Title

The anaerobic threshold: 50+ years of controversy

Journal

The Journal of Physiology, 599(3)

ISSN

0022-3751

Authors

Poole, David C
Rossiter, Harry B
Brooks, George A
et al.

Publication Date

2021-02-01

DOI

10.1113/jp279963

Peer reviewed

J Physiol 0.0 (2020) pp 1–31

TOPICAL REVIEW

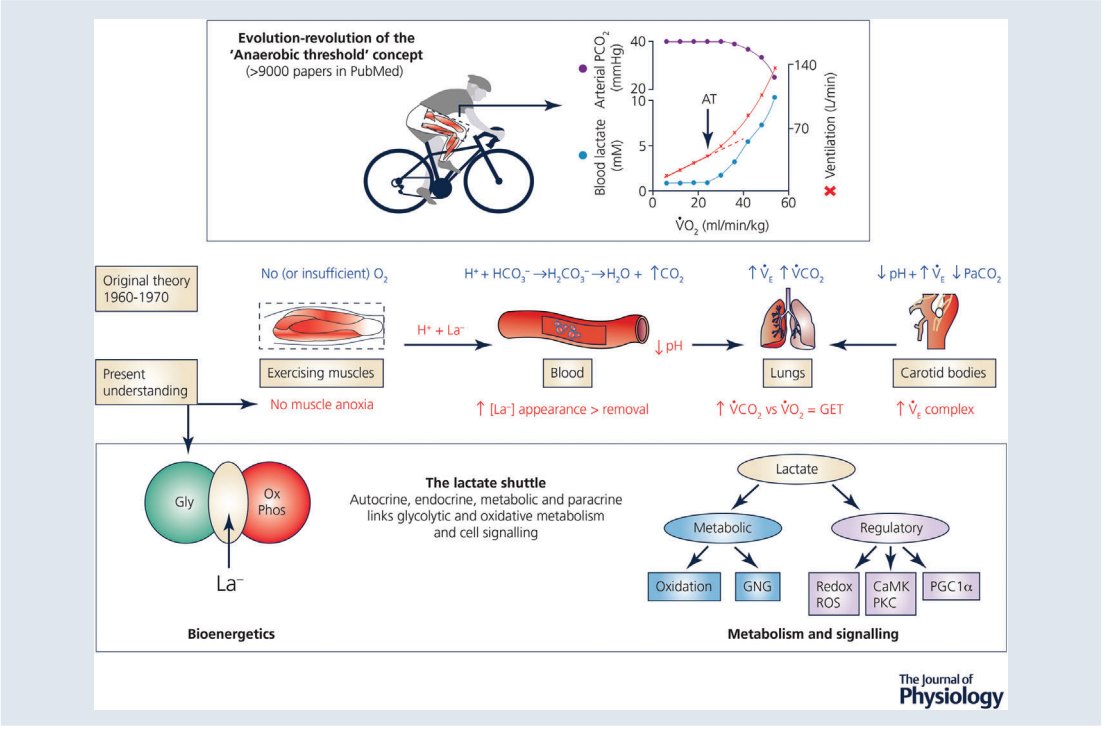
The anaerobic threshold: 50+ years of controversy

David C. Poole[1], Harry B. Rossiter[2], George A. Brooks[3] and L. Bruce Gladden[4]

[1]*Departments of Kinesiology and Anatomy and Physiology, Kansas State University, Manhattan, KS, USA*
[2]*Rehabilitation Clinical Trials Center, Division of Respiratory and Critical Care Physiology and Medicine, and The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA, USA*
[3]*Department of Integrative Biology, Exercise Physiology Laboratory, University of California, Berkeley, CA, USA*
[4]*School of Kinesiology, Auburn University, Auburn, AL, USA*

Edited by: Ian Forsythe & Michael Hogan

Linked articles: This article is highlighted in a Perspectives article by Hogan. To read this article, visit https://doi.org/10.1113/JP280980.



David C. Poole (left) is University Distinguished Professor and Coffman Chair in the Departments of Kinesiology, and Anatomy & Physiology, and co-director of the Clarenburg Cardiorespiratory Laboratory at Kansas State University. **Harry B. Rossiter** (second from left) is an Investigator at The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Centre and Professor at the David Geffen School of Medicine at University of California, Los Angeles (UCLA). **George A. Brooks** (third from left) is a Professor in the Department of Integrative Biology at the University of California, Berkeley and *Docteur Honoris Causa de l'Université Montpellier*. **L. Bruce Gladden** (right) is a Distinguished Professor of Education in the School of Kinesiology at Auburn University. Together they are interested in exercise bioenergetics, metabolic thresholds, lactate metabolism, exercise gas exchange and rapid changes in energy demand and supply upon onset of muscle contraction.

DOI: 10.1113/JP279963

Abstract The anaerobic threshold (AT) remains a widely recognized, and contentious, concept in exercise physiology and medicine. As conceived by Karlman Wasserman, the AT coalesced the increase of blood lactate concentration ($[La^-]$), during a progressive exercise test, with an excess pulmonary carbon dioxide output ($\dot{V}_{CO_2}$). Its principal tenets were: limiting oxygen (O_2) delivery to exercising muscle→increased glycolysis, La^- and H^+ production→decreased muscle and blood pH→with increased H^+ buffered by blood $[HCO_3^-]$→increased CO_2 release from blood→increased $\dot{V}_{CO_2}$ and pulmonary ventilation. This schema stimulated scientific scrutiny which challenged the fundamental premise that muscle anoxia was requisite for increased muscle and blood $[La^-]$. It is now recognized that insufficient O_2 is *not* the primary basis for lactataemia. Increased production and utilization of La^- represent the response to increased glycolytic flux elicited by increasing work rate, and determine the oxygen uptake ($\dot{V}_{O_2}$) at which La^- accumulates in the arterial blood (the lactate threshold; LT). However, the threshold for a sustained non-oxidative contribution to exercise energetics is the critical power, which occurs at a metabolic rate often far above the LT and separates heavy from very heavy/severe-intensity exercise. Lactate is now appreciated as a crucial energy source, major gluconeogenic precursor and signalling molecule but there is no *ipso facto* evidence for muscle dysoxia or anoxia. Non-invasive estimation of LT using the gas exchange threshold (non-linear increase of $\dot{V}_{CO_2}$ *versus* $\dot{V}_{O_2}$) remains important in exercise training and in the clinic, but its conceptual basis should now be understood in light of lactate shuttle biology.

(Received 29 June 2020; accepted after revision 16 October 2020; first published online 28 October 2020)
Corresponding author L. B. Gladden: School of Kinesiology, 301 Wire Road, Auburn University, Auburn, AL 36849-5323, USA. Email: gladdlb@auburn.edu

Abstract figure legend As originally posited and defended most ardently by Karlman Wasserman and Brian J. Whipp, the anaerobic threshold (AT) concept represents the epitome of integrative physiological control. The graph at the upper right portrays blood $[La^-]$, arterial CO_2 partial pressure (P_{CO_2}) and ventilation ($\dot{V}_E$) as a function of $\dot{V}_{O_2}$ increasing from rest to maximum ($\dot{V}_{O_2max}$). The AT is identified (black arrow) by the departure of $\dot{V}_E$ from linearity without P_{CO_2} decreasing. As shown rightwards from the 'Original theory' box, the AT held that lack of (or very low) muscle O_2, via the Pasteur effect, increased muscle and blood lactic acid, then H^+ dissociated from the lactic acid and was buffered by bicarbonate thus increasing CO_2 evolution from the blood; this stimulated the 'extra' $\dot{V}_E$ at AT simultaneous with the increase in $[La^-]$. Currently we understand that neither anoxia nor dysoxia underpins increased muscle La^- production and efflux, but rather its increase in the blood is attributed to the rate of La^- appearance being greater than disappearance. Also, as long appreciated, the variability in, and complexities of, the control of breathing dictate that the $\dot{V}_{CO_2}$ *versus* $\dot{V}_{O_2}$ relation (gas exchange threshold) more reliably approximates the lactate threshold than does the ventilatory profile. At the bottom, the modern understanding of metabolism is that La^- is a central element linking glycolysis (Gly) and oxidative phosphorylation (OxPhos) energetics. Via cell signalling, La^- also plays roles in short- and long-term adaptations in both health and disease.

Rationale and outline

This review is sequenced into four sections that are broadly informative on the fascinating exercise physiology paradigm known as the anaerobic threshold (AT). Whereas it may be read in its entirety we have allowed sufficient redundancy across sections to facilitate selective reading of individual sections at the reader's discretion. 'Introduction and brief history' presents the early discovery of lactate (La^-)/lactic acid in exercising muscles, development of a threshold concept and the presumption that increased blood La^- concentration ($[La^-]$) was *ipso facto* evidence for muscle anoxia/dysoxia. 'The lactate shuttle and the "anaerobic threshold"' details the rapid progression in our understanding of the biochemistry of La^- and its multivariate and complex roles as a major energetic substrate, signalling molecule and gluconeogenic precursor. Rather than a 'dead end product of metabolism' that was blamed for causing contractile dysfunction, La^- is now perceived as an integral biomarker of physiological and metabolic strain with the substantial therapeutic potential to mitigate morbidity. Lest the reader consider that these discoveries have made the AT concept passé, 'The gas exchange threshold: uses and limitations' takes the current non-invasive analogue of the lactate threshold (LT), specifically the gas exchange threshold (GET) and evaluates its present-day utility in sports, science and medicine. Despite discounting its erroneous mechanistic

basis in obligatory muscle anoxia/dysoxia this section demonstrates that both the LT and the GET retain substantial power to provide unique physiological insights. The final section, 'Common threshold concepts', addresses the utility of threshold concepts to partition discrete domains of exercise intensity. Against the backdrop of a confusion of such concepts and profligate nomenclature, the GET/LT is presented as usefully discriminating the moderate to heavy intensity exercise transition. However, it is the critical power (CP)/critical speed (CS), which constitutes the heavy–severe exercise intensity domain boundary, above which there is an obligatory non-oxidative energetic contribution to the exercise energetics. However, as for the LT, there is no necessity or compelling evidence to support the presence of muscle anoxia/dysoxia even for severe intensity exercise (i.e. >CP/CS).

Introduction and brief history

Definition of 'threshold': 'The magnitude or intensity that must be exceeded for a certain reaction, phenomenon, result, or condition to be manifested' – Oxford English Dictionary (https://www.lexico.com/en/definition/threshold).

The Swedish apothecary and chemist Carl Wilhelm Scheele (1742–1786) is credited with discovering lactate/lactic acid in sour milk in 1780 (see Benninga, 1990; Ferguson *et al*. 2018) but it was Jöns Jacob Berzelius (1779–1848) who reported in the early 19th century that [La^-] in the muscles of exhausted deer was greater than in the muscles of animals with partially paralysed extremities (Von Muralt, 1950). In 1861, Louis Pasteur observed that the growth of yeast per gram of sugar consumed was much greater in the presence of oxygen (O_2) than in its absence (Pasteur, 1861; Racker, 1974; Barnett, 2003). Thereafter, most researchers focused on the measurement of the products of fermentation (alcohol for yeast and La^- for skeletal muscle) under aerobic (O_2 present) *versus* anaerobic (O_2 absent) conditions (Racker, 1974). In yeast, sugar consumption decreased upon the introduction of O_2 and there was a concomitant reduction in alcohol production. Later, this same phenomenon was found in skeletal muscle in the form of a decrease in glycogen breakdown and a decrease in La^- formation under aerobic as compared to anaerobic conditions (Meyerhof, 1930*a*; Barnett, 2003). In 1926, Warburg named this phenomenon the 'Pasteur effect' (Warburg, 1926; Krebs, 1972). Thus, from a very early date, the 'Pasteur effect' was a great influence on hypotheses surrounding the role of O_2 in skeletal muscle metabolism.

At the end of the 19th century and the beginning of the 20th century, several avenues of research promoted and reinforced the notion of hypoxia as the necessary cause of La^- accumulation. In the 1890s, both Araki and Zillessen (see (Kompanje *et al*. 2007) demonstrated that interruption of the O_2 supply to the muscles of mammals and birds promoted an increase in [La^-]. In their landmark study, Fletcher & Hopkins (1907) reported that [La^-] was greatly increased during stimulation of muscles to fatigue; and notably, when fatigued muscles were placed in environments containing O_2, the La^- disappeared. This La^- disappearance in the presence of O_2 was in sharp contrast to a further increase in muscle [La^-] when the muscles were incubated in an anaerobic atmosphere. Subsequently, the pioneering exercise physiology studies of A. V. Hill, Long and Lupton (e.g. Hill *et al*. 1924*b*,*c*,*d*) as well as Krogh & Lindhard (1913) determined that strenuous exercise increases blood [La^-] in humans. Otto Meyerhof also observed La^- accumulation in isolated amphibian muscles in the absence of O_2 and its removal upon the return of O_2 (Meyerhof, 1930 *b* ; Krebs, 1972). Therefore, it is not surprising that in the context of the Pasteur effect in skeletal muscle, Hill *et al*. (1924*c*) postulated that La^- increased during muscular exercise because of a lack of O_2 to remove the requisite La^- produced by the contracting muscles. From a historical perspective, while early muscle physiologists and biochemists are to be remembered, and lauded for their efforts, they were insular in their biological perspective because Otto Warburg showed, contemporaneously, that fully oxygenated cancer cells produced La^- at astonishing rates (Warburg, 1926). Today we know that La^- is the inevitable product of glycolysis and that the 'Warburg effect' of aerobic glycolysis is typical of normal metabolism rather than a peculiarity of cancer (Brooks, 2018; Ferguson *et al*. 2018).

