of multiple data collected in the field would positively impact training and competition strategies, as happens in several other sporting disciplines. Among the sensors that we discussed, transmission pulse oximetry and inertial sensor technology seem to have the greatest potential for further technical improvement and innovative uses. The former could give feedback on available oxygen stores (with the limitations outlined above), and the latter on factors influencing oxygen consumption rate, possibly identifying the most economical swimming technique. Therefore, we expect them to produce the greatest impact in the future.

2.6 References

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3 Effects of water immersion on the internal power of cycling

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3.1 Abstract

Introduction. Water immersion is known to add additional drag and metabolic demand for limb movement with respect to air. We tested the hypothesis that this effect would translate into an increased internal metabolic power ($\dot{E}_{int}$) during cycling.

Methods. 12 healthy subjects (4 females) pedalled on a waterproof cycle ergometer in an experimental pool that was either empty (DRY) or filled with tap water at 30.8 ± 0.6 °C (WET). Four different pedal cadences (f_p) were studied (40, 50, 60 and 70 rpm) at four different workloads. The metabolic power at steady state was measured via open circuit respirometry and $\dot{E}_{int}$ was calculate as the metabolic power extrapolated for 0 W.

Results. $\dot{E}_{int}$ was higher in WET in for all investigated f_p except 40 rpm. $\dot{E}_{int}$ increased with the third power of f_p both in WET and DRY.

Conclusions. Cycling in the water increases $\dot{E}_{int}$, however, limb unloading via the Archimedes' principle could potentially be a confounding factor. A simple formula was developed to predict the increase in mechanical power in in dry conditions needed to match energy expenditure of underwater cycling: $43^* f_p^3 - 7$ W, where f_p is expressed in Hz.

3.2 Introduction

During exercise on a cycle ergometer, the steady-state net metabolic power ($\dot{E}$) is a linear function of the external mechanical power ($\dot{W}$) set by the ergometer's resistance, whose slope is the reciprocal of the delta efficiency ($\Delta\eta$) and y-intercept the internal metabolic power ($\dot{E}_{int}$) (Gaesser and Brooks 1975; Francescato et al. 1995; Ferretti et al. 2017). From a theoretical standpoint $\dot{E}_{int}$ reflects the fraction of $\dot{E}$ used to keep the limbs in motion without generating external forces. Since there is not consensus between biomechanical models on the estimation of internal mechanical power and its interdependence with $\dot{W}$ (Kautz and Neptune 2002; Hansen et al. 2004), $\dot{E}_{int}$ was suggested as the golden standard reference measurement for the internal power (Hansen et al. 2004). Several studies dissected the determinants of $\dot{E}_{int}$, showing that it is a power function of pedalling rate (f_p) (di Prampero et al. 1979; Francescato et al. 1995; Minetti 2011; Formenti et al. 2015) as well as a linear function of limb mass (Francescato et al. 1995) and gravity acceleration (Girardis et al. 1999; Bonjour et al. 2010).

Additional energy is also necessary to overcome the resistance to leg movement by the surrounding fluid. This is negligible for air, due to its very small density, and this factor was neglected in all previous models. However, it is not negligible in water, the density of which is 780 times greater than for air at STPD (Fitz-Clarke 2018). The performance of exercise on a cycle ergometer during water immersion shifts the Ė-Ẇ relationship upward with respect to air (Costill 1971; Perini et al. 1998; Almeling et al. 2006). Ė varies with the third power of f_p (Morlock and Dressendorfer 1974; Shapiro et al. 1981; Sogabe et al. 1987), as predicted by hydrodynamic analysis of underwater cycling (Morlock and Dressendorfer 1974; Garzon et al. 2015). However, a comprehensive analysis of $\dot{E}_{int}$ during immersed cycling has never been performed, while usually water cycling experiments simply assume a 25 W increase in Ẇ load to account for increased internal power (Perini et al. 1998).

Therefore, the aim of this study is to quantify the effect of water resistance on the $\dot{E}_{int}$ of cycling at different f_p in the light to moderate intensity domain and to provide predictive equations to estimate the increase in metabolic and mechanical demand.

3.3 Methods

Participants

Twelve healthy subjects (4 females, 28 ± 4 years, 73 ± 13 kg, 173 ± 8 cm) were recruited from the university community through local advertisement. After reviewing the consent document with an investigator, they provided written informed consent. Then, they complete the physical activity readiness questionnaire (2017 PAR-Q+). Females provided a urine sample for a pregnancy test. A positive result in the questionnaire or in the pregnancy test resulted in exclusion from the study. The study was approved by the University at Buffalo Institutional Review Board.

Instrumental setup

All tests were conducted on an electrically braked cycle ergometer (Pedalmate, Collins, Braintree, MA) adapted to underwater exercise. Continuous air supply from a pressurized tank ensured positive pressure inside the crank case, thus avoiding water infiltration. Subjects were fitted with a two-way non-rebreathing T-shape valve (Hans Rudolph, Shawnee, KS) directing expired air into a mixing chamber via plastic tubing, where oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were calculated employing a heated pneumotachograph, a paramagnetic oxygen analyser, and an infrared carbon dioxide analyser (TrueOne 2400, Parvo Medics, Salt Lake City, UT). Subjects wore also an elastic chest strap for heart rate (HR) monitoring Polar Electro Oy, Kempele, Finland). The ergometer was positioned in an experimental pool that was either empty (DRY condition) or filled with tap water (WET condition) at 30.8 ± 0.6 °C to ensure thermoneutrality for a short period of rest and low to moderate-intensity exercise (Craig A.B. and Dvorak 1969; Veicsteinas et al. 1982; Perini et al. 1998). Room air temperature was 23.4 ± 0.6 ° C, with a relative humidity of 43 ± 2% and 50 ± 1% in DRY and WET, respectively. In both conditions, subjects wore neoprene dive boots; in WET, a 10-kg weight belt was used to avoid buoyancy.

Protocol

The subjects came into the laboratory on three occasions. In the first visit, weight and height were assessed and subject completed a familiarization trial on the ergometer. The height of the saddle was adjusted and recorded, in order to be comfortable and allow water level to be around mid-sternum. Then, several short pedalling bouts were performed to experience all the combinations of $\dot{W}$ and f_p required in the protocol. Lower limbs mass was estimated as 0.32 body mass (Winter 2009), while volume was estimated with the disc method (Kaulesar Sukul et al. 1993). The second and third visits consisted in cycling sessions in DRY and WET conditions, the order of which was randomised and balanced. Visits were separated by at least 1 h. Subjects were instructed to be properly hydrated and avoid caffeine and alcohol in the previous12 h and to eat a light meal 2-3 h before arriving at the laboratory. Sessions started with 15 min of rest sitting on the cycle ergometer for instrumentation and recording of the resting metabolic rate. Then, subjects performed four repetitions of incremental exercise, consisting of four consecutive steps of 5 minutes: 25, 50, 75 and 100 W. Each repetition involved a different f_p: 40, 50, 60 and 70 revolution per minute (rpm), corresponding to 0.67, 0.83, 1.00 and 1.17 Hz, the order of which was again randomised and balanced. Capillary blood lactate [La] was assessed immediately after the last workload and, if higher than 3.5 mM, after 15 min (Nova Biomedical, Waltham MA). Repetitions were separated by 15 min of passive recovery or until lactate fell below 2.0 mM. An acoustic metronome was used to help maintaining the cadence, that was also checked at the 1st minute of each workload by recording the time needed to perform 20-35 revolutions.

Data treatment and statistical analyses

Steady-state measurements of HR, $\dot{V}O_2$, $\dot{V}CO_2$, minute ventilation and tidal volume were calculated as the average of the last 4 min of rest and the last 2 min of each exercise step. $\dot{E}$ was calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ with standard equations correcting the energy equivalent of $\dot{V}O_2$ as a function of the respiratory quotient (Jeukendrup and Wallis 2005). $\dot{E}_{int}$ was calculated as the y-intercept of the relationship between net (above resting) $\dot{E}$ and $\dot{W}$. Data are expressed as mean ± standard deviation. Paired sample t-test was used to compare descriptive physiological variables of the different steps between WET and DRY. Two-way ANOVA for repeated was used to compare the effect of f_p and water immersion on $\dot{E}_{int}$ and $\Delta\eta$, and pairwise comparison with Bonferroni adjustment was performed to locate significant difference. The relationship between $\dot{E}_{int}$ and f_p was treated as a power function with least-squares regression. The level of significance was set at $p < 0.05$. The statistical package Prism (GraphPad Software, La Jolla, CA) was used.

3.4 Results

Eight subjects completed the protocol in the same day, the remaining four within two days. Difference between measured and target f_p was 0.1 ± 0.5 rpm (range +2 to -2 rpm). A clear $\dot{V}O_2$ steady state was always attained in all steps. Descriptive physiological data are reported in Table 1. Resting $\dot{V}O_2$ and $\dot{E}$ were the same between DRY and WET ($p = 0.26$, $r = 0.91$). During exercise, at all f_p and at all $\dot{W}$, $\dot{V}O_2$ and $\dot{E}$ were higher in WET than in DRY. Average [La] was always lower than 2 mM, except in at 100 W in WET, where nevertheless it was still below than 4 mM. The net $\dot{E}$-$\dot{W}$ relationships (Figure 1) in WET are shifted upward with respect to those in DRY. There was

a significant effect of f_p, water immersion, and their interaction on $\dot{E}_{int}$, whereas $\Delta\eta$ was marginally (2%) affected by water immersion (Table 2). $\dot{E}_{int}$ was higher in WET than in DRY at all f_p except for 40 rpm (Table 2). $\dot{E}_{int}$ was not significantly related to lower limb mass or volume.

Table 1. Descriptive physiological data of exercise steps of all tests. $\dot{V}O_2$, oxygen consumption; RER respiratory exchange ratio; $\dot{E}$, metabolic power; HR, heart rate; [La], capillary blood lactate concentration; WET, cycling in water; DRY, cycling in air; *, $p < 0.05$ vs DRY

			$\dot{V}O_2$ (l mn^{-1})	**$\dot{V}CO_2$** (l mn^{-1})	**RER**	**$\dot{E}$** (W)	**HR** (bpm)	**[La]** (mM)
Rest		DRY	0.264 ± 0.050	0.216 ± 0.039	0.82 ± 0.05	90 ± 17	74 ± 12	0.9 ± 0.3
		WET	0.271 ± 0.048	0.224 ± 0.033	0.83 ± 0.04	93 ± 16	65 ± 7 *	1.0 ± 0.3
40 rpm	**25 W**	DRY	0.582 ± 0.083	0.457 ± 0.071	0.78 ± 0.05	198 ± 28	85 ± 12	-
		WET	0.646 ± 0.065 *	0.500 ± 0.058 *	0.77 ± 0.04	220 ± 22 *	89 ± 8	-
	50 W	DRY	0.856 ± 0.087	0.715 ± 0.090	0.83 ± 0.05	291 ± 30	97 ± 14	-
		WET	0.936 ± 0.092 *	0.788 ± 0.089 *	0.84 ± 0.04	319 ± 32 *	101 ± 9	-
	75 W	DRY	1.130 ± 0.097	0.983 ± 0.105	0.87 ± 0.05	390 ± 34	110 ± 16	-
		WET	1.229 ± 0.103 *	1.082 ± 0.106 *	0.88 ± 0.04	425 ± 36 *	113 ± 12	-
	100 W	DRY	1.404 ± 0.112	1.243 ± 0.132	0.88 ± 0.105	486 ± 39	124 ± 18	1.1 ± 0.3
		WET	1.560 ± 0.102 *	1.362 ± 0.143 *	0.87 ± 0.06	540 ± 37 *	127 ± 16	1.3 ± 0.9
50 rpm	**25 W**	DRY	0.641 ± 0.091	0.509 ± 0.078	0.79 ± 0.04	219 ± 31	89 ± 14	-
		WET	0.815 ± 0.080 *	0.644 ± 0.087 *	0.79 ± 0.05	278 ± 28 *	95 ± 11 *	-
	50 W	DRY	0.914 ± 0.091	0.780 ± 0.082	0.85 ± 0.05	311 ± 31	100 ± 15	-
		WET	1.101 ± 0.089 *	0.956 ± 0.116 *	0.87 ± 0.06	376 ± 32 *	106 ± 14 *	-
	75 W	DRY	1.187 ± 0.107	1.040 ± 0.105	0.88 ± 0.04	409 ± 37	114 ± 18	-
		WET	1.389 ± 0.112 *	1.243 ± 0.133 *	0.89 ± 0.05	479 ± 39 *	118 ± 16 *	-
	100 W	DRY	1.459 ± 0.135	1.313 ± 0.127	0.90 ± 0.05	504 ± 46	126 ± 21	1.2 ± 0.2
		WET	1.686 ± 0.159 *	1.543 ± 0.194 *	0.91 ± 0.05	583 ± 56 *	130 ± 20 *	1.4 ± 0.8
60 rpm	**25 W**	DRY	0.711 ± 0.095	0.570 ± 0.084	0.80 ± 0.04	243 ± 33	93 ± 13	-
		WET	1.080 ± 0.114 *	0.903 ± 0.096 *	0.84 ± 0.04 *	370 ± 39 *	107 ± 13 *	-
	50 W	DRY	0.982 ± 0.107	0.846 ± 0.100	0.86 ± 0.04	323 ± 36	105 ± 14	-
		WET	1.353 ± 0.128 *	1.213 ± 0.133 *	0.90 ± 0.05 *	452 ± 44 *	118 ± 14 *	-
	75 W	DRY	1.252 ± 0.136	1.108 ± 0.121	0.89 ± 0.03	432 ± 47	117 ± 15	-
		WET	1.635 ± 0.139 *	1.501 ± 0.151 *	0.92 ± 0.05 *	566 ± 48 *	129 ± 17 *	-
	100 W	DRY	1.523 ± 0.173	1.382 ± 0.157	0.91 ± 0.04	527 ± 59	130 ± 18	1.1 ± 0.2
		WET	1.940 ± 0.139 *	1.832 ± 0.174 *	0.94 ± 0.06 *	674 ± 48 *	143 ± 18 *	1.9 ± 1.2 *
70 rpm	**25 W**	DRY	0.787 ± 0.093	0.640 ± 0.092	0.81 ± 0.04	269 ± 32	96 ± 15	-
		WET	1.461 ± 0.167 *	1.312 ± 0.184 *	0.90 ± 0.06 *	505 ± 58	125 ± 16 *	-
	50 W	DRY	1.054 ± 0.090	0.922 ± 0.092	0.85 ± 0.04	363 ± 31	108 ± 16	-
		WET	1.805 ± 0.191 *	1.682 ± 0.218 *	0.91 ± 0.05 *	626 ± 67 *	137 ± 18 *	-
	75 W	DRY	1.340 ± 0.098	1.213 ± 0.106	0.91 ± 0.05	463 ± 34	121 ± 18	-
		WET	2.080 ± 0.178 *	1.994 ± 0.232 *	0.96 ± 0.06 *	723 ± 64 *	148 ± 20 *	-
	100 W	DRY	1.616 ± 0.115	1.482 ± 0.131	0.92 ± 0.04	559 ± 40	134 ± 21	1.3 ± 0.4
		WET	2.376 ± 0.205 *	2.312 ± 0.260 *	0.97 ± 0.06 *	827 ± 73 *	160 ± 22 *	3.6 ± 1.8 *