Perhaps the report most relevant to the idea of a La^- threshold in muscle and exercise physiology is that of Warren Harding Owles in 1930 that blood [La^-] increases only above a certain 'critical metabolic level' (known as Owles's point) that differs among subjects and exercise modalities (Owles, 1930). These results were based on experiments with two participants, Owles himself and his mentor, C. G. Douglas. Figure 1*A* illustrates a typical response that has been observed during incremental exercise over the numerous years since Owles's report. Crucially, Owles noted that this increased blood [La^-] was associated with a decrease in the CO_2-combining power of the blood. This observation laid the physiological foundation for Wasserman & McIlroy (1964) to propose non-invasive determination of their so-called 'anaerobic threshold' (AT) by means of the ventilatory and gas exchange profiles measured across steadily increasing work and metabolic rates (see also Naimark *et al*. 1964; Wasserman *et al*. 1973). As reported previously (Brooks & Gladden, 2003; Ferguson *et al*. 2018), Wasserman provided a historical perspective on his proposal in a letter of 21 February 2000 to George A. Brooks. Briefly, in late 1960 Wasserman's postdoctoral mentor at the

University of California, San Francisco Cardiovascular Research Institute, Julius H. Comroe, urged Wasserman to develop procedures for the early detection of cardiovascular disease. Quoting from Wasserman's letter to Brooks:

> [Evaluation] would be best done during exercise when the heart was being stressed … The first sign of heart failure would be reflected in the failure of the circulation to deliver adequate O_2 to the metabolizing tissues (exercising muscles). Since the muscle O_2 requirement would be markedly increased by exercise, the failure of the heart to transport O_2 adequately would result in lactic acidosis (*Pasteur Effect*) [our emphasis].

As presaged by the work of Owles (Owles, 1930), Wasserman thought that he might be able to detect the oxygen uptake ($\dot{V}_{O_2}$) at which lactic acidosis occurred during exercise via non-invasive gas exchange methods because the buffering of lactic acid by bicarbonate (HCO_3^-) would yield excess carbon dioxide (CO_2) (Wasserman *et al.* 1973). Similar ideas were advanced independently by Hollmann, Kindermann, Keul and others (see Hollmann, 2001), but did not receive recognition equivalent to Wasserman's efforts, perhaps because much of the research was published in German. Also, their results and explanations were based on blood La^- determinations and did not benefit from breath-by-breath gas exchange technology. After Wasserman moved to Stanford, he and William L. Beaver with the cooperation of Varian Associates developed breath-by-breath gas exchange methods and published the first studies employing these methods during exercise (Wasserman, 2002). During this period when Wasserman's ideas were evolving, he also published what he described as his 'springboard paper' (Wasserman, 2002), which addressed the interaction of physiological mechanisms to provide a normal exercise response. It was in this 1967 'springboard paper' (Wasserman *et al.* 1967) that he introduced his widely used gear model for the interaction of the muscular, cardiovascular and respiratory systems (Fig. 2).

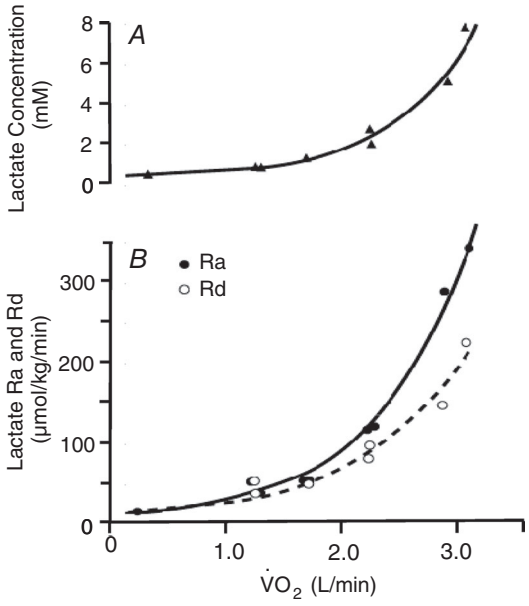


Figure 1. Arterial La^- concentration (*A*) and rates of La^- appearance (R_a) and disappearance (R_d) (*B*) plotted as a function of oxygen uptake ($\dot{V}_{O_2}$) in one participant during a continual, progressive leg cycle ergometer test
Adapted from Stanley *et al.* (1985) and Brooks *et al.* (2019).

O_2 and the anaerobic threshold

When Wasserman & McIlroy first coined the term 'anaerobic threshold', an increase in blood [La^-], according to the Pasteur Effect but not the Warburg effect, was considered *ipso facto* evidence that, for all higher metabolic rates, the body had transitioned energetically to an obligatory anaerobiosis (Naimark *et al.* 1964; Wasserman & McIlroy, 1964). Notably, Wasserman credits D. B. Dill, famously known as the Director of the Harvard Fatigue Laboratory, with inspiring the term 'anaerobic threshold'. According to Wasserman (Wasserman's letter of 21 February 2000 to Brooks), upon Dill seeing Wasserman's data, Dill observed '… that I was detecting the threshold of anaerobic metabolism during exercise by measuring exercise gas exchange. Thus, he put into words succinctly what I was trying to measure.' This view was firmly ensconced into the exercise metabolism literature by the 1973 paper of Wasserman, Whipp, Koyal & Beaver (Wasserman *et al.* 1973) in which breath-by-breath measures were made during incremental exercise to provide 'noninvasive indicators of the onset of metabolic acidosis (anaerobic metabolism)'. With 1541 citations (Web of Science 27 September 2020) this paper is the eighth most cited paper of all time in the *Journal of Applied Physiology*.

As discussed later (see 'Coincidence of exercise thresholds and their mechanistic bases'), numerous methods have been proposed for determining a threshold in exercise metabolism with ensuing debate about which is best. However, the most controversial aspect of the 'anaerobic threshold' is the underlying mechanism implied by its name. At its core, the concept of anaerobiosis implies dysoxia (Connett *et al.* 1990), i.e. O_2-limited cytochrome turnover or quite literally insufficient O_2 molecules to accept electrons from cytochrome *c* in the electron transport chain. While there is no doubt that dysoxia leads to increased La^- production, is this in fact what happens during increasing exercise intensities? Here, we argue that the majority of evidence indicates that *if* dysoxia plays any role at all in the LT, it

is not the primary factor. There are at least three lines of evidence against dysoxia as the cause of the LT; each of these will be described in turn.

First, in the 1960s, Welch and Stainsby (e.g. Welch & Stainsby, 1967) reported a surprising finding for that time; isolated, blood-perfused, highly oxidative canine skeletal muscle *in situ* initially released La^- during contractions but then reverted to lower release or even La^- uptake as the contractions continued (Fig. 3)! Perhaps these authors would have been less surprised had they fully appreciated Ole Bang's report from 1936 (Bang, 1936) or the results of Rubner & Frey in 1885, as described by Barnard & Holloszy (2003). Bang showed that, during prolonged exercise in humans (~40 min at moderate intensity), the blood [La^-] reached a maximum after about 10 min of exercise and then declined, sometimes to pre-exercise [La^-], whether the exercise ceased or not. As reviewed by Barnard & Holloszy (2003), in the 1880s Rubner & Frey perfused mammalian muscles with oxygenated blood and observed La^- release despite the presence of apparently sufficient O_2. Similar results have been observed frequently over the past approximately 100 years, e.g. see net La^- release and uptake in Fig. 4*C*.

Regardless, subsequently Stainsby reasoned that if the initial La^- release from contracting canine muscles was caused by a lack of O_2, then the NAD^+/NADH couple should become more reduced. On this basis, Jöbsis & Stainsby (1968) employed the surface fluorometry technique of Chance, Jöbsis and colleagues (Chance *et al.* 1962) on Stainsby's canine muscle preparation at the onset of contractions, a time during which La^- release had previously been measured (e.g. Welch & Stainsby, 1967). Their results (Jöbsis & Stainsby, 1968) were antithetical to the idea of hypoxia/dysoxia; during muscle contractions, the NAD^+/NADH couple became oxidized, not reduced! While these techniques and results are not immune from criticism (Gladden, 1996; Ferguson *et al.* 2018), they nevertheless focused attention on alternative explanations to dysoxia as the proximate cause of increasing La^- production during muscle contractions and exercise. Additionally, the reversal of net La^- efflux with continued contractions/activity at the same metabolic rate remains a

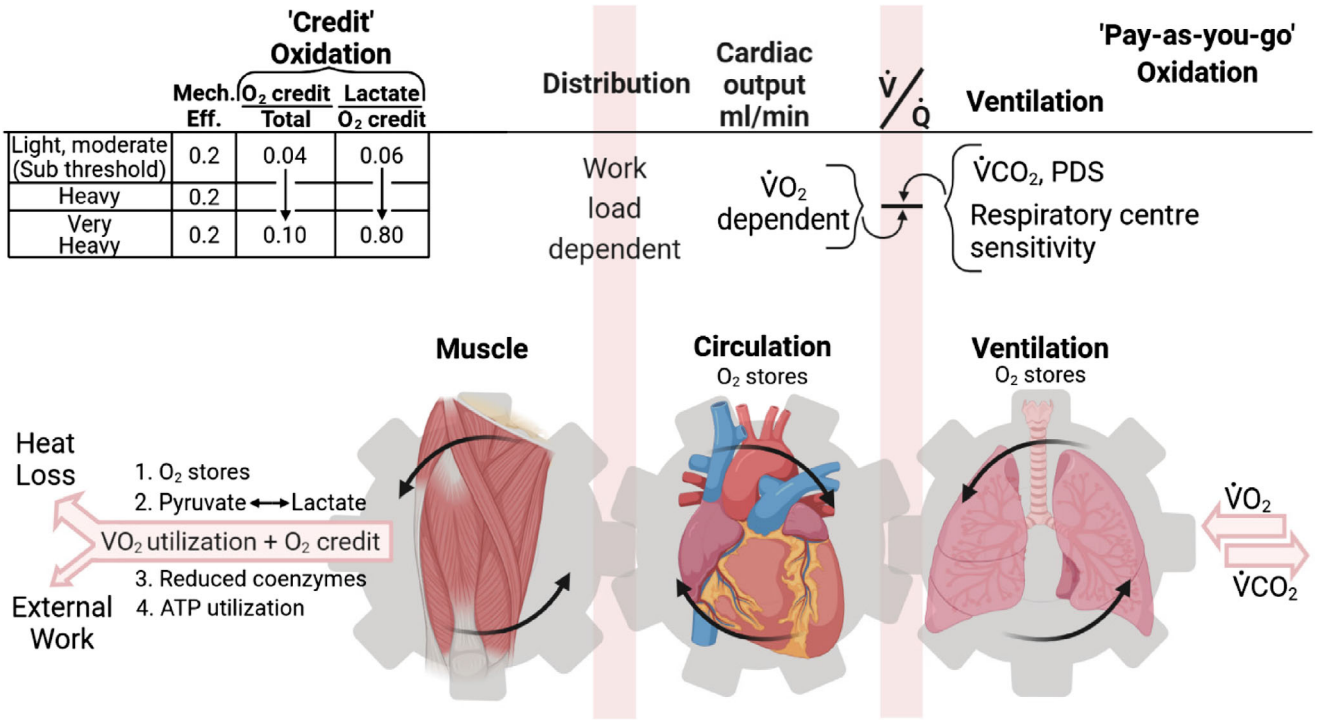


Figure 2. Schematic illustration of Wasserman's concept of the interaction of muscle, and the cardiovascular and respiratory systems
Redrawn from Wasserman *et al.* (1967), the paper that Wasserman described as his 'springboard paper' (Wasserman,2002). In the terminology of Wasserman *et al.*, energy for muscular work was provided by the oxidative system ($\dot{V}_{O_2}$ = 'pay-as-you-go' oxidation) and 'credit' oxidation (conversion of pyruvate to La^- = La^- accumulation, use of muscle ATP and phosphocreatine (PC) stores, reduction of coenzymes, desaturation of oxymyoglobin, decrease in venous O_2 concentration and decreased dissolved O_2 in body fluids). Muscle work led to increased cardiac output and redistribution of blood flow and subsequently ventilation was increased in response to the increased metabolism, but also due to the increased evolution of CO_2 from the blood as the result of lactic acid buffering. This figure was created with BioRender.com and was exported under a paid subscription.

strong argument opposing dysoxia. The conclusion that anoxia/dysoxia was not present in contracting skeletal muscle was also supported by evidence that muscle bulk blood flow (humans, Grassi *et al*. 1996; dogs, Grassi *et al*. 1998) and capillary haemodynamics (rat, Kindig *et al*. 2002) increase so rapidly, following the onset of contractions, that muscle microvascular and interstitial P_{O_2} do not plummet below those appropriate for the subsequent steady-state response (which, if this occurred, would be evidence of dysoxia; rev. Poole, 2019). Importantly, as with the dog gracilis muscle contracting *in situ*, it was found that in human exercise, muscle La^- release increases at exercise onset, but then, depending on training state (and mitochondrial density, *vide infra*), may revert to uptake as exercise continues (Stanley *et al*. 1986; Bergman *et al*. 1999*b*) (Fig. 4).

Second, at both rest and during muscle contractions/exercise at a maintained submaximal metabolic rate, net La^- release will reverse to net La^- uptake when the arterial [La^-] is elevated. This phenomenon was elegantly shown in resting rabbit muscles of differing oxidative capacity by Pagliassotti & Donovan (1990). Subsequently, Brad Zinker, working in David Wasserman's laboratory, showed simultaneous glucose and La^- uptake by muscle in running dogs (Zinker *et al*. 1995). Gladden's laboratory also reported corroborating results for canine skeletal muscle contracting at several different metabolic rates (Gladden, 1991; Gladden *et al*. 1992, 1994) (Fig. 5). Analogous results were reported for exercising humans (e.g. Kjaer *et al*. 1991). Critically, the primary fate of this net La^- uptake is oxidation to CO_2 and H_2O (Kelley *et al*. 2002). The key point here is that contracting muscle would not be capable of La^- oxidation in the face of any significant dysoxia, or stated differently, these findings negate the possibility of any global dysoxia.

A third route of investigation regarding the role of O_2 in the LT was to evaluate the question: how much O_2 is enough? In other words, what critical intramyocyte P_{O_2} is limiting for cytochrome *c* oxidase utilization of O_2, i.e. dysoxia? Multiple studies have suggested that a P_{O_2} below approximately 2 mmHg would be limiting to oxidative phosphorylation by mitochondria (Chance & Williams, 1956; Wilson *et al*. 1988; Connett *et al*. 1990; Rumsey *et al*. 1990; Gnaiger *et al*. 1995; Gnaiger & Kuznetsov, 2002). So, what is the intracellular P_{O_2} in La^--producing skeletal muscle? Connett *et al*. (1983, 1984, 1986) used myoglobin cryomicrospectroscopy to measure myoglobin saturation and thereby the distribution of P_{O_2} in muscle fibres in contracting, isolated, perfused canine gracilis muscle. La^- concentration was measured in approximately the same

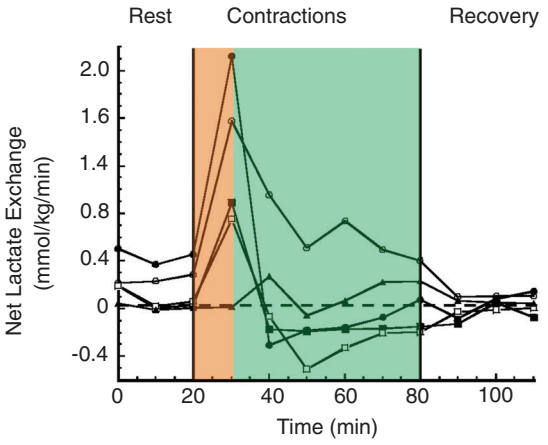


Figure 3. Net La^- exchange for isolated, blood-perfused, canine gastrocnemius muscles that were stimulated *in situ* for a period of 60 min
The interesting result was that although net La^- release occurred during the first period of the contractions (orange shading), this release decreased and sometimes reversed to net La^- uptake (green shading). Redrawn from Welch & Stainsby (1967).

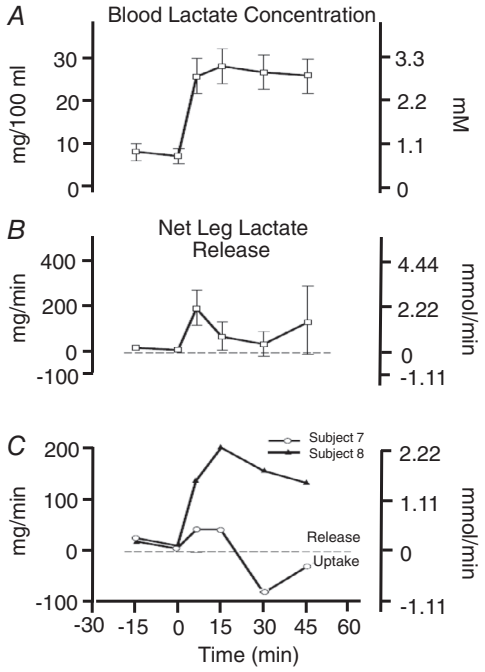


Figure 4. Blood [La^-] and net La^- exchange during exercise
A, mean arterial blood [La^-] (±SEM) in six men during rest and continuous leg cycle ergometer exercise at 50% $\dot{V}_{O_2max}$. *B*, net leg La^- release (= (v − a)[La^-] × Leg blood flow) in the same participants. Note that after 15 min the legs are releasing much less La^- even though exercise continues and arterial [La^-] is constant. *C*, leg net La^- exchange in two participants. When exercise begins, legs of both participants increase La^- release, but only transiently. Further, individual participants show great variability, with the endurance trained participant (subject 7) showing a switch from La^- release to consumption, while working legs of the untrained participant (subject 8) continue to release La^- on a net basis. Training-related increases in intramuscular La^- shuttling of the trained participant (subject 7) cause leg net La^- exchange to decline in the population studies. Adapted from Brooks *et al*. (1991) and Brooks *et al*. (2019).

cell populations. At rest and during submaximal twitch contractions at ∼10% and ∼70% of peak twitch $\dot{V}_{O_2}$ in this muscle, P_{O_2} remained greater than 2 mmHg during the first seconds of the transition from rest to contractions, and in the steady state. Over the same time course, muscle [La^-] rose from 1.2 mmol kg^{-1} at rest to 2.0 mmol kg^{-1} at the 10% $\dot{V}_{O_2peak}$ contraction rate and to 5.7 mmol kg^{-1} at the 70% $\dot{V}_{O_2peak}$ contraction rate. Thus, there was no evidence that dysoxia was the cause of the increased La^- production during increasing metabolic rate. Later work (Voter & Gayeski, 1995) revealed that the muscle O_2 catchment volume was larger than what Connett *et al.* had thought, but this did not invalidate their prior conclusions.

Ultimately, studies on humans (Richardson *et al.* 1995, 1998; Mole *et al.* 1999) provided additional support for the results of the isolated canine muscle studies. Proton magnetic resonance spectroscopy was used to determine myoglobin saturation during single-leg, maximal effort, quadriceps contractions, leading to the same conclusion that increased La^- efflux from the exercising muscle did not result from inadequate intramuscular P_{O_2} (P_{iO_2}) (Richardson *et al.* 1998). Even when the participants breathed a hypoxic gas (12% O_2), local O_2 tension was never low enough to unequivocally limit oxidative phosphorylation (Richardson *et al.* 1998). Furthermore, net blood La^- efflux from the exercising quadriceps did not correlate with P_{iO_2} across a range of metabolic rates from 50 to 100% of $\dot{V}_{O_2max}$.

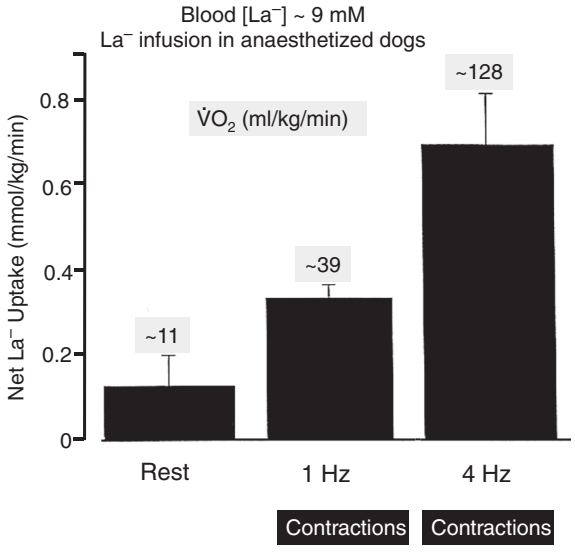


Figure 5. Net La^- uptake during rest and contractions in isolated canine skeletal muscle
La^- was infused into anaesthetized dogs to a level of about 9 mM. Subsequently, the isolated, blood-perfused, gastrocnemius muscles were either at rest or stimulated to contract at either 1 Hz or 4 Hz. The figure shows net La^- uptake *versus* contractile condition with the corresponding $\dot{V}_{O_2}$ above each histogram. Based on steady state data from Gladden (1991).

Evidence against the concept that frank dysoxia is the cause of La^- production during exercise/muscle contractions, such that the 'anaerobic' term of the AT is a misnomer, does not mean that La^- production during exercise is unaffected by reduced O_2 availability. There remains the tantalizing fact that P_{iO_2} does decrease with increasing work rate/contraction intensity (Connett *et al.* 1983, 1984, 1986; Richardson *et al.* 1995, 1998; Mole *et al.* 1999). Of course, increasing the arterial–cellular O_2 gradient has the effect of increasing the drive for O_2 delivery. Regardless, both the increase in work rate and the decrease in P_{iO_2} lead to signals that stimulate glycolysis [e.g. ↓ATP, ↑ADP, ↑AMP, ↑P_i, ↑Ca^{2+}; Gladden, 1996). As discussed in detail in other reviews (Clanton *et al.* 2013; Ferguson *et al.* 2018), the small 'extra' decrease in ATP, and increases in ADP, AMP and P_i, could provide an additional stimulus to glycolysis and thereby enhance La^- production due to the declining P_{iO_2} even without actual dysoxia. This could explain, for instance, the lower La^- levels during incremental exercise when breathing a hyperoxic as opposed to normoxic gas mixture (Hogan *et al.* 1983). In other words, a declining P_{iO_2} with increasing work rate might serve as an ancillary stimulus for La^- production in the absence of dysoxia.

Understanding of O_2–La^- interactions in muscles and other tissues was greatly enhanced by discovery that the cellular respiratory apparatus was not arranged in discrete mitochondrial organelles, but rather as an extensive network, a mitochondrial reticulum (Bakeeva *et al.* 1978; Kirkwood *et al.* 1986, 1987). As such, the mitochondrial reticulum (i.e. mitochondrial power grid) allows for transmission of the trans-inner mitochondrial membrane chemiosmotic gradient from the cell surface to deep within muscle fibres and other cells, thus forming an intracellular power grid (Glancy *et al.* 2015). In this way, potential effects of a declining P_{iO_2} on phosphorylation of ADP to ATP are minimized. Also, Clanton (2019) proposed the idea of a myoglobin/nitric oxide 'shield' working in combination with the mitochondrial power grid to decrease the potential for low P_{O_2} to limit mitochondrial oxidative phosphorylation (OxPhos) activity. Clanton's idea (Clanton, 2019) is that in areas of potentially limiting P_{iO_2}, myoglobin (Mb) becomes more deoxygenated, which decreases the action of Mb to scavenge nitric oxide (NO). A resulting higher [NO] in that locale would competitively restrict cytochrome *c* utilization of O_2. Local loss of OxPhos activity would be mitigated by the mitochondrial power grid as described above. If, or as, P_{iO_2} increases, the return of Mb to a more oxygenated state would increase NO scavenging, removing inhibition of cytochrome *c* activity and permitting local OxPhos to rise.

Overall, as brilliantly educed and championed by George Brooks (Brooks, 1985), the increase in blood [La^-] at and above the LT merely reflects a higher rate

of La^- appearance in the blood (R_a) in opposition to disappearance from the blood (R_d) and, as discussed extensively above, there is no evidence of anoxia (i.e. $P_{iO_2} = 0$ mmHg) or dysoxia ($P_{iO_2} \leq 2$ mmHg). In addition, above LT there is a broad range of metabolic rates (i.e. the heavy exercise intensity domain) at which blood [La^-] can be stabilized, albeit at an elevated level (Whipp & Wasserman, 1986; Poole *et al.* 1988, 1990; rev. Poole & Jones, 2012). When blood (and muscle) [La^-] is (are) not increasing there is no net 'anaerobic' ATP production from glycolysis. Under this condition, some exercising muscles, and perhaps other tissues, are producing La^- while other muscles, both resting and contracting, along with other tissues are simultaneously oxidizing La^-. Note that glycolysis is neither 'aerobic' nor 'anaerobic' in terms of O_2 availability since La^- is the obligatory product of glycolysis (Brooks, 2018; Ferguson *et al.* 2018; Brooks *et al.* 2019). Glycolysis is only 'anaerobic' in the sense that O_2 is not required for the function of this pathway.

In our opinion, skeletal muscle mitochondrial reticulum volume density, not dysoxia, is the major factor in the relationship between exercise intensity and increases in muscle and blood [La^-]. A key foundational concept here is that increasing exercise intensity elicits biochemical and hormonal signals that stimulate glycolysis. Then, due to the high activity of lactate dehydrogenase, La^- production must increase, i.e. La^- is the end product of glycolysis (Rogatzki *et al.* 2015; Brooks, 2018; Ferguson *et al.* 2018; Glancy *et al.* 2020). When La^- production by the glycolytic pathway accelerates, [La^-] obligatorily increases unless there is sufficient mitochondrial mass to siphon off La^- via the NADH shuttles (Glancy *et al.* 2020), the mitochondrial lactate oxidation complex (Hashimoto *et al.* 2006, 2008; Hashimoto & Brooks, 2008) and pyruvate via the mitochondrial pyruvate carrier (Bricker *et al.* 2012; Herzig *et al.* 2012), respectively (Brooks, 2020 *a* ; Glancy *et al.* 2020). Lactate concentration will always be a competitive balance between the glycolytic rate and the subsequent mitochondrial metabolism of pyruvate and La^-. A greater volume density of the mitochondrial reticulum permits lower [La^-] at greater glycolytic rates, i.e. greater metabolic clearance rates (Donovan & Brooks, 1983; Bergman *et al.* 1999*a*; Messonnier *et al.* 2013) that result from greater muscle power outputs.