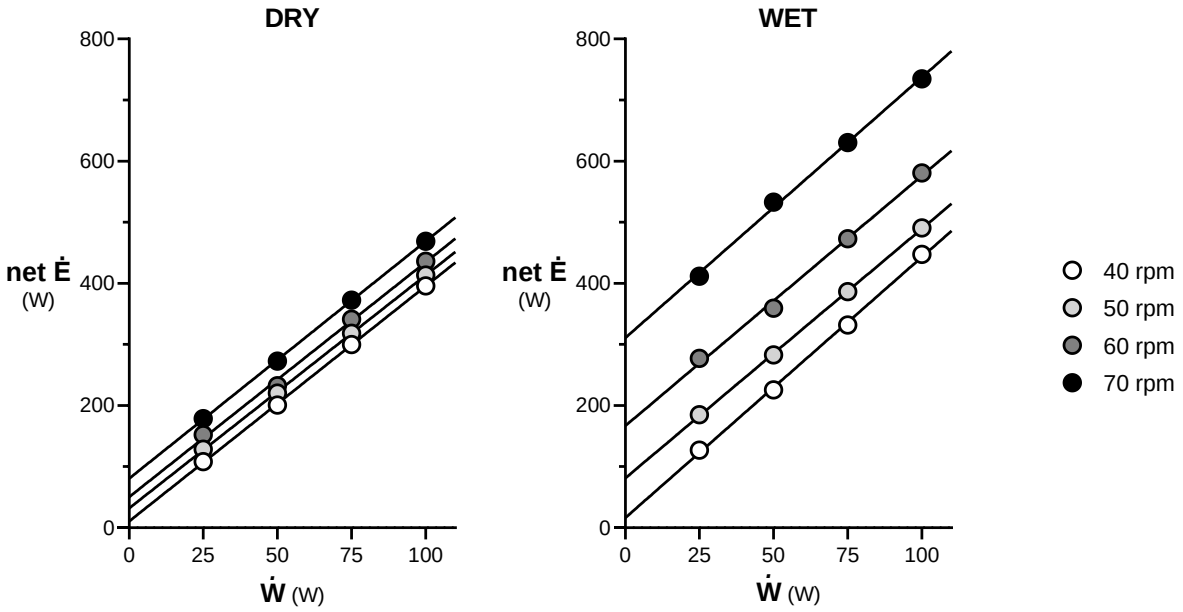

Figure 1. Average net (above resting) metabolic power (net Ė) of cycling in air (DRY) and during head-out water immersion (WET) as a function of ergometer's mechanical power (Ẇ). The y-axis intercept represents the internal metabolic power, while the reciprocal of the slope the delta-efficiency.

Table 2. Average internal metabolic power ($\dot{E}_{int}$) and delta efficiency ($\Delta\eta$) in the various conditions. *, $p < 0.05$ vs DRY; #, $p < 0.05$ vs all pedal cadences (f_p); †, $p < 0.05$ vs all f_p except for 50 and 60 rpm.

f_p		40 rpm	50 rpm	60 rpm	70 rpm
$\dot{E}_{int}$ (W)	DRY	11 ± 17 #	32 ± 30 †	50 ± 29 †	81 ± 30 #
	WET	16 ± 5 #	81 ± 31 * #	167 ± 35 * #	311 ± 51 * #
$\Delta\eta$	DRY	0.26 ± 0.02	0.27 ± 0.05	0.27 ± 0.05	0.26 ± 0.03
	WET	0.24 ± 0.02 *	0.25 ± 0.05 *	0.25 ± 0.03 *	0.24 ± 0.03 *

3.5 Discussion

As predicted, f_p increased $\dot{V}O_2$ and Ė in each Ẇ, and HR followed coherently. $\Delta\eta$, calculated from the slope of the linear relationship between Ė and Ẇ (Gaesser and Brooks 1975), was only marginally affected by f_p and fluid density. The small decrease in $\Delta\eta$ in WET is easily explained by the energy dissipated in small rhythmical upper displacements of the body centre of mass at the highest workloads due to buoyancy, and to the increased work of immersed breathing (Held and Pendergast 2013). In contrast, $\dot{E}_{int}$ (the y-intercept of the net Ė–Ẇ relationship) significantly increased with f_p, and, at the same f_p, it was higher in WET than in DRY. Therefore, the effect of fluid density on the total energy expenditure during cycling was almost exclusively explained by $\dot{E}_{int}$.

It is known that, independently of the type of locomotion, the variation in mechanical energy of limbs with respect to the body centre of mass is proportional to the third power of their velocity

(di Prampero et al. 1979; Zamparo et al. 2002; Minetti 2011; Formenti et al. 2015), i.e. the kinetic component of $\dot{E}_{int}$ ($\dot{E}_k$) is:

$$\dot{E}_k = k_k \, f_p{}^3 \quad (3.1)$$

Where k_k is a proportionality constant including lower limb mass, muscular efficiency, and a conversion factor from angular to linear velocity. This is well described in Figure 2, DRY**.** In the case of stationary underwater cycling, additional force is needed to accelerate the surrounding mass of water (the inertial drag, D) in analogy with the "wasted external work" in swimming (Zamparo et al. 2002, 2020), while viscous drag forces are negligible (Morlock and Dressendorfer 1974). Since in the current model $\dot{E}_{int}$ is assumed as the y-intercept of the $\dot{E}$-$\dot{W}$ relationship, D is necessarily considered among "internal" forces, at variance with swimming, where it is computed among "external" ones. From a physical standpoint, D is proportional to the square of speed, and the related power is proportional to its cube (Morlock and Dressendorfer 1974). Therefore, also the drag component of $\dot{E}_{int}$ ($\dot{E}_D$) should increase with the cube of f_p:

$$\dot{E}_D = k_D \, f_p{}^3 \quad (3.2)$$

Where k_D is a proportionality constant including fluid density, lower limb projection area in the direction of movement, drag coefficient, muscular efficiency, and a conversion factor from angular to linear velocity. Equation 3.2 is in agreement with previous experimental data (Morlock and Dressendorfer 1974; Shapiro et al. 1981; Sogabe et al. 1987) and current results (Figure 2, WET).

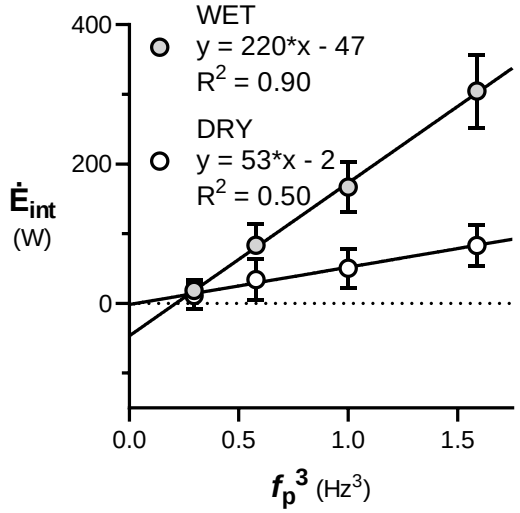

Figure 2. $\dot{E}_{int}$ as a function cubic pedal cadence ($f_p{}^3$) in WET and DRY conditions (grey and white dots, respectively).

The negative y-intercept of -47 W in WET (Figure 2) can be interpreted as a factor accounting for limb unloading due to buoyancy. In fact, investigating the gravitational component of $\dot{E}_{int}$ ($\dot{E}_g$). Bonjour et al. (2010) found that:

$$\dot{E}_g = \varphi' \, M_L \, a_g \, f_p{}^2 \quad (3.3)$$

where M_L is the mass of the legs, a_g is the gravity acceleration and φ' a proportionality constant. For an average leg relative density of 1.06 (Winter 2009), water immersion results in a final net acceleration of 0.06 a_g, so that $\dot{E}_g$ become closes to 0 at all f_p in WET. Alternatively, the negative y-intercept in WET can be due to a small bias in $\dot{E}_{int}$ estimations, especially at lower cadences, where the reduced Δη could underestimate $\dot{E}_{int}$. Nevertheless, it can be included as an empirical factor, proportional to fluid density.

Another internal force opposing to movement is the frictional resistance of anatomical structures, that recently has been characterized as mostly viscous (therefore proportional to speed) and load-dependent (Minetti et al. 2020). Therefore, internal frictional power ($\dot{E}_f$) is proportional to the square of speed. $\dot{E}_f$ has been hypothesised to be of greater importance over $\dot{E}_k$ during cycling (Minetti et al. 2020), however since $\dot{E}_f$ is proportional to f_p^2, it cannot replace entirely $\dot{E}_k$, which is indeed proportional to f_p^3. Interestingly, the $\dot{E}_f$ theory is compatible with the relationship with f_p^2 found in hyper-gravity by Bonjour et al. (2010) (Equation 3.3), suggesting that gravity-induced limb loading influences internal frictions, contrary to previous hypothesis (Girardis et al. 1999).

In conclusion, the internal power of cycling can be partitioned into several components ($\dot{E}_k$, $\dot{E}_f$, $\dot{E}_g$, $\dot{E}_D$), however their reciprocal interdependences make it impossible to express $\dot{E}_{int}$ as a mere sum of these components. In air on Earth, fluid density tends to 0 (in fact 0.0013 kg l^{-1} at sea level in STPD), therefore k_D tends to 0 and $\dot{E}_D$ can be neglected. In microgravity, also a_g is 0, therefore $\dot{E}_g$ becomes nihil and probably also $\dot{E}_f$ is reduced by some extent. In fact, according to Girardis et al. (1999), their subject who was able to keep the same f_p at all workloads had $\dot{E}_{int}$ of 28 W at 0 G, which can represent the "unloaded" $\dot{E}_f$. In water immersion a_g tends to 0 (in fact, 0.06 m s^{-2}) while fluid density is 1.0 kg l^{-1}, therefore a similar disappearance of $\dot{E}_g$ and a reduction in $\dot{E}_f$ are expected, while $\dot{E}_D$ becomes predominant.

Practical applications

A practical application of this study is evident. When there is the need to quantify the equivalent mechanical power of cycling in water, it is possible to use the empirical relationship in between the increase in $\dot{E}$ in WET with respect to DRY and f_p^3 (Figure 3). Assuming a muscular efficiency of 25% (grand average of our Δη values), this could result in a mechanical power of:

$$\dot{W}_{WET} - \dot{W}_{DRY} = 43\, f_p{}^3 - 7 \qquad (3.4)$$

Where f_p is expressed in Hz. Meaning that, for a cadence of 60 rpm (1 Hz), water immersion corresponds roughly to an increase in external load of 36 W. Decreasing to 50 and 40 rpm (0.83 and 0.21 Hz), it becomes 18 W and 6 W, respectively. Increasing to 70 or 80 rpm (1.67 and 1.33 Hz) it goes up to 62 W and 95 W, respectively. The traditional finding that water cycling corresponds to a 25 W increase in $\dot{W}$ (Perini et al. 1998) proves therefore true only for a f_p of 55 rpm and can be better refined by Equation 3.4 taking into account f_p.

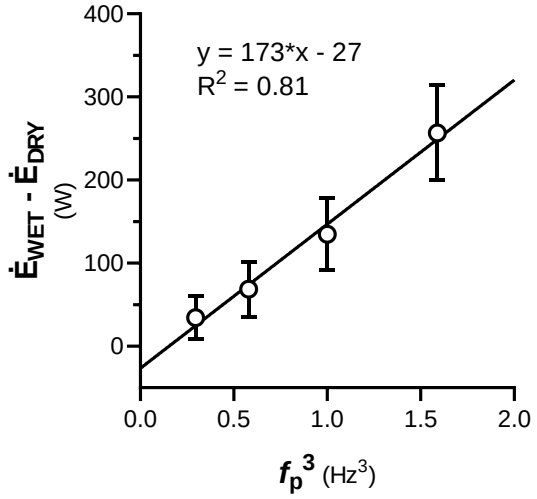

Figure 3. The average difference in metabolic power between of cycling in air and during head-out water immersion, ($\dot{E}_{WET} - \dot{E}_{DRY}$) as a function of the third power of pedal cadence (f_p^3).

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4 Single-breath oxygen dilution for the measurement of total lung capacity: technical description and validation

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4.1 Abstract

Introduction. Total lung capacity (TLC) assessment outside of a research laboratory is challenging. We aimed to describe and validate a novel method for measuring TLC that is both simple and based only on portable equipment.

Methods. We developed an open circuit system to administer a known amount of oxygen to a subject in a single maximal inspiratory manoeuvre. Oxygen fraction and expired and inspired flows were continuously monitored to allow a precise computation of the mass balance. Values of TLC and functional residual capacity (FRC) were compared with standard methods (body plethysmography and multiple-breath helium dilution). Twenty healthy subjects participated to the study, eleven of which performed the manoeuvre twice to assess test-retest reliability.

Results. There was high agreement in TLC between the proposed method and the two standard methods ($R^2 > 0.98$, bias not different from 0, and 95% limits of agreements $< \pm 0.4$ l for both). Test retest reliability was high (intraclass correlation coefficient >0.99 and no bias). Results were similar for FRC, with a slightly higher variability due its sensitivity to changes in posture or breathing pattern.

Conclusions. Single-breath oxygen dilution is valid and reliable to assess TLC and FRC in healthy subjects. The technique is appealing for time- or resource-limited settings, such as field physiological research expeditions or mass screenings.