Conclusions of this section. Based on best evidence, the term 'anaerobic threshold' should be discarded for other than historical purposes. While cutting edge at the time of inception, the concept that the AT is the result of anoxia/dysoxia is not supported by contemporary evidence. Nevertheless, there remains significant importance of and interest in the metabolic threshold during exercise that is associated with sustained elevations of muscle and blood [La^-] (i.e. LT). Various physiological variables have been employed to identify the LT invasively or non-invasively and are detailed below in 'Coincidence of exercise thresholds and their mechanistic bases'.

The lactate shuttle and the 'anaerobic threshold'

Originally conceived as a means to shuttle reducing equivalents among cells, tissues and organs, we now know that La^- shuttling fulfils three basic functions: (1) La^- is a major, sometimes preferred, energy substrate; (2) La^- is the primary gluconeogenic precursor; and (3) La^- is a signalling molecule (Brooks, 1986, 2018, 2020*b*). Workers in diverse fields such as brain metabolism, sepsis, cancer and diabetes research, are employing lactate shuttle theory in an effort to better understand normal and disrupted metabolism in different tissue and cell types. Briefly, in the early 1980s, as evidence against anoxia/dysoxia as the primary cause of La^- production evolved, a paradigm shift occurred in our understanding of La^- metabolism during exercise. This paradigm shift was conceived by Brooks (1985) on the basis of evidence gathered from numerous sources including his own work on La^- and glucose metabolism using isotopic tracers in laboratory rats (Donovan & Brooks, 1983). Critical support for the idea of a cell-to-cell lactate shuttle came from several isotopic tracer studies in humans by the Brooks laboratory, which tracked the flow of carbon intermediates during rest, exercise and recovery in combination with arteriovenous measures of net La^- exchange, and muscle biopsies (Mazzeo *et al.* 1986; Stanley *et al.* 1986, 1988; Bergman *et al.* 1999*b*, 2000; Brooks, 2002). The evidence for La^- as a major player in the coordination of whole-body metabolism has since grown rapidly. Numerous studies over the intervening period have shown that La^- is not merely a dead-end waste product of anaerobic metabolism, but is instead a continuously produced and removed metabolite that circulates among and within essentially all cells of the body (Brooks, 2018). Both the evidence and the concept have been extensively reviewed previously (Gladden, 1996, 2004*a*,*b* , 2008; Brooks, 2000, 2002, 2007, 2009, 2016, 2018, 2020*b*; Brooks & Gladden, 2003), and the reader is referred to those summaries.

Lactate as a biomarker of physiological and metabolic strain. Rising blood [La^-] indicates that an individual is not in a metabolic steady state, and for exercise performance, rising [La^-] predicts a curtailed or diminished performance. Reiterating from prior discussion, rising or extremely high blood [La^-] means only that La^- R_a is greater than the La^- R_d, possibly due to increased La^- production, inadequacy of clearance mechanisms or both. Conversely, lower than predicted blood [La^-] in individuals engaged in heavy or severe

exercise means enhanced La^- clearance capacity, as the result of endurance training (Messonnier *et al.* 2013), genetics or both (San-Millan & Brooks, 2018).

Driver and recipient sites of blood lactate shuttling during graded and continuous exercise. Seminal studies using isotope tracers in laboratory animals (Donovan & Brooks, 1983) and humans (Stanley *et al.* 1985; Mazzeo *et al.* 1986) were informative of whole body La^- kinetics during rest and exercise, but the data provided minimal information on the sites of La^- release and uptake. Knowledge of the sites of La^- production and removal required the addition of carbon-labelled isotope tracers along with simultaneous arteriovenous difference, $(a - v)\Delta$ and blood flow measurements. Fortunately, Brooks *et al.* were able to make those simultaneous measurements across resting and working skeletal muscles (Stanley *et al.* 1985; Bergman *et al.* 1999*b*) and brain (Glenn *et al.* 2015). Brooks and others also made similar tracer, blood flow and $(a - v)\Delta$ measurements across the beating heart (Gertz *et al.* 1981, 1988; Bergman *et al.* 2009), and liver and kidneys (Gerich *et al.* 2001; Meyer *et al.* 2002*a*,*b*). The findings strikingly show that all tissues, including working muscles (Brooks *et al.* 1991), simultaneously produce and consume La^- and that $(a - v)\Delta$ measurements alone in the absence of tracer isotopic enrichments are insufficient to understand the full extent of La^- shuttling *in vivo*. False interpretations ensue unless it is understood that tissues simultaneously consume and produce La^-.

With even a contemporary view of La^- metabolism, one might think that increments in blood $[La^-]$ seen during exercise arise from working skeletal muscles. However, this may not be universally true; consider, for instance, results from participants before and during constant power output exercises. In both healthy individuals and those with chronic disease, resting blood $[La^-]$ is almost always in the range of 0.5–1.5 mM. Resting muscles simultaneously consume, produce and release La^- (Stanley *et al.* 1986; Bergman *et al.* 1999*b*), with net La^- release from resting, fully circulated and oxygenated muscles, including endurance trained muscles often being observed (Brooks *et al.* 1991; Bergman *et al.* 1999*a*,*b*) (Fig. 4*B*). Moreover, while at the onset of submaximal steady rate exercise there occurs a burst of muscle La^- release, net release declines over time (Fig. 4*B*), particularly in endurance trained individuals (Fig. 4*C*) (Brooks *et al.* 1991; Bergman *et al.* 1999*b*). These dynamic changes in La^- metabolism occur while working muscles take up glucose and La^- (Bergman *et al.* 1999*a*,*b*). As submaximal exercise at a constant power output continues, and whole body La^- exchanges come into balance, both arterial blood $[La^-]$ (Fig. 4*A*) and whole body La^- turnover rates (Fig. 6) become constant both before and after endurance training (Fig. 6).

Figure 6 illustrates the effects of endurance training on blood La^- turnover, showing continuous, but balanced, La^- production and disposal during rest and exercise. Figure 4*B* shows an example of very little net La^- release from working muscles despite active turnover (Stanley *et al.* 1986). If exercising muscles are not a net source of La^- production, particularly in endurance trained individuals (Fig. 4*C*), the question arises: what is the source of La^- in the scenario of continuous, but balanced La^- production and disposal as shown in Figs 4 and 6? Net La^- release from the heart, liver, kidneys or brain is unlikely, but where else might the La^- load come from? One potential site is the integument, a large organ that stores glycogen and has the potential to release La^- in response to sympathetic stimulation (Johnson & Fusaro, 1972). In addition to the integument, perhaps another unstudied source of blood La^- might be white adipose tissue or gut, a major site of fermentation by the microbiome (Brooks, 2018, 2020*a*).

As an aid to summarizing the effects of endurance training on blood La^- kinetics, refer to Fig. 6. Men were studied at two continuous exercise power outputs (45 and 65% $\dot{V}_{O_2max}$) before and after 2 months of supervised endurance training (Bergman *et al.* 1999*b*). After training, participants were studied at the same exercise power output that elicited 65% $\dot{V}_{O_2max}$ before training (about 50% of post-training $\dot{V}_{O_2max}$), as well as at a power output that elicited 65% of the post-training $\dot{V}_{O_2max}$. There was good correspondence between La^-R_a, R_d and oxidation (R_{Ox}) (Fig. 6*A*, *B* and *D*), which are expected to be similar during constant power output exercise, both before and after training with oxidation accounting for the majority of La^- disposal. Not shown is that endurance training also increased the capacity for gluconeogenesis from La^- (Bergman *et al.* 2000; Emhoff *et al.* 2013*b*). Perhaps most distinctive among the panels is Fig. 6*C*, which illustrates the effects of endurance training on La^- metabolic clearance rate ($MCR = R_d/[La^-]$). Clearance capacity is a term less frequently used, but to those familiar with the terms 'insulin action' or 'sensitivity', the MCR term may resonate because, by definition, clearance relates La^- removal to its concentration. Before training, exercise at the higher, 65% $\dot{V}_{O_2max}$ metabolic rate was accomplished despite a low baseline MCR capability. However, after training, increased La^- MCR was observed, a result attributable to increased muscle mitochondrial respiratory capacity and correlated with muscle La^- (monocarboxylate) transporter abundance (Dubouchaud *et al.* 2000).

Flexibility of tissues in terms of taking up or releasing La^-, or both simultaneously producing and removing La^-, depending on conditions, forces us to consider the mechanisms allowing and regulating such behaviour. As well, such observations give rise to another concept of the utility of La^- shuttling with regard to the conservation

of carbon energy sources because La^- is secreted mainly as CO_2 (Mazzeo *et al*. 1986; Stanley *et al*. 1988; Bergman *et al*. 1999*b*; Emhoff *et al*. 2013*a*) (there being no reports of extensive urinary La^- excretion in humans).

Beyond exercise: what do elevations in blood [La^-] mean? Aside from exercise, lactataemia also occurs in other conditions such as brain trauma (Brooks & Martin, 2014; Wolahan *et al*. 2018), sepsis (Marik & Bellomo, 2016), dengue (Somasetia *et al*. 2014), cancer (Warburg, 1956; Semenza, 2008; San-Millan & Brooks, 2017), hepatitis and pancreatitis (Hoque *et al*. 2014). In this regard it is important to note that in only one condition, physical exercise, are there definitive data on the significance and meaning of La^- accumulation in a stressful condition; specifically, blood [La^-] rises during exercise when blood R_a is greater than R_d (Stanley *et al*. 1985; Mazzeo *et al*. 1986; Brooks *et al*. 1991; Bergman *et al*. 1999*b*; Messonnier *et al*. 2013). In other conditions, particularly severe clinical conditions such as sepsis, Marik & Bellomo (2016) emphasized that there are no data to support the traditional idea that lactataemia is due to O_2 insufficiency. Classical papers on the 'anaerobic threshold' (Wasserman & McIlroy, 1964; Wasserman *et al*. 1985; Casaburi *et al*. 1989) also failed to consider factors affecting La^- R_a/R_d balance. Regrettably, the reflexive interpretation of lactataemia as tissue O_2 lack is a problem because it deflects from the potential use of La^- therapy in sports and emergency medicine (Garcia-Alvarez *et al*. 2014; Marik & Bellomo, 2016). Indeed, La^- therapy has potential for use in traumatic brain injury (Brooks &

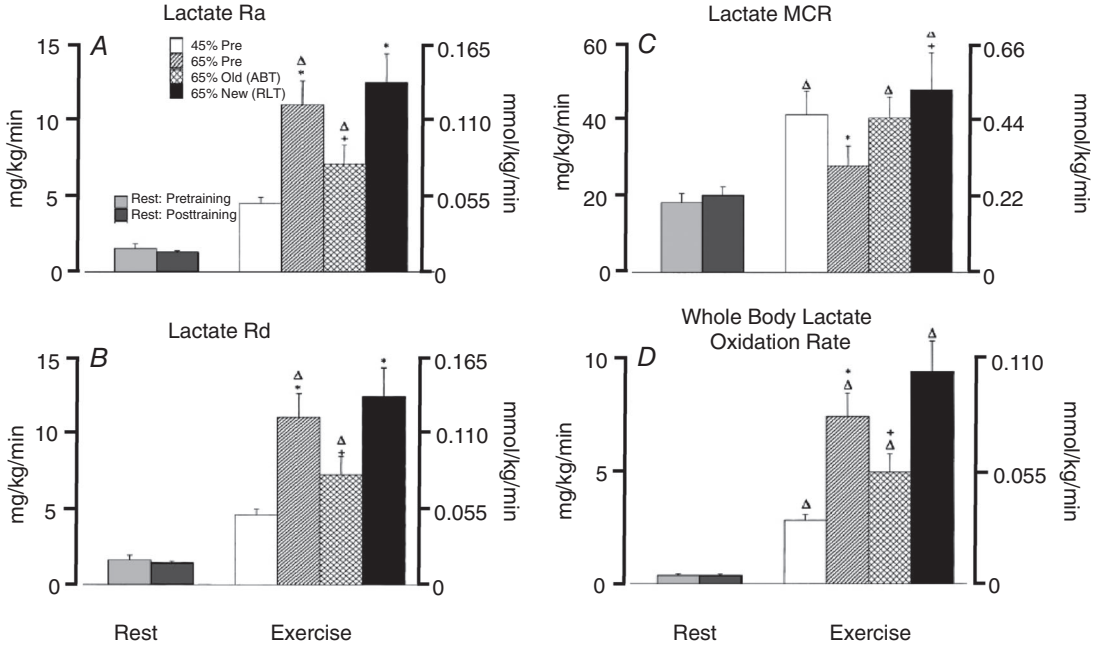


Figure 6. La^- rate of appearance, rate of disappearance, metabolic clearance rate, and oxidation rate during rest and exercise
A, effects of exercise intensity and training on La^- rate of appearance (R_a) during two levels of continuous exercise before and after 2 months of supervised endurance exercise training. Values are means (±SEM for 8–9 participants). *B*, effects of exercise intensity and training on La^- rate of disappearance (R_d). Values are means (±SEM) for the same participants. *A* and *B* look similar because during constant, submaximal exercise $R_a \approx R_d$. *C*, effects of exercise intensity and training on La^- metabolic clearance rate (MCR). Again, values are means (±SEM) for the same participants. *D*, effects of exercise intensity and training on whole body La^- oxidation rate; means (±SEM) for the same participants. *B* and *D* look similar because most La^- disposal occurs via oxidation both before and after endurance training. In *C*, La^-MCR is suppressed during hard 65% $\dot{V}_{O_2max}$ exercise before training, but is improved during hard 65% $\dot{V}_{O_2max}$ exercise post-training. The post-training response is associated with increased sarcolemmal and mitochondrial La^- (monocarboxylate) transporter abundance and mitochondrial respiratory capacity (Dubouchaud *et al*. 2000). Figure from Bergman *et al*. (1999*b*). For *A*, 45% Pre: 45% of pre-training $\dot{V}_{O_2peak}$; 65% Pre: 65% of pre-training $\dot{V}_{O_2peak}$; 65% Old (ABT): absolute work rate eliciting 65% of pre-training $\dot{V}_{O_2peak}$; 65% New (RLT): 65% of post-training $\dot{V}_{O_2peak}$, i.e. same relative intensity as 65% Pre.