4.2 Introduction

The estimation of total lung capacity (TLC) is of great value in answering both clinical (Wanger et al. 2005) and physiological questions (Cogo et al. 1997; Janssens et al. 1999; Ferretti 2001). Nowadays multiple-breath helium dilution (Brown et al. 1998) and body plethysmography (Coates et al. 1997) are well-established methods, with computed tomography emerging as a complementary approach (Tantucci et al. 2016). Albeit with different principles and pitfalls, there is experimental and theoretical consensus that those methods provide minimal differences in normal subjects (Tantucci et al. 2016). Conversely, in respiratory patients gas dilution methods

progressively underestimate TLC with increasing the degree of airflow obstruction, due to difficulty in gas equilibration among poorly ventilated lung regions (Brown et al. 1998; Tantucci et al. 2016).

In situations such as mass screening or scientific expeditions in unusual environments, both time and instrumentation availability are limited. Several simplified techniques involving only a single breath have been proposed, based on nitrogen washout after inspiring pure oxygen (Buist and Ross 1973; Chiang and Yang 1973), the dilution of a known inspired quantity of helium (Mitchell and Renzetti 1968) or methane (Pesola et al. 2007). As expected, single-breath techniques further exacerbate the underestimation of TLC in subjects with airway obstruction while preserving accuracy in healthy subjects (Teculescu 1971; Chiang and Yang 1973; Rodarte et al. 1976; Pino and Teculescu 1980; Punjabi et al. 1998; Pesola et al. 2007). Among sensors, and gas mixtures available, those regarding oxygen are the most widespread. For example, multiple-breath open circuit methods based on oxygen *wash-in* (the mirror image of a nitrogen *washout*) have been developed and validated for the measurement of functional residual capacity during spontaneous breathing (Maisch et al. 2007) and in the intensive care setting (Weismann et al. 2006; Heinze et al. 2007; Patroniti et al. 2008), where oxygen supply is already present. An unexpected advantage characterizing of oxygen dilution is that it is independent of the calibration of the oxygen sensor (Weismann et al. 2006). In fact, since gas fractions are always directly measured, they appear in the equation for the calculation of TLC always in the form of a quotient of subtractions (see Appendix for details), therefore cancelling out the sensitivity error (quotient) and the offset error (subtraction). Besides intensive care departments, oxygen supply and related sensors are also frequently utilized during field studies in respiratory physiology. Under some circumstances, for instance in breath-holding studies in which both pure oxygen is used and knowledge of lung volumes is important (Overgaard et al. 2006; Schiffer et al. 2013; Fagoni et al. 2015, 2017; Bain et al. 2017; Taboni et al. 2019, 2020), the availability of a simple and standardized procedure for assessing TLC involving only portable equipment would be beneficial.

The aim of our study was to develop a single-breath oxygen dilution method for TLC measurement, as well as to validate it against multiple-breath helium dilution and body plethysmography in healthy subjects.

4.3 Methods

Subjects

Twenty healthy young subjects (11 females, 9 males, 26 ± 5 years, 173 ± 9 cm, 67 ± 12 kg, body mass index 22.3 ± 2.9 kg/m2) volunteered to participate in the study by written informed consent. They were all non-smokers, free of chronic medical conditions, medications, and acute pulmonary disease in the past five years. The study conformed to the Declaration of Helsinki and was approved by the local ethical committee.

Theory

The test relies on the principle of mass conservation during the introduction of a known amount of oxygen from a Douglas bag, net of some losses in the instrumental dead spaces, in an alveolar space of unknown volume but known pre-and post-manoeuvre oxygen fractions (F_AO_{2pre}

and F_AO_{2post}, respectively). From the same manoeuvre, both TLC and functional residual capacity (FRC) can be mathematically derived. Complete calculations are described in the Appendix.

Experimental setup

The instrumental setup consisted of an open circuit system as schematically represented in Figure 1. The subject breathed through a rubber mouthpiece connected to a non-rebreathing T valve (AFT21, BIOPAC® Systems Inc., Goleta, CA, USA). The T valve with mouthpiece had a dead space of 0.08 l. Care was taken to insert the mouthpiece always in the same position in order to maintain the dead space constant among subjects. The inspiratory port of the T valve was sealed together with three-way stopcock (dead space 0.05 l) capable of switching between ambient air or an oxygen supply system. The oxygen supply system consisted of the following element sealed in series: a 50 l Douglas bag (De Marco Engineering, Geneva, CH), a pneumotachograph (TSD107B, BIOPAC® Systems Inc., Goleta, CA, USA), and a rubber tube connecting the Douglas Bag to the three-way stopcock. The whole oxygen supply system was flushed 2-3 times with medical oxygen coming from a high-pressure tank connected with the Douglas bag, that acted as a pressure buffer and did not need to be completely inflate Then, the Douglas bag was gently squeezed and immediately the three-way stopcock was turned in the "ambient air" configuration, in order ensure that the whole system was completely filled with oxygen until the 3-way stopcock dead space. The oxygen tank was closed well before the pressure of the Douglas bag exceeded ambient pressure. The expiratory port of the T valve was connected to another pneumotachograph (TSD107B, BIOPAC® Systems Inc., Goleta, CA, USA) via a rubber tube. Oxygen fraction (FO_2) was continuously sampled at the mouthpiece via Nafion drying capillaries (AFT20, BIOPAC® Systems Inc., Goleta, CA) routing to a paramagnetic oxygen analyser (O2100C, BIOPAC® Systems Inc., Goleta, CA, USA). The signals from the twin pneumotachographs and the oxygen sensor were sampled at 200 Hz by a 16-bit A/D converter (MP150 with AcqKnowledge 5.0 software, BIOPAC® Systems Inc., Goleta, CA, USA), displayed on-line on a personal computer and finally stored for subsequent analysis.

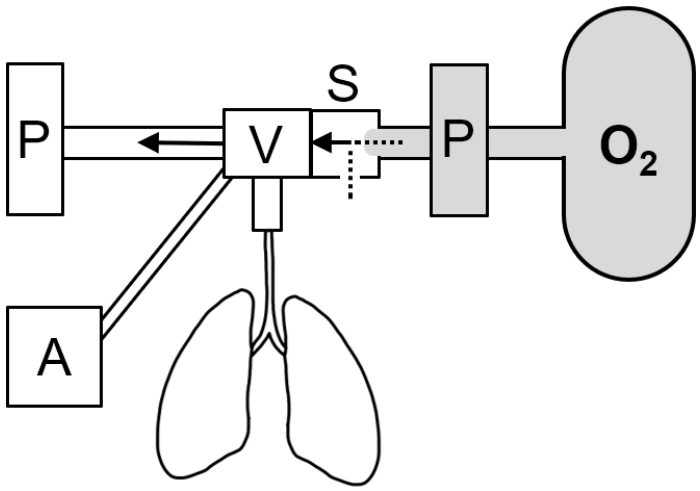

Figure 1. Experimental setup. Grey colour depicts the oxygen delivery system. V, non-rebreathing T valve. P, pneumotachograph; S, three-way stopcock; A, oxygen analyser; O_2, Douglas bag. Arrows indicate the direction of the valves. Dashed lines represent the two configurations of the three-way stopcock.

Calibrations

Flow signals were calibrated by means of a 3-l syringe temporarily connected at the mouth port of the T valve. The syringe was discharged four times at low flows (0.5–1 l s^{-1}) and four times at medium flows (1.5–2.0 l s^{-1}) to mimic experimental manoeuvres. The manoeuvre was firstly performed with the stopcock in the "ambient air" configuration, in order to calibrate the expiratory flowmeter with ambient air. Then it was repeated with the stopcock in the "oxygen configuration" to calibrate the inspiratory flowmeter in oxygen. Finally, an additional flushing of the system with oxygen (always including the rubber tube and the connected 3-way stopcock) was performed to counteract possible leakages during the calibration procedure. Even if not necessary (see Appendix for details), the oxygen analyser was calibrated with ambient air and pure medical oxygen. Ambient temperature and barometric pressure were recorded before each test. The Douglas bag oxygen content was assumed in thermal and barometric balance with ambient air, and dry (ATPD). Empirical measurements showed that expired air at the level of the expiratory flowmeter at steady state was 25° C, saturated with water vapor.

Manoeuvres

The subject seated comfortably on a chair with the back straight. The height of the T-valve, hanging from a perch, was regulated accordingly. Then he/she fitted the mouthpiece between the teeth and the lips and wore a noseclip, with the inspiratory three-way stopcock set to ambient air and breathed quietly for two minutes to allow the attainment of FRC. At the end of a tidal expiration identified at the flow trace on the screen, the experimenter switched the stopcock to the oxygen delivery system and contemporarily signalled the subject to perform a maximal inhalation (inspiratory capacity, IC), followed by a 5-s breath hold and then a slow, maximal exhalation, during which the stopcock was turned back to ambient air before the subject resumed normal breathing. A familiarization trial was performed in advance, consisting in the same sequence of manoeuvres except for the turning of the stopcock, which remained on ambient air.

Data treatment

FO_2 trace was corrected for the time delay between the pneumotachograph signals and the gas analyser. After correction for the calibration factors, inspired and expired flows were integrated over time to obtain the inspired volume, and converted to body temperature, pressure, saturated (BTPS), starting from ATPD (volume inspired from the Douglas Bag), ambient air (volume inspired from the small stopcock's dead space) and 25°C, ambient pressure and saturated (expired volume). Therefore, all volumes used for calculations were expressed in BTPS. Instrumental dead spaces volumes were taken into account. The anatomic dead space was estimated from predictive equations (Hart et al. 1963). The FO_2 of the Douglas bag was measured as the highest 0.5-s average of FO_2 during the IC manoeuvre. $F_AO_{2(pre)}$ was measured as the lowest end-tidal FO_2 of the five breaths preceding the IC manoeuvre. $F_AO_{2(post)}$ was retained as the FO_2 of a large virtual alveolar gas sample (50-60% of VC) calculated by integral mean during the phase III (plateau) of the FO_2-expired volume relationship, similarly to the alveolar volume determination in single-breath lung carbon monoxide uptake in the lung (Graham et al. 2017). All values were inserted in Equation 4.6 to obtain TLC or in Equation 4.7 to obtain FRC.

Experimental validation

On the same day on which they underwent the experimental tests with the single breath oxygen dilution method described above (TLC_{O2} and FRC_{O2}), the subjects performed also a baseline spirometry and the validation tests. These consisted of body plethysmography (TLC_{pleth} and FRC_{pleth}) and multiple-breath helium dilution (TLC_{He} and FRC_{He}). A subgroup of 11 subjects repeated the single-breath oxygen dilution test within 15 minutes in order to assess test-retest reliability. In this case, only the first trial was compared with other methods. Spirometry, helium dilution, and plethysmography were performed according to clinical guidelines (Coates et al. 1997; Brown et al. 1998; Miller et al. 2005) using certified medical equipment as previously described (Tantucci et al. 2016). To account for spurious differences between FRC_{He} and FRC_{pleth} due to positional factors, FRC_{He} was corrected as previously described (Tantucci et al. 2011). All measures were carried out in the morning and were completed within two hours avoid circadian variation of lung volumes (Rubini et al. 2011), in the seated position.

Statistical analysis

Data were tested for normality with the Shapiro-Wilk test, and resulted normally distributed. One-way ANOVA for repeated measures was used to assess the effect of the measurement method on TLC and FRC. In case of a significant effect, pairwise comparison with the Bonferroni correction was performed. Linear regression and Bland-Altman plot were used to assess correlation and agreement between methods, respectively. One-sample t test was used to check if the bias was different from 0. Intraclass correlation coefficient (ICC) was calculated with a two-way mixed-effect, single measures model (Shrout and Fleiss 1979) to asses test-retest reliability. The level of significance was set at $p < 0.05$. The statistical package Prism (Version 7.00, GraphPad Software Inc., La Jolla, CA, USA) was used.

4.4 Results

Baseline spirometry was normal in all subjects. In particular VC was 4.78 ± 1.19 l (104% ± 15% of predicted value, range 136%–87%), forced expiratory volume in 1 s (FEV_1) was 4.08 ± 0.83 l (107% ± 12% of predicted value, range 136%–91%) and the FEV_1/VC ratio was 0.87 ± 0.08 (104% ± 9% of predicted value, range 116%–90%).

There was no effect of measurement method on TLC and FRC (Table 1). Volumes obtained with oxygen dilution correlated well with those obtained by the other two techniques, especially TLC (Figure 2). The TLC_{O2} bias was not significantly different from 0 with both TLC_{He} and TLC_{Pleth} (0.02 ± 0.16 l and −0.02 ± 0.17 l, respectively). The corresponding 95% limits of agreement were −0.29 to 0.34 l and −0.36 to 0.33 l, respectively (Figure 3). Seventeen out of 20 TLC_{O2} values (85%) were within ± 0.2 l of TLC_{Pleth} and TLC_{He}. Similar agreements were found for FRC_{O2} and FRC_{He}/FRC_{Pleth} (bias 0.13 ± 0.31 and 0.09 ± 0.311 both non significantly different from 0 and 95% limits of agreement from −0.47 to 0.73 and −0.52 to 0.69 l, respectively). Test-retest reliability was high for both TLC_{O2} and FRC_{O2} (ICC 0.997 and 0.992, bias 0.00 ± 0.14 and 0.02 ± 0.13 l, 95% limits of agreement −0.27 to 0.28 and −0.23 to 0.26 l, respectively).

Table 1. Comparisons between lung volumes measurements. TLC; total lung capacity; FRC functional residual capacity; He, multiple-breath helium dilution; Pleth, body plethysmography; O_2 single-breath oxygen dilution.