Martin, 2014), heart failure (Bergman *et al*. 2009), sepsis (Garcia-Alvarez *et al*. 2014; Marik & Bellomo, 2016), dengue (Somasetia *et al*. 2014), hepatitis and pancreatitis (Hoque *et al*. 2014) and other conditions (Brooks, 2018).

Conclusions of this section. While the 'anaerobic threshold' concept was an important and arguably laudable application of late 19th and early 20th century biology, the mechanistic basis for the concept is inadequate in terms of what is known today (Brooks, 2018; Ferguson *et al*. 2018). La^- is produced continuously under fully aerobic conditions (Rogatzki *et al*. 2015) and is an important energy source (Mazzeo *et al*. 1986; Stanley *et al*. 1986; Bergman *et al*. 1999*b*; Messonnier *et al*. 2013), the major gluconeogenic precursor (Bergman *et al*. 2000; Meyer *et al*. 2002*a*), and an important signalling molecule that works by changing cellular redox (Brooks, 2002), allosterically binding to receptors (Ahmed *et al*. 2010), signalling via a transforming growth factor β_2 signalling cycle (Takahashi *et al*. 2019) and gene expression by lactylation of histones (Zhang *et al*. 2019). Further, recent evidence suggests that a gut microbiome-to-host La^- shuttle exists that may enhance endurance exercise performance (Scheiman *et al*. 2019). Classic as well as contemporary findings in biology lead to the conclusion that La^- turnpoints or high blood [La^-] provide little or no information on adequacy of tissue oxygenation, but rather that the balance between La^- production and disposal is perturbed for reasons that are not always understood. We know that during physical exercise La^- disposal is accomplished mainly by oxidation (75–80%) in working muscle (Bergman *et al*. 1999b), the heart and elsewhere (Gertz *et al*. 1988), and the remainder mainly by gluconeogenesis (Bergman *et al*. 2000). Historically, rising or high blood [La^-] under conditions of physiological or metabolic stress have been misinterpreted (Brooks, 2018; Ferguson *et al*. 2018). La^- production is an important strain response, the purpose of which is to mitigate stress. Understood in this light, blood [La^-] can be an important biomarker of physiological stress/strain relationships, and ironically, as now appreciated by investigators conducting a variety of clinical experiments and clinical trials, La^- supplementation can be an important adjunct to therapy across a broad array of physiological or life-threatening conditions (Brooks, 2018).

Above, the benefits of La^- shuttling and La^- therapy are proposed. However, it is also important to recall that lactataemia can be a harbinger of poor outcomes in critical conditions such as infection, sepsis and cancer (Shapiro *et al*. 2005; Martinez-Outschoorn *et al*. 2011; Gohil & Brooks, 2012; San-Millan & Brooks, 2017). Additionally, lactataemia gives rise to metabolic inflexibility because it suppresses white adipose tissue lipolysis via hydroxycarboxylic acid receptor 1 binding and cAMP response element-binding protein activation, and limits mitochondrial uptake of activated fatty acids via malonyl-CoA formation and inhibition of carnitine palmitoyl transferase 1 (Brooks, 2018). Therefore, researchers and clinicians should be cognizant of basic principles of physiology as expressed by Selye regarding the alarm reaction to stress, resistance development and exhaustion (i.e. some stress can be beneficial, but too much stress can result in exhaustion) (Selye & Fortier, 1950), i.e. hormesis (Southam & Erlich, 1943). When considering circulating levels of La^- or any metabolite, appreciation of the underlying reasons for changes in metabolite R_a, R_d and MCR are important (Brooks, 2018). Consider for instance the Warburg effect of aerobic glycolysis in cancer cells (Warburg *et al*. 1927; Racker, 1972). From recent epidemiology we know that regular physical exercise is beneficial for reducing the risks of many common cancers including those of breast, colon, bladder, endometrium, oesophagus, kidney, lung and stomach (Powell *et al*. 2019). Interestingly, those organs appear to play minimal roles in sustaining exercise; consequently, an emerging hypothesis is that exercise-stimulated myokine release is involved in the protective effect of exercise on these tissues during exercise (San-Millan & Brooks, 2017). Alternatively, it may be that exercise-induced upregulation of gut *Veillonella* populations play a role in promoting colon and general health (Scheiman *et al*. 2019). However, consistent with biological principles underlying stress–strain relationships that too much of an efficacious substance (e.g. insulin or La^-) can have negative effects, the need to further explore and understand the regulation of La^- metabolism in all of its facets is essential. For example, it was recently shown that endogenously produced or exogenously added La^- can provoke oncogenesis in cancer cells (San-Millan *et al*. 2019). This raises the question of whether lactataemia following high intensity interval exercise training could provoke carcinogenesis in cancer-susceptible, but as yet untransformed, cells (San-Millan & Brooks, 2017). Considering the importance of maintaining homeostasis in physiology, the deleterious effect of prolonged, continuous La^- accumulation, as occurs in cancer by comparison to the intermittent La^- stimulus during exercise, deserves further exploration (San-Millan *et al*. 2019).

The mechanistic basis of the AT, i.e. dysoxia, has been disproven, and the lactate shuttle is now recognized as the appropriate paradigm for the description of La^- metabolism. Nevertheless, application of physiology of La^- R_a and R_d that are reflected in LT remains valuable in sport, science and medicine. Development of non-invasive estimation of LT by gas exchange has greatly enhanced the application of concepts in La^- metabolism to both physical training and clinical settings, as described in the next section.

The gas exchange threshold: uses and limitations

Identifying the gas exchange threshold. The GET is defined as the metabolic rate at which excess CO_2 (i.e. in addition to the metabolic CO_2 output ($\dot{V}_{CO_2}$) associated with a given $\dot{V}_{O_2}$) is evolved proportional to the rate at which muscle and blood [bicarbonate] decrease, consequent to buffering of a metabolic acidosis, and which derives from non-hyperventilatory mechanism(s). Or, to put it another way, GET is identified by an increase in the $\dot{V}_{CO_2}/\dot{V}_{O_2}$ relationship (the V-slope plot) with evidence of absence of a frank hyperventilation. GET occurs proximal to the increase of arterial blood [La^-] (LT). Identifying such threshold behaviour is essential to understanding the performance and control of the physiological system(s) under investigation. Presented below in 'Common threshold concepts' is the evidence that critical power (CP) may be a better index of the threshold than the LT for an obligatory non-oxidative contribution to exercise energetics, and a stronger correlate of physical performance. While methods to measure CP (or CS) during e.g. cycling or running using a single laboratory visit have been developed (Vanhatalo *et al.* 2007; Pettitt *et al.* 2012; Murgatroyd *et al.* 2014), these approaches depend upon a sustained maximal effort. Effort-independent methods for measurement of physiological thresholds add construct validity to, and reduce potential variability of, the measured variable, because they do not rely on participant perceptions and are likely preferable for testing vulnerable populations, such as the elderly or patients. Using direct arterial La^- sampling, Wasserman *et al.* (1967) distinguished metabolic rates that, if sustained for 15 min or more, result in arterial [La^-] remaining at resting values (termed moderate intensity exercise) from those that do not (heavy and very heavy/severe intensity) (Fig. 7). Later, they developed non-invasive techniques, validated against more direct invasive measures, that increased accessibility and utility of this assessment (Beaver *et al.* 1986). Estimation of LT using non-invasive GET criteria is likely now the most widely used method to estimate metabolic rates that result in arterial blood [La^-] above baseline (Wasserman *et al.* 1967; Beaver *et al.* 1986) (Fig. 7; see also Figs 8 and 9). There exists a small difference between the onset of arterial La^- accumulation (measured by sampling of arterial blood La^-; LT), and the buffering of the metabolic acidosis resulting in increased CO_2 exchange into the alveolar compartment (measured by sampling of arterial bicarbonate; sometimes called the lactic *acidosis* threshold) and therefore detection by GET. This small difference is due to contributions by non-bicarbonate mechanisms of buffering the proton load (Stringer *et al.* 1992). Although it is physiologically correct to distinguish these mechanisms, differences among them turn out to not be clinically meaningful.

Valid identification of GET relies on the fact that the incremental exercise stimulus (Whipp *et al.* 1981; Davis *et al.* 1982; Zhang *et al.* 1991) is sufficiently rapid that the onset of a metabolic acidosis is dissociated from that of compensatory hyperventilation. Without this, it is not possible to distinguish whether excess, non-metabolic, $\dot{V}_{CO_2}$ – which occurs at LT – results from bicarbonate buffering of metabolic acidosis or from frank hyperventilation to drive down arterial P_{CO_2} (Whipp *et al.* 1987). Our knowledge of the mechanism causing this dissociation is incomplete, but it is thought to be due to a relatively slow response to changes in arterial pH of carotid body neural outflow stimulating ventilation (Buckler *et al.* 1991; Whipp & Ward, 1991). The intervening 'window' between LT and respiratory compensation is termed the 'isocapnic buffering region', because during this range of metabolism, end-tidal and arterial P_{CO_2} are stable, despite ventilation increasing out of proportion to $\dot{V}_{O_2}$ and a rise in end-tidal and arterial P_{O_2} (Fig. 8) (Whipp *et al.* 1989). The existence of isocapnic buffering, and its identification in breath-by-breath gas exchange measurements during ramp incremental exercise, therefore provides a means to determine the onset of a metabolic acidosis, i.e. the LT.

Sensitivity, specificity and construct validity of the gas exchange threshold. In many regards, the estimation of LT using GET is advantageous. Not only is it non-invasive and effort-independent, but also modern instruments can

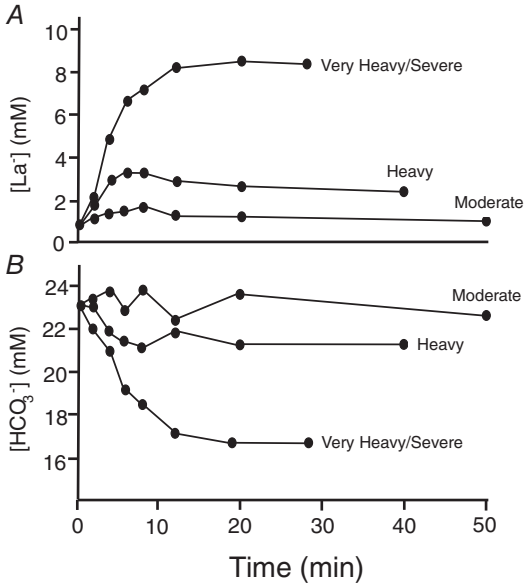


Figure 7. Blood [La^-] and blood [HCO_3^-] during different exercise intensities
A, arterial blood [La^-] during constant power exercise at moderate, heavy or very heavy/severe intensity exercise over ∼30–50 min. *B*, blood bicarbonate concentration ([HCO_3^-]) during the same exercise as *A*. Redrawn from Wasserman *et al.* (1967).

sample gas exchange on a breath-by-breath basis, meaning that the sampling frequency around the metabolic rate at which LT occurs is much greater than could be reasonably obtained from sampling the arterial blood. Specifically, gas exchange is measured in the range of approximately once every 2–4 s, depending on the breathing frequency of the participant at GET. GET occurrence is independent of ramp-incremental protocol, although a false negative (i.e. the false observation that GET was absent) can occur if the rate of increase in power output is slow relative to the participant's aerobic fitness. A slow ramp rate reduces the sensitivity to identify the onset of threshold behaviour in the V-slope plot. However, it is important to note that GET occurs at the same $\dot{V}_{O_2}$ whether the ramp is rapid (completed within 5 min) or slower (completed within 10–15 min) (Agostoni *et al*. 2005; Bowen *et al*. 2012).