	He	**Pleth**	**O_2**	**ANOVA**
FRC (l)	3.53 ± 0.89	3.57 ± 0.88	3.66 ± 0.86	p = 0.11
TLC (l)	6.55 ± 1.42	6.59 ± 1.43	6.57 ± 1.48	p = 0.39

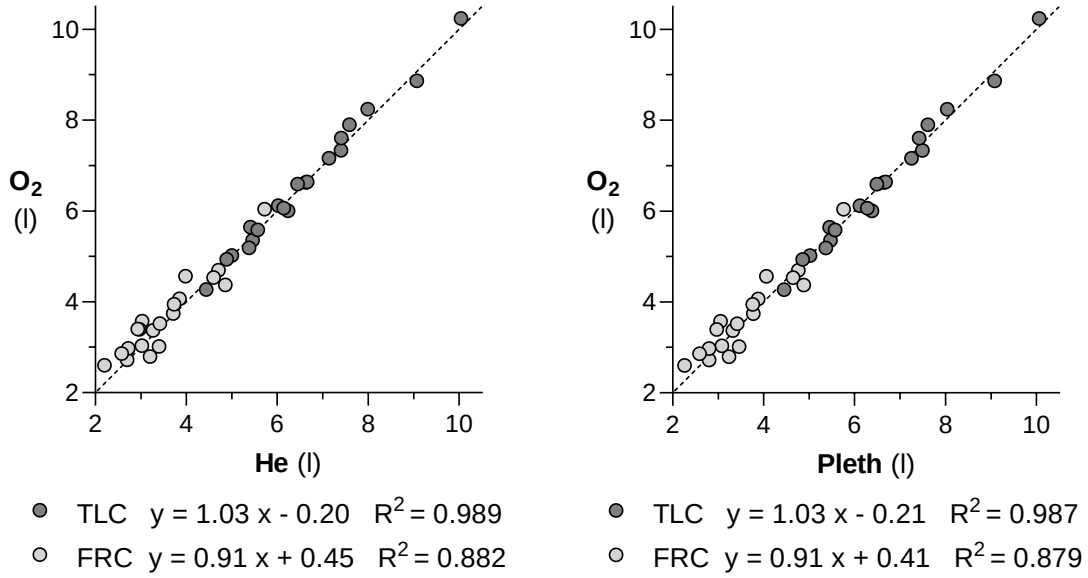

Figure 2. Correlation between volumes measured by single breath oxygen dilution (O_2) with helium dilution (He) and with body plethysmography (Pleth). For clarity, only the identity line is shown. TLC; total lung capacity; FRC functional residual capacity.

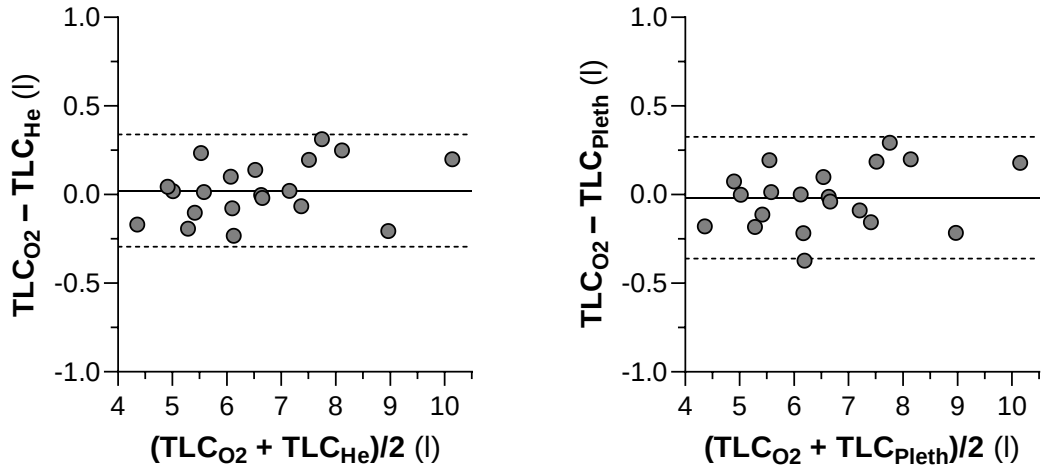

Figure 3. Bland-Altman plot comparing of total lung capacity measured by single-breath oxygen dilution (TLC_{O2}) against helium dilution (TLC_{He}, left panel) and body plethysmography (TLC_{Pleth}, right panel). Continuous line, bias; dashed lines, 95% limits of agreement.

4.5 Discussion

Validation results are satisfactory and in line with previous literature on other single-breath techniques in healthy subjects (Teculescu 1971; Chiang and Yang 1973; Rodarte et al. 1976; Punjabi et al. 1998; Pesola et al. 2007), with an added value of a negligible bias and optimal reliability. The slightly, yet non significantly lower agreement of FRC with respect to TLC was expected since FRC is sensible to small changes in posture or breathing pattern. Therefore, the single-breath oxygen dilution technique is feasible and valid for measuring TLC and FRC in healthy subjects. In fact, despite oxygen is a gas that can diffuse across the alveolar-capillary barrier, the duration of the manoeuvre (a single inspiration and expiration) is so short, that oxygen diffusion effects on TLC and FRC measures are definitely negligible. Similarly, also the multiple-breath oxygen dilution technique recently proposed for intensive care (Weismann et al. 2006) seems not to underestimate FRC as compared to body plethysmography in spontaneously breathing lung disease patients (Maisch et al. 2007) or computer tomography in a swine model of pleural effusion (Graf et al. 2010). This could be explained by the fact that different oxygen fractions were breathed until steady state (Weismann et al. 2006; Maisch et al. 2007), thus allowing for complete equilibration of the mixture also in poorly ventilated lung zones, at variance to single-breath techniques. However, multiple-breaths techniques are time consuming, not a great issue in bedridden critical patients, where serial assessment of functional alveolar volume is of ever increasing importance (Marini and Gattinoni 2020), but not compatible with the aims of the current study, which is designed for the out-of-hospital – or even out-of-laboratory – setting.

The method is appealing under several methodological aspects. First, the test starts at FRC and not from residual volume as the original single-breath nitrogen washout (Buist and Ross 1973), therefore there is no need to perform a preliminary expiratory vital capacity (that is physically and time demanding), and the closing volume (Buist and Ross 1973) is not reached, ensuring a more uniform distribution of the tracer gas. The absence of an initial deep expiratory manoeuvre makes the determination of FAO2pre challenging, however using the lowest end-tidal FO2 of the previous five breaths seems a valid and effortless alternative. Concerning the FAO2post, the volume chosen for the virtual sample (50-60% of VC), starting as soon as the alveolar plateau has been reached during expiration, has revealed optimal. Adding the volume expired before the reaching the alveolar plateau (dead spaces and intrinsic response time of the oxygen sensor), this implies an expiration of approximately 80% of VC, therefore a complete expiratory manoeuvre is not needed, further easing the testing procedure. Moreover, the last portion of the expired alveolar air may contain a reduced oxygen fraction due to diffusion into bloodstream. Also, we emphasize the fact that final expiration is not to be forced, because high flows may overcome the intrinsic response time of the oxygen sensor, altering the FO2 vs time relationship upon which FAO2post is calculated. As previously noted, another advantage of this technique is the independence of calibration of the oxygen sensors. Conversely, calibration of the inspiratory flowmeter is crucial since the determination of the inspired volume represents the greatest source of error in this protocol. Since flow and viscosity of the gas were relatively constant during the manoeuvres, the inherent non-linearity of the inspiratory pneumotachograph was minimized, and a correct calibration could be accomplished in less than one minute. The use of two separate flowmeters, a choice performed to minimize the dead space volume, can represent a practical limitation. In case of limited instrumentation availability, the setup can be conveniently modified to host a single

flowmeter between the mouthpiece and the T-valve. In this case, however, the dead space between valve and mouth would be much bigger. Expiratory flowmeter calibration was more challenging, since gas viscosity and temperature were more variable with respect to calibration conditions: however, we used expired flow only to weigh the average FO2 and thus obtain a valid estimate of FAO2(post). Therefore, there was no need of obtaining correct absolute values of expired volumes, which would have required more sophisticated sensors such as mass flowmeters. Another option would be to refrain from using any expiratory flowmeter, therefore calculating FAO2(post) only from the simple average of the FO2 time course (assuming constant flow) or with an a-priori defined weighting accounting for a decreasing flow. This would have increased portability, however in spite of accuracy and precision.

In conclusion, the portability, simplicity, and reliability of single-breath oxygen dilution technique make it appealing to measure lung volumes of healthy subjects in limited resources environments. The validity of this method in clinical populations is not granted and still needs to be tested, especially for obstructive lung disease, while there are clues that single breath techniques conserve their validity in pure restrictive lung disease (Mitchell and Renzetti 1968; Cliff et al. 1999). Nonetheless, the proposed method is already apt for use in studies pertaining normal human physiology.

4.6 Appendix

During the single-breath oxygen dilution test, the gas flow and balance is assumed as follows. The volume of oxygen in the alveoli at the beginning of the manoeuvre is equal to the functional residual capacity (FRC), minus the anatomic dead space ($V_{D(A)}$) times the corresponding alveolar oxygen fraction ($F_{AO_2(pre)}$):

$$\Delta\left(FRC - V_{D(A)}\right) \cdot F_{AO_2(pre)} \tag{4.1}$$

Then, the subject performs the inspiratory capacity (IC) manoeuvre. Before receiving the Douglas bag's content, the alveolar space receives an amount of oxygen from the air coming from the overall dead space volume of the system, consisting in $V_{D(A)}$, the T-valve ($V_{D(V)}$) and the three-way stopcock ($V_{D(S)}$). The air residing in $V_{D(A)}$ and ($V_{D(V)}$) is already in BTPS because it comes from the last quiet expiration before the manoeuvre. $V_{D(S)}$ contains ambient air and upon entry in the alveolar space is heated and humidified to BTPS conditions, therefore increasing of a factor *c*. Hence, the volume of oxygen in the overall dead space of the system is equal to:

$$\left(V_{D(A)} + V_{D(V)}\right) \cdot F_{AO_2(pre)} + c \cdot V_{D(S)} \cdot F_{IO_2(amb)} \tag{4.2}$$

where $F_{IO_2(amb)}$ is the ambient oxygen fraction.

Finally, the portion of the Douglas bag's oxygen volume effectively reaching the alveolar space is the volume exiting from the Douglas bag measured at its exit point (V_I, ambient temperature and dry conditions, ATPD), net of its losses in the aforementioned dead spaces, multiplied by its measured oxygen fraction ($F_{IO_2(bag)}$). Moreover, when trespassing $V_{D(A)}$, the air is also heated to body temperature and humidified (BTPS), therefore expanding by a factor *d*

$$\left[d\left(\mathrm{V_I}-\mathrm{V_{D(S)}}-\mathrm{V_{D(V)}}\right)-\mathrm{V_{D(A)}}\right]\mathrm{F_{IO_2(bag)}} \tag{4.3}$$

The principle of the mass balance tells that the post-manoeuvre alveolar oxygen volume must be equal to the sum of Equation 4.1, 4.2, and 4.3:

$$\begin{aligned}\left(\mathrm{TLC}-\mathrm{V_{D(A)}}\right)\mathrm{F_AO_{2(post)}} = \\ \left(\mathrm{FRC}-\mathrm{V_{D(A)}}\right)\mathrm{F_AO_{2(pre)}}+\left(\mathrm{V_{D(A)}}+\mathrm{V_{D(V)}}\right)\mathrm{F_AO_{2(pre)}} \\ +\mathrm{V_{D(S)}}\,c\,\mathrm{F_IO_{2(amb)}} \\ +\left[d\left(\mathrm{V_I}-\mathrm{V_{D(S)}}-\mathrm{V_{D(V)}}\right)-\mathrm{V_{D(A)}}\right]\mathrm{F_IO_{2(bag)}}\end{aligned} \tag{4.4}$$

Where all volumes are expressed in BTPS, TLC is the total lung capacity and $\mathrm{F_{AO_2(post)}}$ the final alveolar oxygen fraction. Equation 4.4 can be solved both for TLC and FRC, knowing that TLC = FRC + IC and that IC is the sum of all the volumes in Equations 4.2 and 4.3:

$$\mathrm{IC}=\mathrm{V_{D(V)}}+\mathrm{c\,V_{D(S)}}+d\left(\mathrm{V_I}-\mathrm{V_{D(S)}}-\mathrm{V_{D(V)}}\right) \tag{4.5}$$

Substituting in Equation 4.4 FRC with TLC – IC and then IC with Equation 4.5 we obtain:

$$\mathrm{TLC}=\frac{d\left(\mathrm{V_I}-\mathrm{V_{D(S)}}-\mathrm{V_{D(V)}}\right)\left(\mathrm{F_{IO_2(bag)}}-\mathrm{F_{AO_2(pre)}}\right)-\mathrm{V_{D(A)}}\left(\mathrm{F_{IO_2(bag)}}-\mathrm{F_{AO_2(post)}}\right)+c\,\mathrm{V_{D(S)}}\left(\mathrm{F_{IO_2(amb)}}-\mathrm{F_{AO_2(pre)}}\right)}{\mathrm{F_{AO_2(post)}}-\mathrm{F_{AO_2(pre)}}} \tag{4.6}$$

Substituting in Equation 4.4 TLC with FRC + IC and then IC with Equation 4.5 we obtain:

$$\mathrm{FRC}=\frac{\left[d\left(\mathrm{V_I}-\mathrm{V_{D(S)}}-\mathrm{V_{D(V)}}\right)-\mathrm{V_{D(A)}}\right]\left(\mathrm{F_IO_{2(bag)}}-\mathrm{F_AO_{2(post)}}\right)-c\,\mathrm{V_{D(S)}}\left(\mathrm{F_AO_{2(post)}}-\mathrm{F_IO_{2(amb)}}\right)}{\mathrm{F_AO_{2(post)}}-\mathrm{F_AO_{2(pre)}}}-\mathrm{V_{D(V)}} \tag{4.7}$$

4.7 References

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5 Energy balance and cost of dynamic apnoea

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5.1 Abstract

Introduction. The energy cost (C) of and the energy sources for dynamic apnoea (DA) are largely unknown. The aim of this study is therefore to assess these physiological variables during submaximal DA.

Methods. Twelve freedivers (5 monofin, 7 bi-fins) performed one (50 m, 6 subjects) or two (50 and 100 m, 6 subjects) DA in an Olympic pool. Aerobic energy stores depletion (E_{O2}) was calculated measuring pre- and post-DA alveolar gas composition and peripheral oxygen saturation, with preventive measurement of total lung capacity (O_2 dilution test), haemoglobin mass and blood volume (CO rebreathing). Net capillary blood lactate increase above resting was measured to calculate the contribution of anaerobic lactic metabolism (E_{La}) to the energy expenditure during DA. Anaerobic alactic energy expenditure (E_{PCr}) was calculated by subtracting E_{O2} to the oxygen debt measured during recovery. Selected biomechanical variables were measured with inertia measurement units.

Results. Net (above resting) C was 0.63 ± 0.13 kJ m^{-1} (8.1 ± 1.7 J kg^{-1} m^{-1}) for bi-fins and 0.42 ± 0.08 kJ m^{-1} (5.9 J ± 1.3 kg^{-1} m^{-1}) for mono-fins ($p < 0.05$). No significant differences in fractional energy balance were present between fin types. Fractional contributions for the 50 m trial (E_{O2} 43 ± 11%, E_{La} 9 ± 4%, E_{PCr} 48 ± 12%) were significantly different from the 100 m trial (E_{O2} 35 ± 10%, E_{La} 19 ± 4%, E_{PCr} 46 ± 10%) except for E_{PCr}. The gross O_2 cost per unit of distance (E_{O2}/d), but not C, was significantly inversely related to subjects' personal best.

Conclusions. The C of DA seems halfway between that of eupnoeic underwater (with snorkel) and surface swimming. Anaerobic alactic metabolism plays a major role in the energetic balance during DA. The distance covered per l of O_2 depleted seems the best predictor of performances.

5.2 Introduction

Dynamic apnoea (DA) is a growing sport discipline, wherein divers cover the longest possible horizontal distance, while swimming underwater holding their breath (Schagatay 2010). The absence of compression-decompression sequences of deep dives makes it a simplified model to study human bioenergetics and biomechanics of breath hold diving. However, the only known

bioenergetic feature about DA is the increase in blood lactate concentration with respect to eupnoeic exercise (Olsen et al. 1962; Andersson et al. 2004; Tagliabue et al. 2005; Rodríguez-Zamora et al. 2018), that shows positive correlation with swimming speed (Breskovic et al. 2011) and negative correlation with breath-hold training status (Joulia et al. 2002, 2003). No data are available in the literature either on the comprehensive energy balance of DA, or on the energy cost per unit of distance (C) of this particular type of swimming.

Knowing C is important for breath-hold diving since it's a key factor affecting maximal distance (Schagatay 2010) and depth (Ferretti 2001). In fact, C is the ratio of net metabolic energy utilised over the travelled distance. Since during breath-holding carried out from total lung capacity (TLC) the amount of energy available in the body is finite and fix for any given diver, the maximal distance covered by a diver is inversely proportional to C (Ferretti 2001). In non-apnoeic underwater swimming with the SCUBA asset and fins, C ranged from 35 to 55 ml of O_2 m^{-1} (Goff et al. 1957; Pendergast et al. 1996, 2003a, b), depending primarily on speed and secondarily on anthropometric characteristics. More relevant to DA, the C of underwater swimming with fins with legs only (breathing through a snorkel) was found to be 43 ml of O_2 m^{-1} (0.89 kJ m^{-1}) at 1 m s^{-1} (Pendergast et al. 2005). This value was ~65% higher underwater than at surface This is not surprising given in underwater swimming the over-water arm recovery is impossible, therefore propulsion is generally provided totally from a flutter type leg kick, which *per se* has a lower efficiency and a higher C with respect to surface front crawl, where propulsion is primarily provided by the arms (Holmér 1974).

Therefore, the aim of this study was to assess for the first time C during submaximal DA through a complete computation of the energy balance encompassing all three main energetic metabolisms: aerobic (E_{O2}), anaerobic lactic (E_{La}), and anaerobic alactic (phosphocreatine depletion, E_{PCr}) (Ferretti 2015). Secondary aims were to preliminary analyse the effects of fin type and biomechanical variables on C and identify the role of C in maximal performances. Since it is known that mono-fins are more efficient than bi-fins when swimming at the surface (Zamparo et al. 2006), we expect this to be true also in DA.

5.3 Methods

Subjects

Twelve healthy breath-hold divers (two females, 40 ± 8 years, 76 ± 9 kg, 176 ± 6 cm) with heterogeneous performance levels in DYN (personal best 132 ± 45 m, range 75-240 m) volunteered for the study, which is part of a larger project (Taboni et al. 2020b, a). Ethical approval was granted by the Cantonal Commission for Research Ethics of Geneva, Switzerland. Subjects were instructed to avoid caffeine, alcohol, and intense exercise in the previous 24 h and to eat a light meal 3-4 h prior the trials. They were equipped with neoprene wetsuit, swimming cap, goggles, noseclip, fins (5 monofin, 7 bi-fin according to individual preference) and a counterweight at the base of the neck in order to maintain neutral buoyancy and counterbalance the leg-sinking torque (Schagatay 2010).

Preliminary measurements

TLC, total blood volume (V_B) and total haemoglobin mass (Hb_{mass}) were measured in dry conditions on the same day of the experimental trials. TLC was measured with the technique of single-breath O_2 dilution previously described (Chapter 4). Briefly, after quiet rest in the supine position while breathing ambient air, subjects performed a maximal inhalation of pure O_2 coming from a Douglas bag, followed by a 5-s breath-hold and a slow maximal exhalation. Inspiratory and expiratory flows were measured at the mouth with an ultrasonic flowmeter (Spiroson; ECO MEDICS AG, Duernten, Switzerland) calibrated with a 3-l syringe. The fraction of O_2 was obtained continuously at the mouth by means of a paramagnetic analyser (O2100C module, BIOPAC Systems, Goleta, CA, USA). All signals were collected and sampled at 200 Hz (MP150 system with AcqKnowledge acquisition and analysis software; BIOPAC Systems, Goleta, CA, USA) and stored on a personal computer for subsequent analysis. TLC was calculated from the principle of O_2 mass conservation in the alveolar space by knowing the volume of O_2 inhaled, net of the losses in the instrumental and anatomical dead spaces, and the pre- and post-manoeuvre alveolar O_2 fraction (Chapter 4).

V_B and Hb_{mass} were measured with the CO re-breathing method, using a fully automated, high-precision system (OpCO, Detalo, Denmark) (Breenfeldt Andersen et al. 2018; Fagoni et al. 2018). Briefly, a 20-gauge catheter placed in the antecubital vein and after 10 minutes of rest, subjects were connected to a closed breathing circuit flushed with 100% O_2 for 4 minutes. In the 3rd minute, a 2-ml venous blood sample was analysed for percent carboxyhaemoglobin (%HbCO) and haemoglobin concentration (GEM4000, Instrumentation Laboratory, Bedford, USA). Then, the circuit was switched into rebreathing mode and a bolus of1.5 ml·kg^{-1} of 99.997% chemically pure CO (CO N47, Air Liquide, Pullach, Germany) was administered into the circuit. After 10 min of re-breathing, an additional 2-ml venous blood sample was obtained and analysed for %HbCO. Hb_{mass} was determined from the ratio of CO inhaled and the change in %HbCO. V_B was derived from Hb_{mass} and haemoglobin concentration.

Experimental trials

Trials were performed at sea level in a 50-m long, 2- to 2.5-m deep indoor Olympic swimming pool (Bella Italia village, Lignano Sabbiadoro, UD, Italy). Water temperature was 26.9 ± 0.5 °C, ambient air temperature was 27.3 ± 1.0 °C, barometric pressure and relative humidity were 764 ± 2 mmHg and 59 ± 6 %, respectively.

Moreover, subjects were instrumented with eight 3-D inertial measurement units (IMU, WaveTrack, Cometa Systems, Bareggio, Italy); each IMU, which operated independently of the others, was positioned according to a specific configuration (Figure 1), to evaluate whole-body kinematics via a simplified model (Nikodelis et al. 2013). The 8 IMU sensors were switched on, synchronized and zeroed in the neutral posture just before immersion.

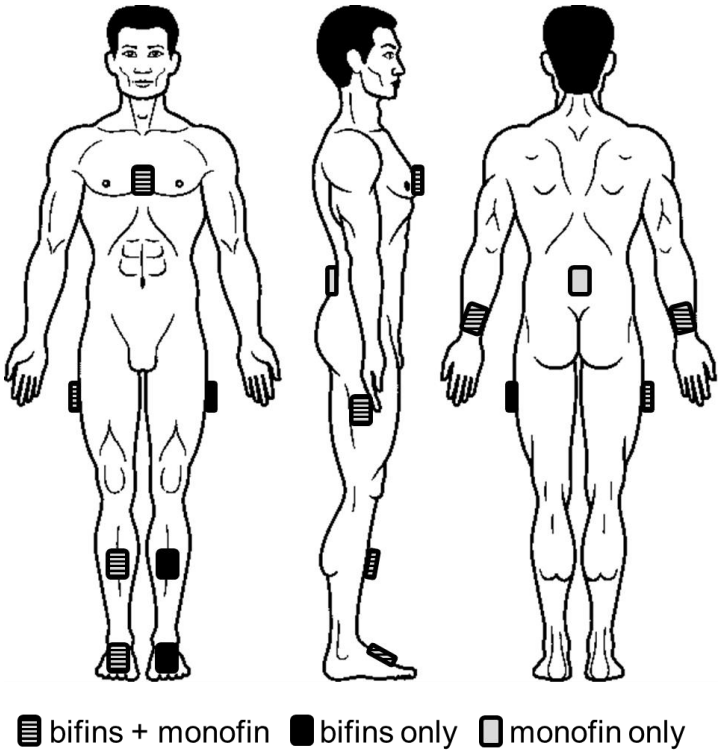

Figure 1. Configuration of the IMU sensors.

Then subjects entered in the pool up to the neck standing on a removable platform and rested for 6 minutes wearing a face mask connected to an breath-by-breath metabolic cart (Quark CPET, COSMED, Rome, Italy) consisting of a zirconium O_2 analyser, an infrared CO_2 meter, and a turbine flowmeter, all carefully calibrated following manufacturer instructions. Oxygen consumption at rest ($\dot{V}O_{2R}$) was calculated from the average of the last 3 minutes.

Then, face mask was removed, subjects wore a noseclip and performed their usual pre-dive routine, consisting of some deep, slow respiratory acts ending with a maximal inspiration, and started the DA. Glossopharyngeal insufflation was not allowed in order to keep the starting lung volume as close as possible to the preliminarily measured TLC. In order to assess pre-apnoea values, a "time zero" DA was first performed. Investigated DA distances were 50 m (all subjects) and 100 m (6 subjects), administered in random order. These distances were always lower than 80% of the subject's personal best. Subjects were instructed to keep the same swimming technique and speed they use in competitions.

At the end of each trial, as well as after the maximal inspiratory manoeuvre in "time zero" DA, noseclip was quickly removed, subjects were again fitted with the face mask connected to the metabolic cart and were carefully instructed to perform a maximal exhalation. Simultaneously, both earlobes were exposed, dried and warmed with a heated towel: one was immediately connected to a pulse oximeter (Nonin Medical, Plymouth, MN, USA) to assess the nadir in peripheral O_2 saturation (SpO_2), the other was used to obtain serial 10 µl capillary blood samples at minute 1, 3, 5, and 7 of recovery to assess peak lactate concentration [La] with an electro-enzymatic method (Lactate Pro, Arkray, Japan). Alveolar O_2 and CO_2 fractions (F_AO_2 and F_ACO_2) were calculated from the maximal expiration at the end of each DA as the average of the phase III (plateau) of the gas fractions raw data sampled at 100 Hz by the metabolic cart. Alveolar O_2 and

CO_2 partial pressures (P_AO_2 and P_ACO_2) were calculated. Oxygen consumption ($\dot{V}O_2$) was then measured breath-by breath during spontaneous breathing for the subsequent 6 minutes of recovery. Total DA duration (t_{DA}) was recorded from the start of the apnoea to face emersion, while extra non-DA breath-hold time was recorded from face emersion to the beginning of the first inspiration, i.e. including the maximal exhalation manoeuvre.

Metabolic data treatment

Post-DA $\dot{V}O_2$ kinetics were modelled with a monoexponential function projecting towards $\dot{V}O_{2R}$, excluding the first 1-3 breaths (Liner and Linnarsson 1994). Excess oxygen consumption was calculated as the time integral above $\dot{V}O_{2R}$ from 0 (the end of the maximal exhalation upon emersion) to $+\infty$. Since during recovery subjects were heavily breathing with the thorax still submerged, the O_2 cost of breathing during water immersion, 3.5 ml per each l of increase in expired ventilation above resting values (Held and Pendergast 2013) was calculated and subtracted, therefore obtaining the oxygen debt contracted during the whole apnoeic time ($DebO_2$). E_{La} was computed multiplying the net increase of [La] by the energetic equivalent of blood lactate accumulation (3 ml O_2 mM^{-1} kg^{-1}) (di Prampero and Ferretti 1999). To obtain total energy expenditure (E_{tot}), E_{La} was added to $DebO_2$. To account for the dynamic part of the apnoea only, a correction coefficient for E_{tot} was included, therefore C of DA was calculated as:

$$C = \frac{E_{tot} \cdot \frac{t_{DA}}{t_{DA} + t_E} - \dot{V}O_{2R} \cdot t_{DA}}{d} \quad (5.1)$$

where t_{DA} is the duration of the DA before emersion, t_E is the time between emersion and the first inspiration (i.e., the time for approaching the lateral border of the pool and completing the maximal exhalation), and d is the swimming distance. An equivalent of 20.9 kJ per l of O_2 (corresponding to a respiratory quotient of 0.96) was used (Zamparo et al. 2006). Then E_{tot} was partitioned in its three components: E_{O2}, E_{PCr} and E_{La}.

E_{O2} was calculated as the sum of the changes in alveolar (ΔV_AO_2), blood (ΔV_BO_2), and muscle O_2 stores (ΔMbO_2), all expressed in ml of O_2 standard pressure and temperature, dry (STPD). Alveolar volume was obtained subtracting from TLC the anatomic dead space (V_D) estimated from predictive equations (Hart et al. 1963). ΔV_AO_2 was calculated taking into account both F_AO_2 changes and the lung shrinkage predicted by assuming constant alveolar N_2 content during breath holding (Otis et al. 1948):

$$\Delta V_AO_2 = (TLC - V_D)\ \left(F_AO_{2(pre)} - F_AO_{2(post)}\ F_AN_{2(pre)}/F_AN_{2(post)}\right) \quad (5.2)$$

ΔV_BO_2 was calculated as the changes in both oxyhaemoglobin (ΔHbO_2) and the O_2 physically dissolved in blood (ΔBO_2). For this purpose, change in mixed venous oxyhaemoglobin saturation (SvO_2) was considered equal to that in SpO_2, conservatively assuming constant arteriovenous oxygen difference:

$$\Delta HbO_2 = 1.34 \cdot Hb_{mass} \cdot \Delta SpO_2 \quad (5.3)$$

where Hb_{mass} is expressed in grams and 1.34 is the Hb O_2 carrying capacity in ml g^{-1}. For ΔBO_2, the change in P_AO_2 (ΔP_AO_2) was considered a proxy of the change in arterial O_2 partial pressure, while for O_2 venous partial pressure we assumed a pre-apnoea value of 40 mmHg and calculated

the post-apnoea value after the Hill equation (Baumann et al. 1987) assuming a final SvO_2 of 75% minus ΔSpO_2 and arterial and venous blood volume fractions 0.25 and 0.75, respectively (Lindholm and Linnarsson 2002).

$$\Delta BO_2 = 3 \cdot 10^{-5} \cdot V_B \left\{ 0.25\, \Delta P_A O_2 + 0.75 \left[40 - 27 \left(\frac{0.75 - \Delta SpO_2}{0.25 + \Delta SpO_2} \right)^{\frac{1}{2.8}} \right] \right\} \quad (5.4)$$

where $3\ 10^{-5}$ is the solubility coefficient of O_2 in blood in ml $mmHg^{-1}$, 27 the p50 of venous Hb in mmHg, and 2.8 the Hill coefficient.