The GET is responsive to interventions: reduced muscle O_2 delivery in healthy subjects lowers GET (Koike & Wasserman, 1992); cardiac resynchronization therapy in heart failure increases GET (Auricchio *et al*. 2002); exercise training in chronic heart or lung disease increases GET (Casaburi *et al*. 1991; Kiilavuori *et al*. 1996). However, when the exercise response does not manifest an isocapnic buffering region, then the validity of the assumption is violated, thus negating the assumption that reaching LT is the sole explanation for the observed GET. This can happen, for example, during exercise at high altitude, where peripheral chemoreceptors may be

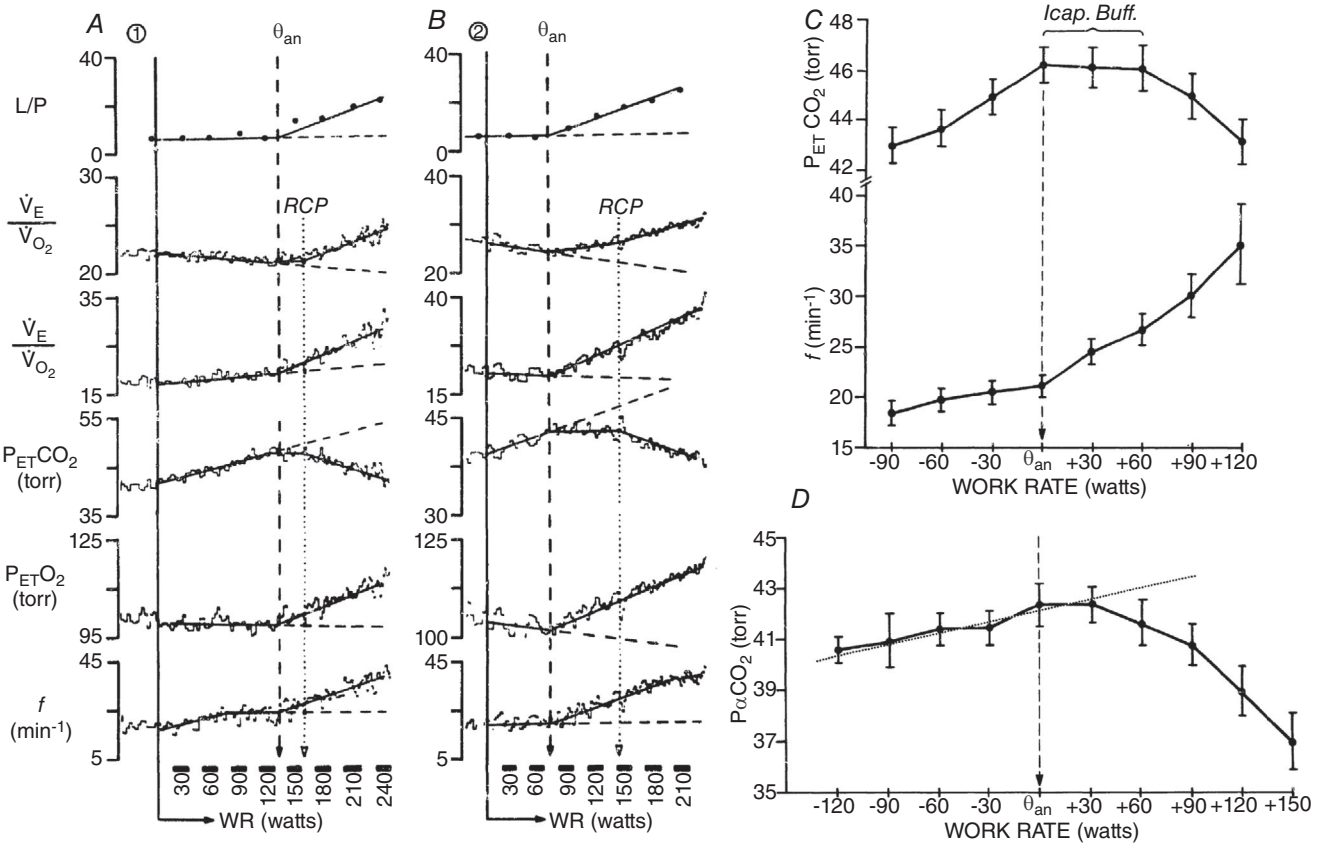


Figure 8. Breath-by-breath responses of the variables used to discern the anaerobic threshold (AT; here abbreviated to *θ*an) and the onset of respiratory compensation (RCP) in a 1 min ramp incremental exercise test to exhaustion
A and *B*, the period of isocapnic buffering (i.e. end tidal partial pressure of CO_2(P_{ETCO_2} becoming constant between *θ*an and RCP) is relatively short in subject 1 (*A*) and is much longer in subject 2 (*B*). *C*, pattern of response of P_{ETCO_2} and breathing frequency (*f*) throughout the sub- and supra-anaerobic threshold range of an incremental exercise test in 24 normal subjects. Note that isocapnic buffering is maintained for an average 60 watts after *θ*an and that *f* begins to increase at a much faster rate as the isocapnic buffering phase is established. *D*, the mean (±1 SD) pattern of response of arterial blood partial pressure of CO_2(P_{aCO_2}), relative to the anaerobic threshold (*θ*an), for 10 normal subjects performing a maximal incremental exercise test. The slope of the increase (represented by the dotted line) up to *θ*an is significant ($P < 0.05$). Note the short isocapnic region that is maintained beyond *θ*an for ∼30 watts. Abbreviations: Icap. Buff., isocapnic buffering region; L, lactate; P, pyruvate; $\dot{V}_E$, ventilation; $\dot{V}_{CO_2}$, carbon dioxide output; $\dot{V}_{O_2}$, oxygen uptake; P_{ETO_2}, end tidal partial pressure of oxygen; WR, work rate. Reprinted with permission from Whipp *et al*. (1989).

sensitized by reduced arterial P_{O_2}, and a frank hyperventilation is manifest at the LT (Agostoni *et al.* 2008). The observation of a ventilatory threshold in McArdle's disease patients is often cited as another example of why the AT concept is flawed (Hagberg *et al.* 1982). McArdle's patients lack the enzyme glycogen phosphorylase and therefore do not metabolize muscle glycogen during exercise; accordingly, with limited glycolysis, they do not accumulate either muscle or blood La^-. However, while these patients respond with a frank hyperventilation, the hyperventilation occurs in the absence of isocapnia, which violates the assumption required for valid LT estimation by GET.

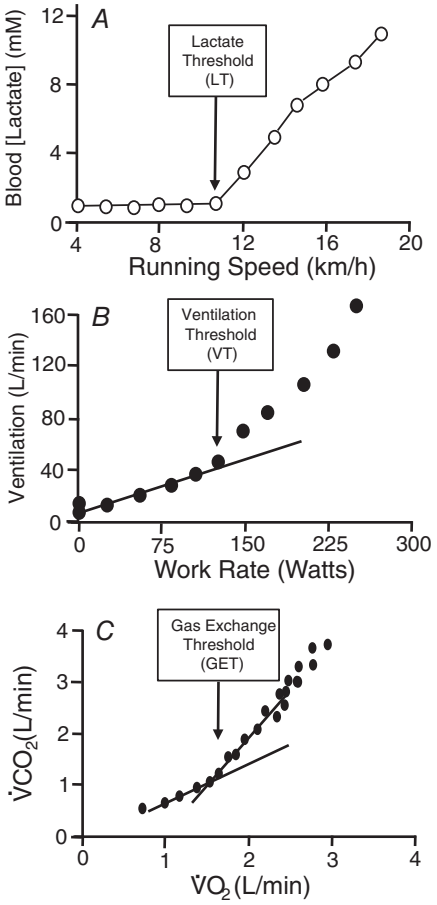


Figure 9. Some of the most established and common variables utilized to detect the 'Anaerobic Threshold' during incremental or ramp increases in running speed (treadmill) or power output (cycle ergometer)
A, initial increase in blood [La^-] above resting baseline. *B*, beginning of a non-linear increase in expired ventilation (here averaged per min rather than breath-by-breath). *C*, non-linear increase in carbon dioxide output ($\dot{V}_{CO_2}$) plotted as a function of oxygen uptake ($\dot{V}_{O_2}$) (commonly referred to as the V-slope; Beaver *et al.* 1986). Note that the advent of ramp protocols, rapid blood La^- sampling and breath-by-breath gas analysis increases the granularity of each technique beyond what is shown in this figure. Also, whereas it is common practice to present thresholds as speeds or power output, so doing incurs a ramp slope-dependence on the threshold. Hence the threshold parameter is expressed more correctly as $\dot{V}_{O_2}$ as demonstrated for the V-slope analysis in *C*.

False positives have also been identified, in which the GET is dissociated from LT. A particularly important example of this behaviour is the response seen following hyperventilation. The study of Ozcelik *et al.* (1999) implemented a 10 min hyperventilatory period prior to incremental exercise testing that reduced end-tidal P_{CO_2} by ∼10 mmHg. Their rationale for this was that participants, particularly those naïve to exercise testing, may be nervous and hyperventilate prior to the exercise test. Hyperventilation would reduce body CO_2 storage, e.g. as carbamino compounds, bicarbonate and dissolved CO_2. If sustained, this hyperventilation could reduce bodily [CO_2] sufficiently to alter the normal dynamics of CO_2 storage and output during an exercise test and dissociate GET from LT (Ozcelik *et al.* 1999). Specifically, the metabolic CO_2 produced in the TCA cycle (e.g. in active muscle) would be directed initially to replenish the CO_2 stores that were lost to prior hyperventilation, and would not be evolved at the mouth at the normal rate. This causes an atypical falling pattern in respiratory exchange ratio (RER) ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) early in exercise. Once the CO_2 stores are refilled, however, the falling RER abruptly changes direction and begins to rise steeply, as the metabolic CO_2 shifts from being stored to being evolved. This manifests as threshold-like behaviour in the V-slope relationship, but is not associated with a buffering of a metabolic acidosis, i.e. as it normally would be at LT. Careful inspection of the lower-than-expected rates of $\dot{V}_{CO_2}$ and/or falling RER may identify the observed GET as a false positive (or pseudothreshold) (Ozcelik *et al.* 1999). A similar behaviour is seen when the ramp rate is too rapid in comparison to the individual's aerobic fitness, and CO_2 storage dynamics can dissociate GET from LT (Ward & Whipp, 1992).

True negatives are also possible, although rare, in which GET is not observed in patients who terminate exercise prior to reaching LT. Such true negative responses are not common, but would include McArdle's disease patients (a hyperventilatory response without an isocapnic buffering region or accompanying acidosis), and those with exercise intolerance due to, e.g. severe dyspnoea, such as some patients presenting with heart failure (Agostoni *et al.* 2013) or chronic obstructive pulmonary disease (Sue *et al.* 1988).

Uses of the lactate or gas exchange threshold. The applications of the LT (here taken as synonymous with GET) for predicting sports performance are presented below (see 'Coincidence of exercise thresholds and their mechanistic bases'). Another intriguing recent observation is that limitations in the rate of energy intake

via the alimentary system may constrain ultra-endurance performance. The maximal rate of nutritional intake in runners competing in the 140-day transcontinental Race Across the USA (RAUSA) was found to be equivalent to ∼2.5–3 times basal metabolic rate (Thurber *et al.* 2019), meaning that exercise performance above this level for days or weeks could not be met by nutritional intake and therefore organismal homeostasis would be lost. This identifies a limit (in endurance athletes) for nutritional intake constraining human exercise performance, i.e. when energy expenditure is increased. Reliance on glycolysis with net La^- accumulation (above LT) for sustained performance depletes carbohydrate substrates at a disproportionately rapid rate compared with exercise below LT, such that sustained exercise above LT would hasten limitation and reduce extreme endurance exercise performance.