Total myoglobin mass was estimated 5 g kg^{-1} of wet muscle (Jansson et al. 1982) with a relative muscle mass of 40% (Frontera and Ochala 2015). We assumed an overall desaturation of 30%, typical of light exercise (Richardson et al. 1995; Molé et al. 1999), at 50m, while a desaturation of 40% at 100 m due to the increased effect of hypoxia and lactate (Richardson et al. 1995; Giardina et al. 1996):

$$\Delta MbO_2 = 5 \cdot 0.4\, m \cdot 1.34 \cdot [0.3(50\text{m});\ 0.4(100\text{m})] \quad (5.5)$$

where *m* is the body mass in kg and 1.34 the myoglobin O_2 carrying capacity in ml g^{-1}. Since O_2 stores depletion play a major role in DA performance, also the gross (i.e., including $\dot{V}O_{2R}$) aerobic-only cost of apnoeic swimming per unit of distance was calculated as E_{O2}/d.

Finally, E_{PCr} was calculated by subtraction:

$$E_{PCr} = E_{tot} - E_{La} - E_{O_2} = DebO_2 - E_{O_2} \quad (5.6)$$

Biomechanical data treatment

Kinematic data were acquired by means of a dedicated software (EMG and Motion Tools 5.0, Cometa Systems, Bareggio, Italy) and exported in c3d standard format for further analyses performed via a custom software (MATLAB R2020a, MathWorks, Natick, MA, USA). From the three-dimensional acceleration values measured at fin level we estimated on average time, the number of strokes, the stroke duration, the stroke rate, and the average time lag when considering bi-fin by means of cross-correlation function. Furthermore, we were able to estimate several additional parameters, according to Nikodelis et al. (2013); in particular, estimating each joint flexion-extension angle as the corresponding helical axis angle identified between the proximal and distal reference systems associated to each segment (with the pelvis movement assessed with respect to the initial position), we performed:

- analysis of peak to peak amplitude, used as an index to describe the mobility of each specific joint;
- autocorrelation analysis, used to underline the periodicity of the joint movement;
- spectral analysis, used to investigate the frequency content with possible functional significance;
- relative phase analysis between the two consecutive joints, to underline possible synergies; relative phase was estimated by considering the phase angle Φ starting from the values corresponding to the angular position α and the angular velocity $\dot{\alpha}$ via the formula $\Phi = \tan^{-1}(\dot{\alpha}/\alpha)$, and then subtracting the phase angle of the proximal from that of the distal joint.

Statistical analysis

Paired and unpaired t test was used to assess differences between conditions. Linear regression analysis was used to correlate pertinent variables. Statistical significance threshold set at $p = 0.05$. To assess reliability of C estimation between the 50 and 100 m trial s, intraclass correlation coefficient (ICC) was calculated with a two-way mixed-effect, single measures model (Shrout and Fleiss 1979).

5.4 Results

Preliminary measurements are shown in **Table 1**. Three subjects did not perform Hb_{mass} measurement due to logistic reasons and were excluded from fractional energy contribution analysis. Table 2 shows data measured at 50 m in all subjects. The differences between monofin and bi-fins were analysed in the 50 m DA, where sample size was greater (**Table 2**). As expected, monofin showed lower C compared to bi-fins (0.42 ± 0.9 vs 0.63 ± 0.13 kJ m^{-1}, $p < 0.01$). Moreover, there was no difference in fractional composition of the energy balance between fin types. Therefore all 6 subjects who completed the both 50 and 100 m DA were grouped in **Table 2** to assess differences in energy balance between 50 m and 100 m. ΔV_AO_2 remained always the major oxygen stores, however increasing d its relative contribution significantly decreased in favour of ΔV_BO_2.With increasing d there was a significant reduction both E_{O2}/E_{tot} and E_{O2}/d, an increase in E_{La}/E_{tot}, while E_{PCr}/E_{tot} slightly decreased. Fractional energy contribution is summarized also in **Figure 2** as well, with a linear extrapolation up to 200 m.

There was good agreement in C between the 50 and the 100 m trials (ICC = 0.79). C, either gross or net, was not significantly inversely related the personal best. However, personal best was significantly inversely proportional to E_{O2}/d (i.e., directly proportional to its reciprocal, d/E_{O2}, the distance covered per l of O_2 depleted) both when assessed from the 50 m trial ($R^2 = 0.67$) and from the longest DA performed ($R^2 = 0.78$) (**Figure 3**).

Average finstroke rate was 54 ± 11 cycles min^{-1}, being 49 ± 14 for bi-fins and 37 ± 8 for monofin ($p = 0.13$). There was no significant relationship between finstroke rate and C. Peak to peak amplitude is shown different finstroke patterns (**Figure 4)**. These pattern influence also autocorrelation (**Figure 5)**, phase plots (**Figure 6**), relative phase (**Figure 7**) and power spectrum of frequencies (**Figure 8**). There was not a clear relationship between kinematic patterns and C.

Table 1. Preliminary and 0-m apnoeas measurements values.

	All (n = 12)	**Bi-fins (n = 7)**	**Monofin (n=5)**
TLC (l)	7.0 ± 1.1	7.1 ± 1.3	6.8 ± 0.7
HB_{mass} (g)	791 ± 129 (n = 9)	805 ± 73 (n = 6)	764 ± 228 (n = 3)
V_B (l)	5.2 ± 0.5	5.2 ± 0.3	5.1 ± 0.9
$\dot{V}O_{2R}$ (ml kg^{-1} min^{-1})	5.2 ± 1.0	5.6 ± 0.6	4.7 ± 1.2
P_AO_2 (mmHg)	122 ± 6	119 ± 3	127 ± 7 *
P_ACO_2 (mmHg)	30 ± 6	31 ± 7	29 ± 5
SpO_2	100 ± 0%	100 ± 0%	100 ± 0%
[La] (mM)	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1

* $p < 0.05$ vs bi-fins

Table 2. Measurements and calculations for the 50 m trial.

50 m	**All (n = 12)**	**Bi-fins (n = 7)**	**Monofin (n=5)**
Speed (m s^{-1})	1.07 ± 0.20	1.03 ± 0.13	1.13 ± 0.28
P_AO_2 (mmHg)	56 ± 9	52 ± 8	60 ± 8
P_ACO_2 (mmHg)	49 ± 6	50 ± 7	48 ± 4
SpO_2	89 ± 4%	88 ± 4%	91 ± 4%
[La] (mM)	2.2 ± 1.4	2.6 ± 1.0	1.3 ± 0.3 *
$DebO_2$ (ml)	1522 ± 365	1695 ± 371	1280 ± 185*
E_{tot} (ml O_2)	1749 ± 429	2028 ± 316	1357 ± 169 **
C (kJ m^{-1})	0.54 ± 0.15	0.63 ± 0.13	0.42 ± 0.8 **
C (J kg^{-1} m^{-1})	7.2 ± 1.9	5.9 ± 1.3	8.1 ± 1.7 *
E_{O2} (ml O_2)	760 ± 182 (n = 9)	799 ± 193 (n = 6)	682 ± 159 (n = 3)
E_{O2}/E_{tot}	43 ± 11% (n = 9)	39 ± 9% (n = 6)	49 ± 14% (n = 3)
E_{O2}/d (ml O_2 m^{-1})	15 ± 4 (n = 9)	16 ± 4 (n = 6)	14 ± 3 (n = 3)
ΔV_AO_2 (ml O_2)	575 ± 129	582 ± 138	564 ± 131
$\Delta V_AO_2/E_{O2}$	77 ± 4% (n = 9)	76 ± 4% (n = 6)	80 ± 3% (n = 3)
ΔV_BO_2 (ml O_2)	115 ± 58 (n = 9)	132 ± 61 (n = 6)	82 ± 41 (n = 3)
$\Delta V_BO_2/E_{O2}$	15 ± 5% (n = 9)	16 ± 5% (n = 6)	11 ± 4% (n = 3)
ΔMbO_2 (ml O_2)	61 ± 7	63 ± 9	58 ± 5
$\Delta MbO_2/E_{O2}$	8 ± 1% (n = 9)	8 ± 2% (n = 6)	9 ± 2% (n = 3)
E_{La} (ml O_2)	226 ± 202	333 ± 201	77 ± 63 *
E_{La}/E_{tot}	12 ± 10%	17 ± 11%	6 ± 5%
E_{PCr} (ml O_2)	872 ± 364 (n = 9)	989 ± 349 (n = 6)	638 ± 299 (n = 3)
E_{PCr}/E_{tot}	47 ± 13% (n = 9)	48 ± 12% (n = 6)	44 ± 18% (n = 3)

Table 3. Paired comparison between the 50 m and the 100 m DA.

	50 m (n = 6)	**100m (n = 6)**
Speed ($m\ s^{-1}$)	1.05 ± 0.11	1.09 ± 0.07
P_AO_2 (mmHg)	61 ± 7	36 ± 6 **
P_ACO_2 (mmHg)	47 ± 6	50 ± 5 **
SpO_2	92 ± 2%	77 ± 4% **
[La] (mM)	1.8 ± 0.4	3.8 ± 1.0 *
$DebO_2$ (ml)	1463 ± 239	2513 ± 436 **
E_{tot} (ml O_2)	1620 ± 312	3123 ± 634 **
C ($kJ\ m^{-1}$)	0.50 ± 0.11	0.51 ± 0.11
C (J kg-1 m-1)	6.7 ± 1.0	6.8 ± 1.3
E_{O2} (ml O_2)	675 ± 101	1051 ± 140 **
E_{O2}/Etot	43 ± 11%	35% ± 10% *
E_{O2}/d (ml $O_2\ m^{-1}$)	14 ± 2	11 ± 1 **
ΔV_AO_2 (ml O_2)	531 ± 78	724 ± 82 **
$\Delta V_AO_2/E_{O2}$	79 ± 3%	69 ± 5% **
ΔV_BO_2 (ml O_2)	84 ± 29	247 ± 80 **
$\Delta V_BO_2/E_{O2}$	12% ± 3%	23% ± 6% **
ΔMbO_2 (ml O_2)	60 ± 7	80 ± 10 **
$\Delta MbO_2/E_{O2}$	9% ± 1%	8% ± 1% **
E_{La} (ml O_2)	157 ± 96	609 ± 235 **
E_{La}/E_{tot}	9 ± 4%	19 ± 4% **
E_{La}/d (ml $O_2\ m^{-1}$)	3 ± 2	6 ± 2 **
E_{PCr} (ml O_2)	788 ± 280	1463 ± 513 **
E_{PCr}/E_{tot}	48 ± 12%	46 ± 10%
E_{PCr}/d (ml $O_2\ m^{-1}$)	16 ± 6	15 ± 5

* $p < 0.05$ vs 50 m, ** $p < 0.01$ vs 50 m

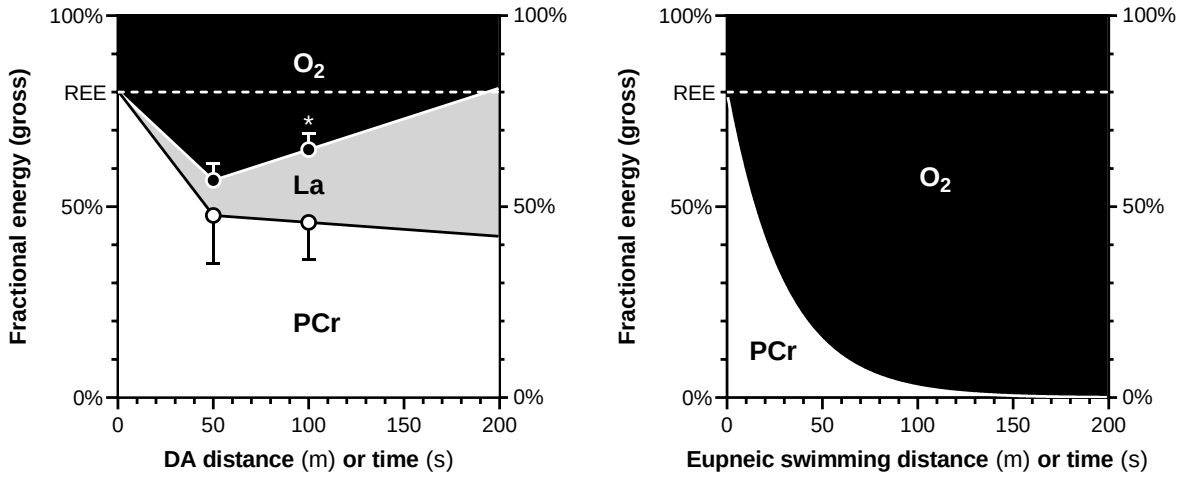

Figure 2. The fractional gross energy contributions as a function of distance are stacked together, assuming fully aerobic pre-apnoea resting energy metabolism (REE, dashed line), which approximately accounts for 20% of total exercise energy metabolism. Since average speed was close to 1 m s^{-1}, distance and time axis have the same numerical values. **A)** Dynamic apnoeas (DA) with experimental points shown. Backward extrapolation was performed resting values. Forward extrapolation was performed by linear regression of experimental points. **A)** The theoretical balance during submaximal eupnoeic swimming at 1 m s^{-1}. O_2, aerobic metabolism; La, lactate metabolism; PCr, phospho-creatine metabolism; *, significantly different from 50 m.

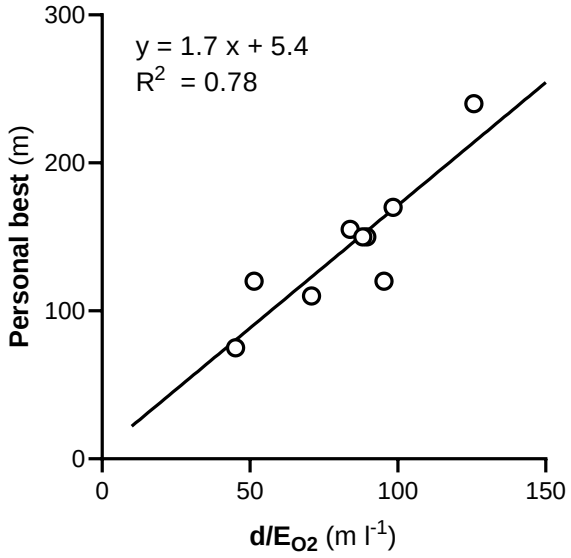

Figure 3. Personal best in dynamic apnoea with fins, expressed as the maximal DA distance, as a function of the reciprocal of the aerobic portion of the gross energy cost of underwater swimming (d/E_{O2}) assessed during the longest distance covered in the experiments.

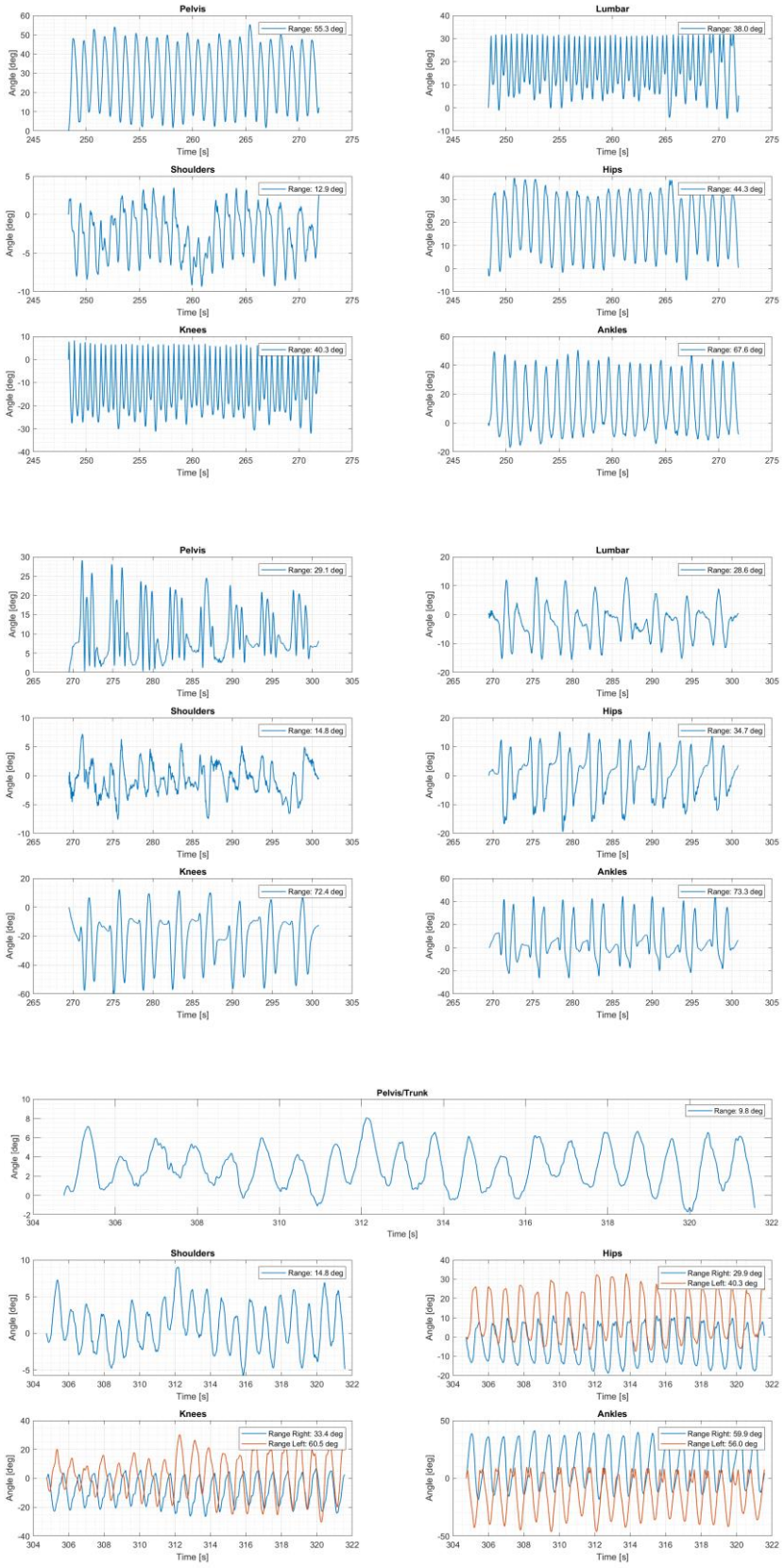

Figure 4. Angular position: comparison between monofin with constant stroke rate (upper panel), monofin with "kick and glide" technique (middle panel), and bi-fins (lower panel) from three representative subjects.

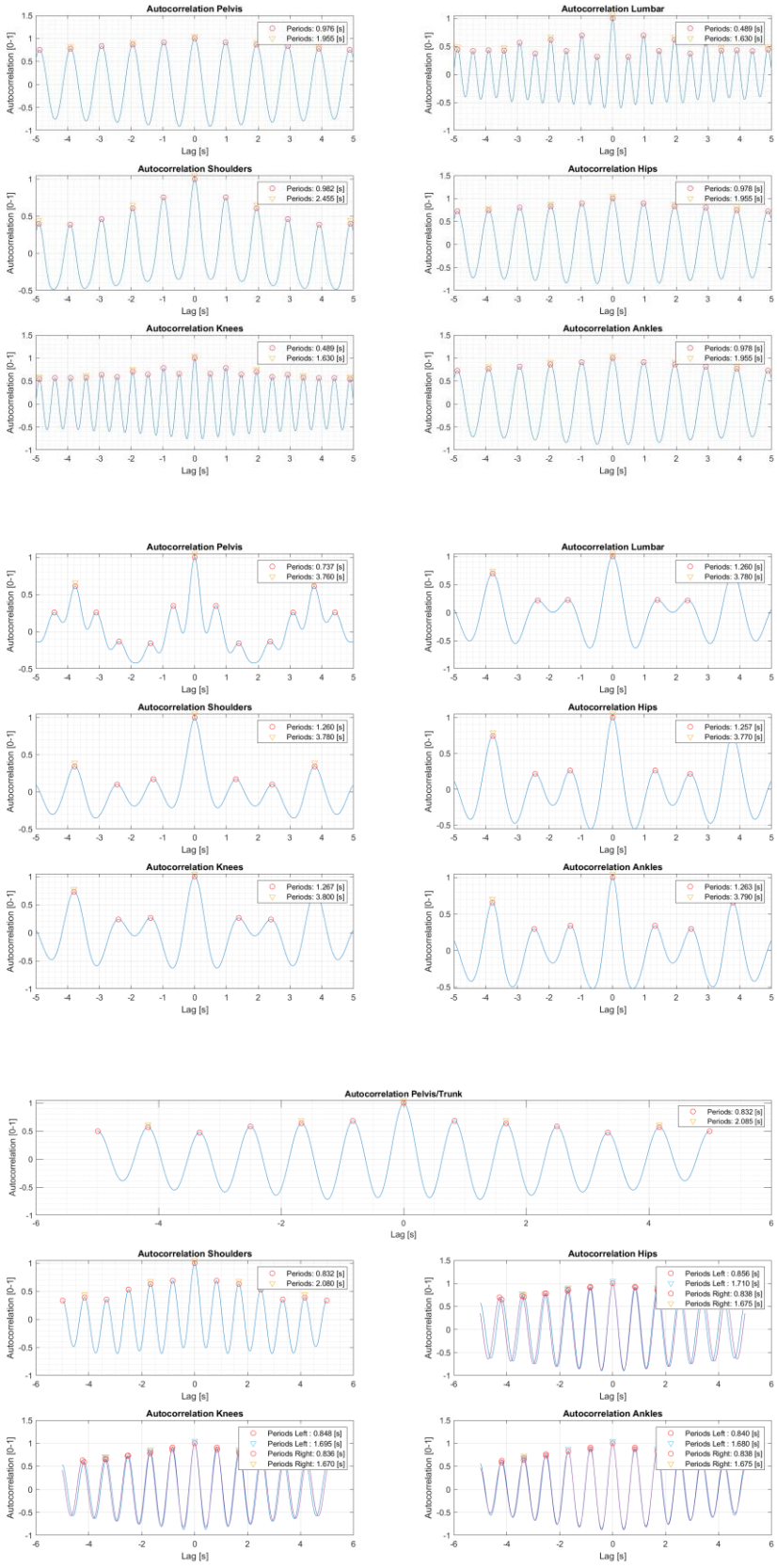

Figure 5. Autocorrelation analysis: comparison between monofin with constant stroke rate (upper panel), monofin with “kick and glide” technique (middle panel), and bi-fins (lower panel) from three representative subjects.

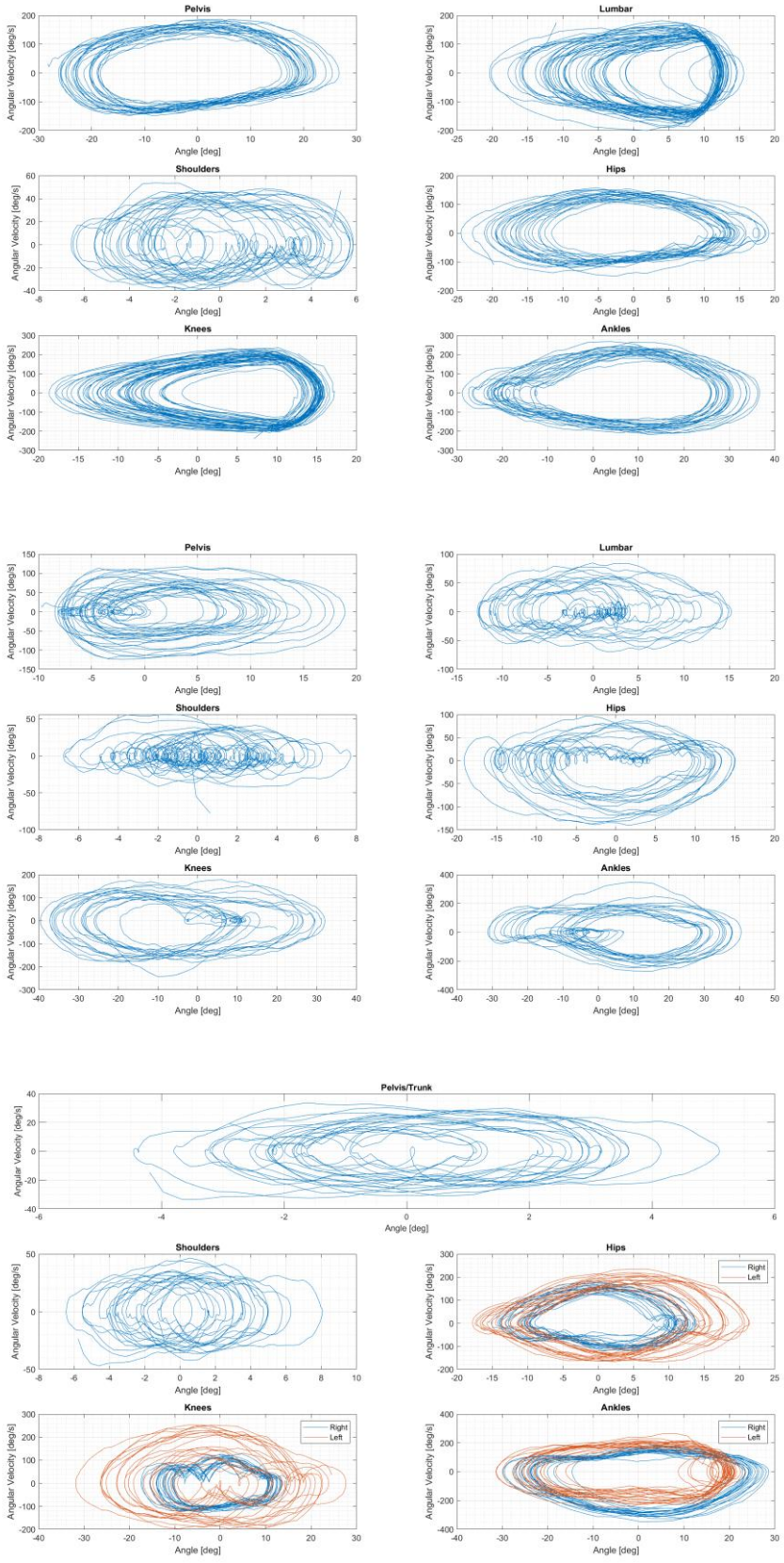

Figure 6. Phase plots: comparison between monofin with constant stroke rate (upper panel), monofin with "kick and glide" technique (middle panel), and bi-fins (lower panel) from three representative subjects.