The effort-independent nature of the LT (as opposed to CP/CS and $\dot{V}_{O_2max}$) lends itself well to diagnostic and prognostic utility. Some current uses of LT include:

- Assessing the normalcy of an individual's integrative systemic function. As originally conceived, the AT was hypothesized to detect 'the failure of the circulation to deliver adequate O_2 to the metabolizing tissues (exercising muscles)' in heart failure patients (Wasserman's letter of 21 February 2000 to Brooks). Although the eventual mechanism was revealed to be more closely related to a reduced La^- clearance consequent to poor systemic circulation rather than dysoxia, the absolute and relative $\dot{V}_{O_2}$ at which GET occurs is extremely useful in evaluating the normalcy or otherwise of an individual's response to the stress of exercise (Wasserman, 1984). A GET that occurs at a low absolute $\dot{V}_{O_2}$, e.g. <1 L/min, corresponds to impaired physical performance because it would mean that even fairly slow walking would exceed the metabolic rate at LT, and necessitate attendant increases in $\dot{V}_{O_2}$ and energetic inefficiencies that occur during exercise above LT in order for exercise to be sustained. Similarly, GET <40% predicted $\dot{V}_{O_2max}$ is commonly considered clinically abnormal and would trigger further investigation (Sietsema *et al.* 2020). For this, GET is referenced to the individual's predicted, rather than measured, $\dot{V}_{O_2max}$; this helps to overcome misinterpretation in those for whom $\dot{V}_{O_2max}$ is unusually low or high. A GET that occurs at a high relative $\dot{V}_{O_2}$, e.g. >50% predicted $\dot{V}_{O_2max}$, is typically associated with an endurance trained state. However, interpretation of relative GET is complicated by the fact that $\dot{V}_{O_2max}$ declines with age more rapidly than GET (Neder *et al.* 1999). Therefore, GET as a fraction of $\dot{V}_{O_2max}$ increases with increasing age, and does so more rapidly in women than in men, reaching an average normal value of 64% of predicted $\dot{V}_{O_2max}$ in 80-year-old women.
- Optimizing the intensity of training and re- and pre-habilitative exercise prescription. In the clinical setting, prescription for cardiac or pulmonary rehabilitation is often preceded by cardiopulmonary exercise testing. Training intensities that overload physiological processes underpinning LT or CP/CS will, at least in normal physiology, result in beneficial adaptation. In patient populations, training intensities above GET, which challenge the mechanisms responsible for La^- production and clearance, unsurprisingly produce enhanced training benefits in terms of reduction of limiting symptoms and increased exercise tolerance compared with endurance training below GET (e.g. Casaburi *et al.* 1989). Training above GET, however, should also account for CP/CS. Most Olympic endurance events are performed above CP/CS, and therefore prescriptions that include training >CP/CS have a strong underlying rationale. However, exercise tolerance >CP/CS causes limitations in training volume, especially in some patient populations. In this case, patients who are prescribed exercise >LT but <CP/CS would likely benefit from the attendant increase in LT (among other adaptations) of heavy intensity training.
- Judging appropriateness to undergo major thoracic or abdominal surgery and triaging a patient post-operatively to the post-surgical ward, high dependency unit or intensive care unit. A report by Older *et al.* (1999) contained the original observation that GET could distinguish patients who had a 4–5 times increased risk of post-surgical mortality. GET <11 ml kg^{-1} min^{-1} provided a pre-surgical, effort independent, measure that predicted those patients at higher risk. Patients with GET <8 ml kg^{-1} min^{-1} experienced a near 8% post-surgical death rate from cardiovascular causes, compared with none of the patients in whom GET was >14 ml kg^{-1} min^{-1}. In the past 20 years, the utility of peri-operative exercise testing has become an area of intense research. Patients can be tested for risk of surgery or for establishing post-surgical triage to an appropriate level of care. Repeatedly, patients' inability to perform exercise or those with GET or LT values ∼9–11 ml kg^{-1} min^{-1} discriminates risk or predicts survival, and often with stronger predictive power than other commonly used clinical risk stratification measures (e.g. Older *et al.* 1999; Snowden *et al.* 2010; Colson *et al.* 2012; Goodyear *et al.* 2013; Lai *et al.* 2013; West *et al.* 2016; rev. Older & Levett, 2017). For example, in 116 patients undergoing major elective surgery, GET <10.1 ml kg^{-1} min^{-1} was a better predictor of post-surgical pulmonary, renal, gastrointestinal,

infective or cardiovascular complication than $\dot{V}_{O_2peak}$, $\dot{V}_E/\dot{V}_{CO_2}$ slope, age, BMI, cardiac risk index, serum creatinine or several other physiological or hospital-based risk stratification scores (Snowden *et al.* 2010). West *et al.* (2016) identified in 723 patients undergoing major colorectal surgery from six centres that the area under the receiver operator characteristic curve was 0.79 for GET <11.1 ml kg^{-1} min^{-1} resulting in post-surgical morbidity, indicating strong sensitivity and specificity. In multivariable logistic regression, GET <11.1 ml kg^{-1} min^{-1} was significantly associated with increased odds of in-hospital morbidity (odds ratio = 7.56; 95% CI 4.44–12.86; $P < 0.001$), providing ∼4 times the sensitivity of the next most strongly associated variable ($\dot{V}_{O_2peak}$ <18.2 ml kg^{-1} min^{-1}; odds ratio = 2.15; 95% CI 1.01–4.57; $P = 0.047$). These data provided further evidence that GET can be used to assess risk prior to elective surgeries.
- As an index of life expectancy in patients with heart disease. Heart failure patients in whom GET is <11 ml kg^{-1} min^{-1} have ∼4 times increased risk of death (Gitt *et al.* 2002). Those in whom GET is <8.5 ml kg^{-1} min^{-1}, or in whom GET could not be detected (true negatives as well as inability to identify GET), also had a further increased risk of death compared to those with GET >11 ml kg^{-1} min^{-1} (Agostoni *et al.* 2013).

As yet, the mechanism that associates LT with mortality in many different populations is unknown. However, it is intriguing that sensitive thresholds for GET around 9–11 ml kg^{-1} min^{-1} are equivalent to ∼2.5–3 times basal metabolic rate, the maximal rate of nutritional intake identified in RAUSA competitors. We speculate whether an LT of <2.5–3 times basal metabolic rate results in a loss of organismal homeostasis, i.e. the metabolic demands of even light activities overcomes the ability for nutritional replenishment of lost energy stores. Like the extreme endurance athletes, any activity that exceeds LT, which would be even very light activities of daily living for some extremely impaired patients, would accelerate rates of glucose and glycogen utilization that may not be easy to replenish by nutritional intake in elderly patients with poor absorption and metabolic function, hastening the loss of organismal homeostasis.

Conclusions of this section. The concept that the AT is a consequence of dysoxia, i.e. in its original conceptualization, is not corroborated by more recent research. Nevertheless, the subsequent steps of the AT concept, such as the notion that early onset of arterial La^- accumulation during exercise signifies a poor capacity to mediate metabolic strain resulting in significant organismal stress, has stood the test of time. The LT, whether measured directly or non-invasively by gas exchange has construct validity and is sensitive and specific to interventions including, but not limited to, endurance exercise training, cardiac resynchronization therapy or manipulations of systemic O_2 delivery. As such, it has gained wide utility, in particular in determining the normalcy of physiological function in chronic disease states and identifying 'fitness' to undergo systemic stressors such as thoracic or abdominal surgery. The prognostic ability of LT exceeds that of many other clinically or physiologically based measures, often by severalfold, even though the mechanisms associating LT and morbidity/mortality are still not well known.

Coincidence of exercise thresholds and their mechanistic bases

In physiology, 'thresholds' are powerful concepts constituting fundamental 'tipping points' that establish boundaries between distinct domains of physiological control, such as the moderate→heavy→very heavy/severe domains of exercise intensity (Fig. 10). Knowledge of certain thresholds offers the promise of, among other things, predicting athletic performance; assessing the ability to perform essential work-related physical tasks and tracking efficacy of training or therapeutic countermeasures to ageing or organic disease; and stratifying patient populations for surgical procedures such as heart transplantation (Table 1, and see section 'Uses of the lactate or gas exchange threshold' above). Unfortunately, problems of nomenclature, definition, measurement and interpretation can lead to confusion and this has certainly happened with the AT. This section will briefly survey the plethora of extant threshold concepts, including the AT. The

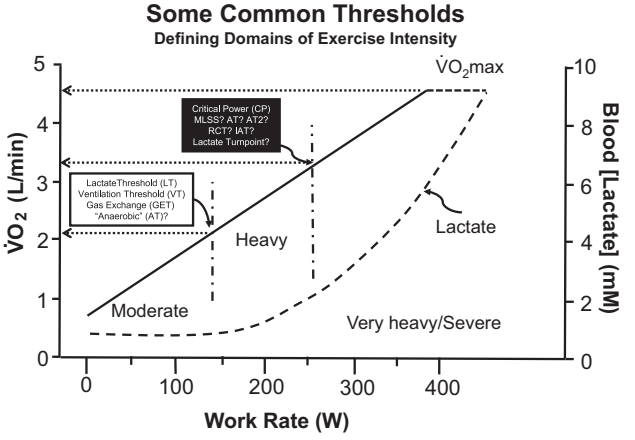


Figure 10. Common thresholds that have been considered, though not unequivocally, to partition the primary exercise intensity domains: moderate, heavy and very heavy/severe
For reviews, see Poole & Jones (2012) and Poole *et al.* (2016). See text for details.

Table 1. Pros and cons of threshold concepts in exercise science

Potential to:	Problems
(1) Teach about physiological mechanisms	(1) Definition and measurement: largely correlational analyses
(2) Establish rigorous exercise intensity domains in which to investigate mechanisms (e.g., exhaustion)	(2) Proliferation of terms breeds confusion
(3) Predict athletic performance	(3) Misleading as regards mechanistic bases ('anaerobic threshold')
(4) Monitor training progress	(4) Complexity: hierarchy of importance needs to be established
(5) Clinically assess patient physiological function/dysfunction: heart failure, diabetes, peripheral artery disease, ageing	(5) Disease changes mechanism (e.g. McArdle's disease)
(6) Determine suitability for surgery/transplant	(6) Misapplication to patients possible (harm > good)
(7) Assess therapeutic efficacy	

arguments will be supported that, rather than detecting the moderate–heavy exercise boundary as the AT does, knowing the heavy–severe boundary, as determined by the CP/CS) concept (see Fig. 11 and 'Critical power' section below), is more relevant to athletic performance (Vanhatalo *et al.* 2011; Poole *et al.* 2016; Craig *et al.* 2018) and in keeping with the original intent of the AT concept. In addition, the case will be made that knowledge of CP/CS is also more germane to unravelling key aspects of physiological control, such as the causes of fatigue and exhaustion, than is the AT. Indeed, CP fulfils far better

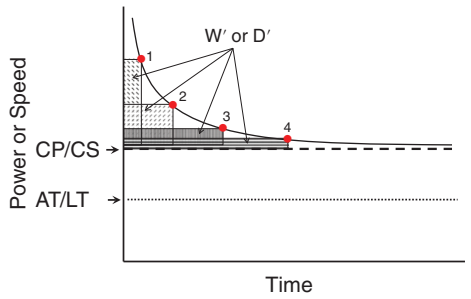


Figure 11. Critical power (CP) or critical speed (CS) is typically resolved using a series of four or more constant-load exercise bouts, each performed on a separate day, over a range of work rates (CP) or speeds (CS) that exhaust the participant between 2 and 15 min (each red circle denotes limit of tolerance for one separate such test)
Note that CP/CS occurs at a power or speed different from, and appreciably exceeding, the so-called anaerobic threshold (AT) or La^- threshold (LT). The parameter W' (or D' for CS) represents a finite energy store that is expended above CP/CS at a rate dependent upon power or speed sustained relative to CP or CS. For cycle ergometry W' is in kJ whereas for running D' constitutes a finite distance. Note that the hatched areas are precisely the same for each exercise bout representing the finite value of W' or D' irrespective of work rate or speed above CP or CS. The asymptote (CP, CS) constitutes the highest rate of oxidative energy expenditure, often expressed as $\dot{V}_{O_2}$, that can be sustained without drawing continuously on W' or D'. Adapted from Poole et al. (2016).

the intent and purpose of the original AT concept than the AT itself! (See Fig. 11 and legend for a description of the CP/CS concept.) Specifically, as detailed in 'The lactate shuttle and the "anaerobic threshold"' section above, an elevated, but stable, level of blood [La^-] can be sustained for constant power exercise above AT (Fig. 7, heavy intensity). It is only for exercise performed above CP/CS that the energy storage component (W', Fig. 11) consisting principally of non-oxidative energy sources (i.e. glycolysis, PCr), is continually depleted and the rate of La^- appearance (R_a) continuously exceeds its rate of disappearance (R_d) in the blood. Exhaustion during very heavy/severe-intensity exercise occurs within a duration that is highly predictable when CP (or CS) and W' (or D') are known. Importantly, almost all Olympic athletic track and other contests from the marathon (∼96% CS), across a plethora of shorter events down to the 800 m are contested in the heavy- (i.e. <CS, marathon) and across the very heavy/severe-intensity domain (i.e. >CS, 10,000 m and shorter; Poole *et al.* 2016; Jones & Vanhatalo, 2017; Craig *et al.* 2018).

Common threshold concepts: partitioning the range of exercise intensities. The AT has been one of the most studied of physiological phenomena (rev. Peinado *et al.* 2014; Jones *et al.* 2018) with attempts to detect the AT, in addition to blood La^- sampling, by a plethora of indices mostly during progressive incremental/ramp exercise (Fig. 9 presents three of these: blood [La^-], ventilation, and $\dot{V}_{CO_2}$ to $\dot{V}_{O_2}$ relation or V-slope). The physiological variables proposed to detect AT include discontinuities in ventilation (Naimark *et al.* 1964; Wasserman & McIlroy, 1964), pulmonary gas exchange (GET; Beaver *et al.* 1986), RER (Wasserman *et al.* 1973), muscle electromyography (Jurimae *et al.* 2007), heart rate (Conconi *et al.* 1980), plasma catecholamines (Davies *et al.* 1974), salivary [amylase] (Chicharro *et al.* 1999), deoxy-[Hb+Mb], oxygen extraction and haemodynamics

(Crisafulli *et al*. 2006; Coquart *et al*. 2017), and rate of perceived exertion (Voorn *et al*. 2014; Coquart *et al*. 2017; Alberton *et al*. 2019) (rev. (Peinado *et al*. 2014). Whereas some have interpreted these responses as a sign of a global central nervous system-coordinated response (e.g. Peinado *et al*. 2014), rigorous prospective interventional studies rather than correlational observations are requisite to unveil mechanistic connections among these disparate variables, if such connections exist. Even the correlational observations available to date are confounded by problems related to detection method, selective sampling (e.g. superficial muscle for EMG and deoxy-[Hb+Mb]), system and transit-time delays, and, often, poor signal-to-noise ratios and among-subject variability. The latter concern is epitomized by the broad span of carotid body sensitivity normally found among the populace and also the high breath-to-breath variability that conflate to challenge accurate detection of the ventilatory threshold.