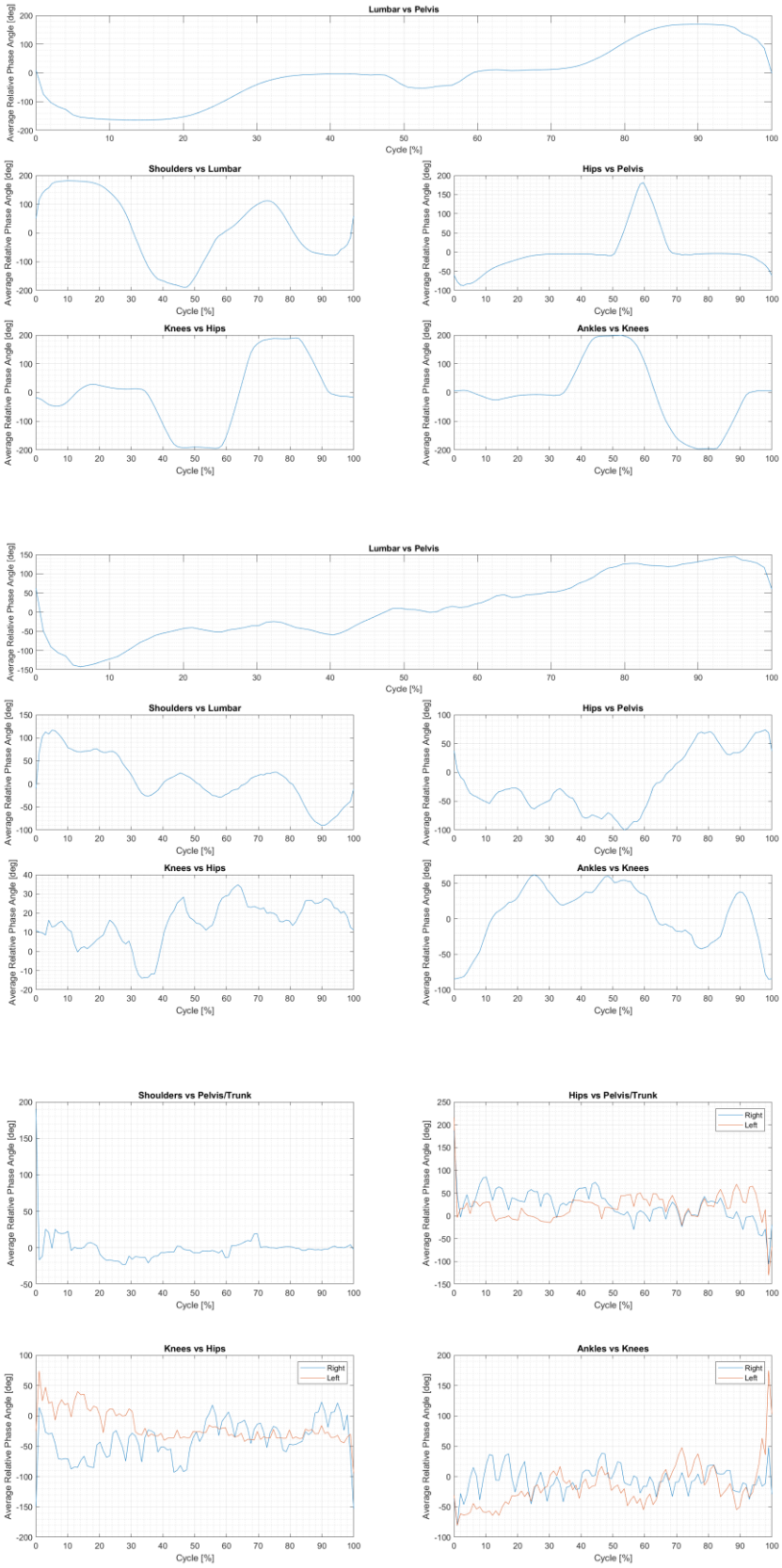

Figure 7. Relative phase analysis: comparison between monofin with constant stroke rate (upper panel), monofin with "kick and glide" technique (middle panel), and bi-fins (lower panel) from three representative subjects.

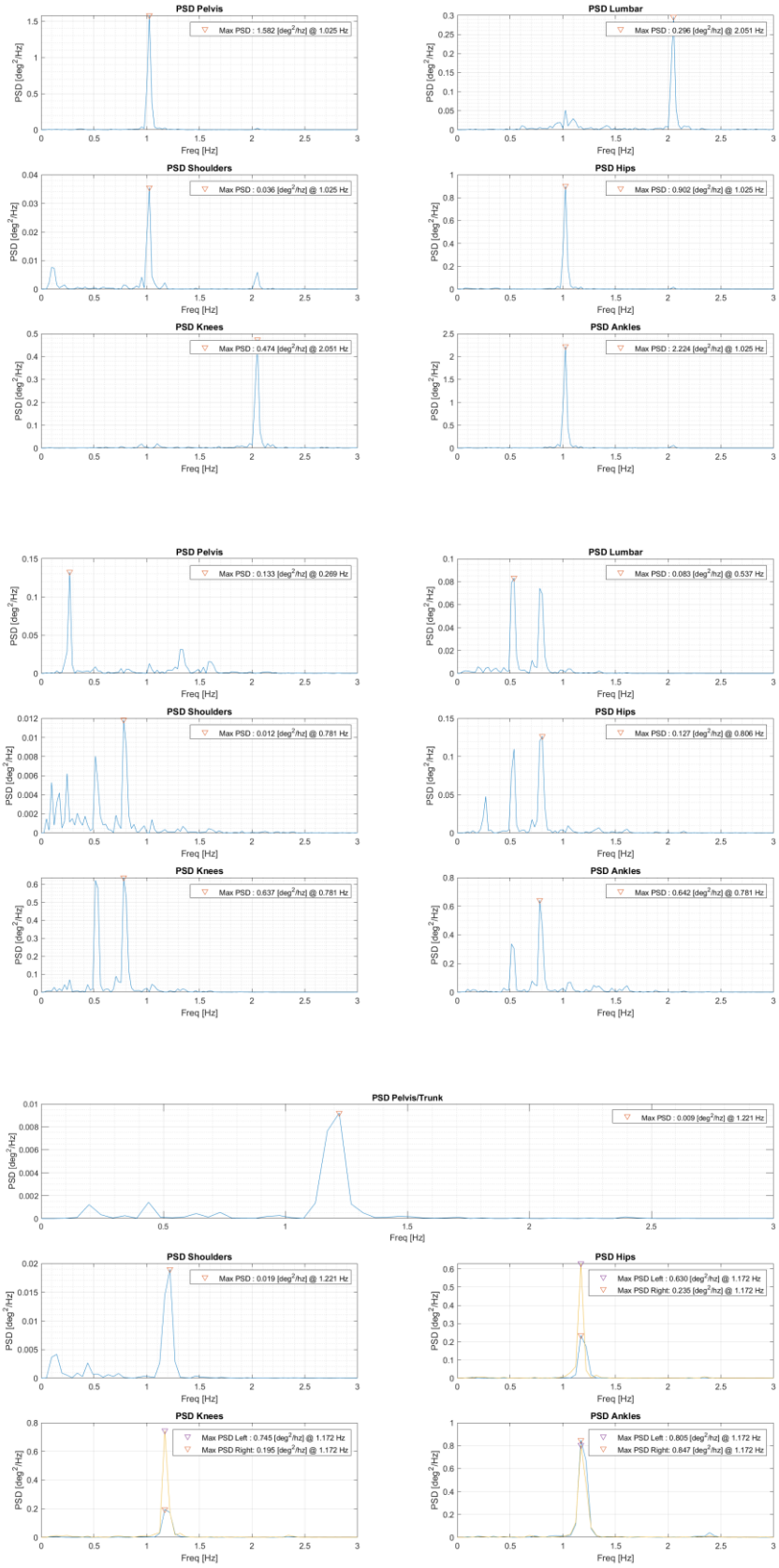

Figure 8. Power spectrum of frequencuies: comparison between monofin with constant stroke rate (upper panel), monofin with “kick and glide” technique (middle panel), and bi-fins (lower panel) from three representative subjects.

5.5 Discussion

This study demonstrated for the first time the feasibility of the bioenergetic approach to study C during DA. Reliability in C measurements was high between 50 and 100 m. Therefore, it seems that the turning manoeuvre had, on average, no effect on C, although individual differences may still occur, being an advantage for some athletes and an hindrance for others (Schagatay 2010). Our C estimates are comparable, though slightly higher, to those measured during surface eupnoeic swimming with fins at 1 m s^{-1} (Zamparo et al. 2006, p 464, Fig. 2): 5.9 vs 5.2 J m^{-1} kg^{-1} for monofin and 8.1 vs 7.4 J m^{-1} kg^{-1} for bi-fins. These differences could be explained by the additional drag imposed by complete water immersion during DA. In fact, our bi-fins values are lower than those reported by Pendergast et al. (2005) during underwater eupnoeic swimming with fins at 1 m s^{-1} (0.63 vs 0.89 J m^{-1}). Surprisingly, C was not able to predict the personal best of our subjects. Since C is an estimator of the economy of the movement, it correctly disregards $\dot{V}O_{2R}$. However, $\dot{V}O_{2R}$ represents an important fraction of the total energy demand during DA, since even water immersion at 27° C with wetsuit can increase $\dot{V}O_{2R}$ (Shiraki et al. 1986). Therefore, gross C, in particular its aerobic fraction (E_{O2}/d), may limit performance more than C alone. In fact, d/E_{O2} turned out a great predictor of maximal performance (Figure 3), stressing again the importance of O_2 conserving mechanisms and strategies. This is evident observing that E_{O2}/E_{tot}, after a sharp initial increase similar to eupnoeic metabolic transient, seems reaching a peak and then start to decrease (Figure 2).

Specularly, E_{PCr}/E_{tot}, decreases rapidly at the beginning, then almost imperceptibly, and the remainder of the energy demand is therefore fulfilled by lactate metabolism. Capillary [La] increased approximately by 1 mM above resting values every 50 m, and E_{La} fractional contribution increased linearly with swimming distance. This occurs despite total metabolic power, expressed in oxygen equivalent, was on average 27 ml kg^{-1} min^{-1}, corresponding to 70% of the maximal oxygen consumption ($\dot{V}O_{2max}$) predicted by standard equations (de Souza e Silva et al. 2018) and probably to a lower fraction of the actual $\dot{V}O_{2max}$ of this athletic cohort. Therefore, the increase in [La] could be attributed to both a reflex peripheral vasoconstriction (the diving response) and to an "early lactate" phenomenon (Cerretelli et al. 1979), typical of intense exercise. Moreover, early lactate and diving response can be interdependent, since early lactate can appear at lower exercise intensities when oxygen delivery is impaired (Lador et al. 2013). Traditionally, E_{La} was believed the most important anaerobic energy source in DA, while E_{PCr} contribution was considered small (Schagatay 2010). Our findings overturn this belief: anaerobic alactic energy contribution plays a major role in DA performance, as previously hypothesised by Pendergast (1987), representing up to 45% of total energy supply (Figure 2). This is not surprising given that PCr represents the obligatory component of the oxygen deficit during exercise transients (di Prampero and Margaria 1968).

It must be stressed that our E_{PCr} estimates are highly dependent on E_{O2} (Equation 5.6), which relies on multiple a priori assumptions, notably the fall of venous O_2 content assumed equal to ΔSpO_2, *i.e.*, constant arteriovenous oxygen difference. This is correct in apnoeas initiated only after 3-4 minutes of moderate exercise, where steady-state pre-apnoea SvO_2 are already much lower than at rest (Lindholm and Linnarsson 2002). But, when apnoea and exercise are initiated together, the fall SpO_2 could have been greater than that in SpO_2. Therefore, E_{O2} could have been

underestimated and E_{PCr} specularly overestimated. Nevertheless, also assuming constant SvO_2 decrease, E_{PCr} would be still more than one third of total energy supply. Moreover, a predominance of E_{PCr} over E_{La} appears convenient in term of acid-balance equilibrium since, contrary to the latter, it is associated with a decrease in pH (Wasserman et al. 1997). Increasing the relative contribution of E_{PCr} in place of E_{La} could be therefore related with increased DA performances. The lower peak lactate concentration after a standardized DA in trained breath-hold divers (Joulia et al. 2002, 2003) points toward this direction. In this context, a low time constant of $\dot{V}O_2$ kinetics at exercise onset could be detrimental by decreasing E_{PCr} metabolism (Ferretti et al. 2017) in favour to an early erosion of the precious O_2 stores. This could explain why the relationship between aerobic fitness and DA performance remains elusive (Schagatay 2010).

For the first time were able to identify several kinematic patterns of DA, however linking those patterns with performance is not possible within the current study. It is known in fact that inter-individual differences in swimming technique are high (Figueiredo et al. 2012) and that overall efficiency does rely on any specific kinematic parameter, but seems to depend on the body movement as a whole (von Loebbecke et al. 2009). Several limitations must be acknowledged, above all, the small sample size, which prevented a deeper analysis of the biomechanical determinants of C and the relationship of C with maximal personal performances. Other notable limitations are the absence of direct measurements of venous O_2 content, which accounts for a substantial proportion of total O_2 stores, and the use of the breath-by breath technique to compute $DebO_2$ after emersion, which may have resented of significant fluctuations in tidal volumes and expired gas concentrations outside usual ranges.

In conclusion, C of DA seems halfway between that of eupnoeic underwater and surface swimming. The distance covered per l of O_2 depleted, but not C, was able to predict performances. Anaerobic alactic metabolism plays a major role in the energetic balance during DA.

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6 General conclusions

6.1 Final summary

Despite breath-hold diving is a widespread and long standing human physical activity, little experimental data are present on its energetics and biomechanics (Chapter 1). However, recent developments in wearable sensor technology, especially in the field of inertia measurement units, have the potential to fill this knowledge gap (Chapter 2). It is well known that water exerts a great resistance to water movement, and that an efficient locomotion plays a major role in every sport, as well as in breath-hold diving performance. First, in this thesis we demonstrated that in a controlled underwater environment, internal work of cycling increased with the third power of speed (Chapter 3). Then, since total lung capacity is a major determinant of breath-hold performance, we developed and validate a portable technique to assess it, with encouraging results (Chapter 4). Finally, we were able to assess bioenergetics and biomechanical data during dynamic apnoeas. (Chapter 5). For the first time, we were able to identify several kinematic patterns of apnoeic swimming, however their relationship with performances was elusive. This confirm that, as for surface swimming, inter-individual differences in swimming technique are high and overall efficiency does rely on any specific kinematic parameter but seems to depend on the body movement as a whole.

6.2 Impact

All these studies can have a major impact on the understanding of human physiology in unusual environments. The systematic review of Chapter 2, by describing the state-of-the art of wearable sensors applied to breath-hold diving, can help the researcher to properly design future studies in the field. The underwater cycling results of Chapter 3 expand current models of underwater biomechanics, as well as on the internal cost of locomotion in general, providing a useful formula of conversion of mechanical power output between dry and water cycling. The portable technique for measurement of total lung capacity described in Chapter 4 is useful for every research group interested in assessing lung volumes in resource-limited environments. The results of Chapter 5 have strong implications in dynamic apnoea physiology and training, arousing the awareness about the importance of anaerobic alactic metabolism.

6.3 Future directions

The approach of Chapter 3 can be used to study the internal work in other fluids with lower or higher densities than water. The technique to assess total lung capacity described in Chapter 4 can be subject to other validation studies involving different environmental settings (i.e., hypobaric and hyperbaric conditions) and different clinical populations (obstructive and restrictive lung disease patients). The setup can also be further miniaturized and automatized into a single marketable medical device. The protocol used un Chapter 5 can be replicated in a larger sample size and different conditions. First, we can manipulate horizontal swimming speed (0.6, 0.8, 1.0 and 1.2 m s^{-1}) in order to assess its effect on the estimated energy cost. Then, other freediving

discipline could be studied, such as constant weight apnoeas with fins, where subjects can perform a series of descents at pre-determined depths (20, 30 and 40 m) at their usual speed. Subsequently also the average vertical speed could be manipulated (0.4, 0.5, 0.6 and 0.7 m s^{-1}). Once established, this protocol could allow the evaluation of other diving activities such as those assisted by a self-contained underwater breathing apparatus (SCUBA). A study of the energetics of deep breath-hold diving and SCUBA diving would be of great interest for a more focused training, a safer occupational environment, and to better understand human limits.