Notwithstanding the above concerns, when the LT and GET are operationally defined and measured carefully, they provide invaluable demarcation of the boundary between the moderate and heavy exercise intensity domains (Figs 7, 8 and 10) (Henson *et al*. 1989; Poole & Jones, 2012). Specifically, moderate exercise is characterized by rapid monoexponential pulmonary $\dot{V}_{O_2}$ kinetics (in most healthy young subjects) and blood [La^-] does not increase above rest, *versus* heavy exercise where $\dot{V}_{O_2}$ kinetics evince a slow component that increases the $\dot{V}_{O_2}$ amplitude, slowing the overall response and decreasing efficiency, and blood [La^-] is elevated (rev. Poole & Jones, 2012).

Problems with the lactate threshold: how to take a straightforward idea and make it hopelessly complex. Whether LT can be detected non-invasively by means of the ventilatory or gas exchange profiles during incremental/ramp ergometry (Naimark *et al*. 1964; Wasserman & McIlroy, 1964) has been a matter of great contention over the past few decades. This question matched the gurus of pulmonary gas exchange headed by Brian J. Whipp, Karlman Wasserman and James A. Davis against biochemistry and metabolism experts such as George Brooks (see Davis (1985) *versus* Brooks (1985)). In linking the non-linear ventilatory response to the excess CO_2 generated by the bicarbonate buffering of the H^+ associated with La^-, both the rate of La^- (and hence H^+) appearance and the sensitivity and time course of the subsequent ventilatory response were crucial. This led to establishing recommendations that participants should reach their maximum tolerance on the incremental/ramp test in ∼10 min or so (Buchfuhrer *et al*. 1983). But the fact that LT, determined from the blood [La^-] response, could be dissociated from ventilation-derived variables using altered pedal frequency and glycogen depletion/dietary modifications (Hughes *et al*. 1982) and/or exercise training (Poole & Gaesser, 1985) eroded faith in the underlying connection between the LT and Ventilatory Threshold. This problem was diminished by removing emphasis on the overall ventilatory response and focusing specifically on detecting the break-point in the $\dot{V}_{CO_2}$ *versus* $\dot{V}_{O_2}$ relationship (i.e. V-slope procedure; Beaver *et al*. 1986; Fig. 9*C*).

Notwithstanding the above concerns, with the modern availability of inexpensive, accurate and convenient rapid blood [La^-] analysis, direct determination of the LT should be relatively straightforward. However, this has not proven to be the case. Although the La^- response to exercise is highly reproducible when conditions are standardized it can be altered by factors such as muscle glycogen concentration, changed acid–base balance, dietary manipulations, sampling site, pharmacological intervention, ambient temperature and likely hydration state (rev. Jacobs, 1986). Furthermore, the rate of ramp increase can alter the work rate (but not the $\dot{V}_{O_2}$) at which the LT occurs (Davis *et al*. 1982; Whipp *et al*. 1982; rev. Faude *et al*. 2009; Jamnick *et al*. 2018). Moreover, as evident in Figs 1, 10 and 12, depending on the subject, incremental/ramp characteristics, site of blood sampling (venous, capillary, arterial), and frequency of [La^-] measurement, the [La^-] profile may be curvilinear without a readily discernible breakpoint or threshold. These considerations, among others, have spawned confusion and a veritable plethora of threshold concepts. An exemplar of this appears in an exhaustive review of LT concepts (Faude *et al*. 2009). These authors identified more than 25 different LT measures, with some detecting the initial rise in [La^-] above resting, others a fixed [La^-] of 2 or 4 mM, for instance, and some the maximal lactate steady-state (MLSS; notionally similar to CP/CS) (Faude *et al*. 2009; see also Sjodin & Jacobs, 1981; Hofmann *et al*. 1994; Jones *et al*. 2019). Figure 12 illustrates how this, perhaps well-intentioned but hopelessly complex and confusing, proliferation of [La^-]-related concepts looks when plotted, perhaps not ideally, against work rate (Jamnick *et al*. 2018). Given the curvilinear nature of the [La^-] *versus* work rate relationship, it should be clear that any method of threshold determination represents an attempt to describe a curve with a single data point. Recognition that the various methods of determination are no doubt highly correlated with one another should temper any tendency towards dogmatic (and perhaps arbitrary) preference for one specific definition of the LT over all others.

Insights into the curvilinear nature of [La^-] *versus* work rate derive from the work of Stanley *et al*. (1985) in which arterial [La^-] and La^- R_a and R_d (Fig. 1) were measured during each step of a progressive exercise test together with cardiac output and $\dot{V}_{O_2}$. Each of these variables responds to the exercise forcing with its own

individual time constant, the slowest of which is the La^- R_d, removing any sharp break points. Continuous ramp, as opposed to progressive step tests, will inevitably evince very different cardiovascular, pulmonary, and La^- R_a and R_d relationships because, during submaximal constant-power output exercises, R_d may rise to match R_a resulting in a constant blood [La^-] that is dependent on the subject's La^- clearance capacity (Messonnier *et al.* 2013). It is likely that a relative infrequency of blood La^- measurement combined with inattention to precise exercise test structure (e.g. step size and step *versus* ramp) and lack of appreciation of R_a and R_d has conspired to create insufficiently granular [La^-] profiles that could be interpreted (or misinterpreted) as distinct thresholds in the literature.

A profusion and confusion of thresholds? It may be argued that the lowest $\dot{V}_{O_2}$ at which there is a continuously increasing blood [La^-] for the tolerable duration of exercise, and thus a sustained non-oxidative contribution to ATP production, fulfils the spirit of Wasserman's AT concept. Thus, it has been demonstrated that, above CP/CS (or, more correctly, their metabolic equivalents), all constant-power output exercise bouts are attended by an inexorably increasing blood [La^-] and a pronounced $\dot{V}_{O_2}$ slow component that drives $\dot{V}_{O_2}$ towards $\dot{V}_{O_2max}$ (Hill *et al.* 2002; Keir *et al.* 2015). Moreover, exhaustion is manifested within a finite time as defined by the power–duration relationship (e.g. CP and W') (Jones & Poole, 2008; Jones *et al.* 2010; Poole *et al.* 2016). Not surprisingly, given the confusion of thresholds identified in Fig. 12, this 'higher threshold' itself (CP/CS) has received multiple alternative names. Specifically, for some it has simply been considered the AT, or, alternatively, the second AT (AT_2), the individual anaerobic threshold, the respiratory (or ventilatory) compensation point/threshold (RCP/RCT), the La^- turnpoint (Smith & Jones, 2001), the aerobic/anaerobic threshold, the MLSS and the fatigue threshold among many others (Figs 10 and 12) (rev. Faude *et al.* 2009; Jamnick *et al.* 2018). Whether these terms actually represent a common metabolic rate has been the topic of much debate and polemic (see Broxterman *et al.* (2018) *versus* Keir *et al.* (2018)). Certainly methodological features of their determination might result in the MLSS underestimating CP or CS and the RCT/RCP

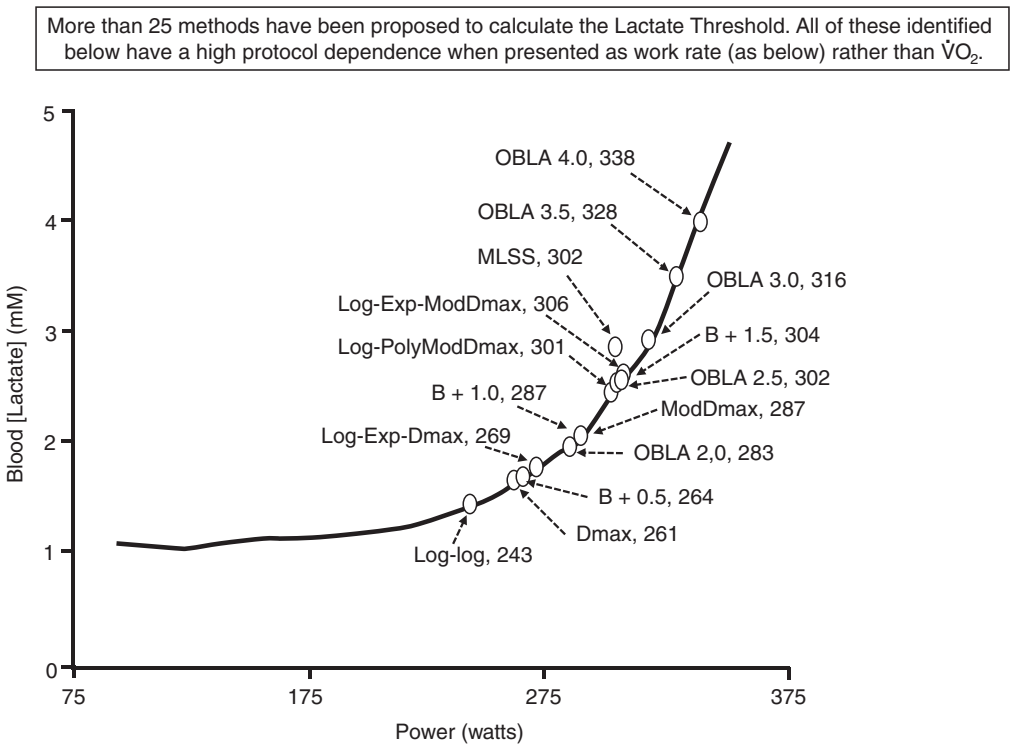


Figure 12. Blood [La^-] response to incremental cycling exercise with 14 different La^- 'thresholds' (LTs) calculated and identified for one participant
Abbreviations: B, baseline [La^-]; MLSS, maximal lactate steady-state, established from a separate series of constant-load tests; OBLA, onset of blood La^- accumulation. Power output for each is noted in watts following each LT. The numbers beside each threshold method indicate the power output for that particular method; note the enormous range of work rates from 243 to 338 W. Adapted from Jamnick *et al*. (2018). This figure emphasizes the complexity of this field. For specialist definitions and methods of calculation for each 'threshold' the reader is referred to the original paper (Jamnick *et al*. 2018).

overestimating the work rate or speed, or the $\dot{V}_{O_2}$ (Barker *et al*. 2006), at which CP or CS occurs, for instance (Jones *et al*. 2019). A recent meta-analysis demonstrates that this is indeed the case (Galán-Rioja *et al*. 2020).

Critical power concept. The curvilinear profile of the power (P)–duration (t) relationship (and its parameters CP, W', Fig. 11; Monod & Scherrer, 1965) defines exercise tolerance for locomotory activity across all species examined including amphibia (salamander, Full, 1986; rodentia, mouse, Billat *et al*. 2005, and rat, Copp *et al*. 2010; and equid, horse, Lauderdale & Hinchcliff, 1999). This is also true for alternative muscle contraction paradigms such as isometric (Monod & Scherrer, 1965) and isotonic (Jones *et al*. 2008; Burnley, 2009) (see also Briggs (1920) for an early identification of the 'fatigue threshold' (Zoladz & Grassi, 2020)) as well as disparate exercise modalities in humans, specifically, running (Hughson *et al*. 1984; Fukuba & Whipp, 1999; Fukuba *et al*. 2003; Broxterman *et al*. 2013), cycling (Poole *et al*. 1988, 1990; Hill, 1993; Neder *et al*. 2000*a*,*b*; Pringle & Jones, 2002; Hill, 2004; Vanhatalo *et al*. 2007), swimming (Wakayoshi *et al*. 1993) and rowing (Hill *et al*. 2003). Consequently, the hyperbolic P–t relationship is highly conserved with its mechanistic underpinnings and close coherence with systemic responses ($\dot{V}_{O_2}$, blood [La^-]) and muscle metabolism supporting its deterministic role in defining exercise performance.

Unlike any of the other threshold concepts considered above, CP (or CS) is the only one that predicts closely the tolerable duration of high intensity exercise that precedes intolerance (i.e. t_{lim}). Thus, t_{lim} is dictated by the proximity of the power output or speed to CP and is dependent upon the size of the energy equivalent parameter W′ (or D′) according to $t_{lim} = W'/(P - CP)$, where t_{lim} is the tolerable duration of exercise in seconds, W' is given in joules, P is the extant power in watts and CP is critical power in watts. In this respect, CP is notionally equivalent to the MLSS but its determination is free from overt measurement bias towards lower metabolic or work rates (Fig. 11) (Jones *et al*. 2019). This being the case, it is instructive to examine the physiological characteristics of exercise performed below, as opposed to just above, CP as these may provide important insights into the very mechanisms of exercise limitation itself, potentially fulfilling the scientific prerogative that was intended by the original development of the AT concept.

As demonstrated in Fig. 13, exercise performed above *versus* below CP elicits a completely different spectrum

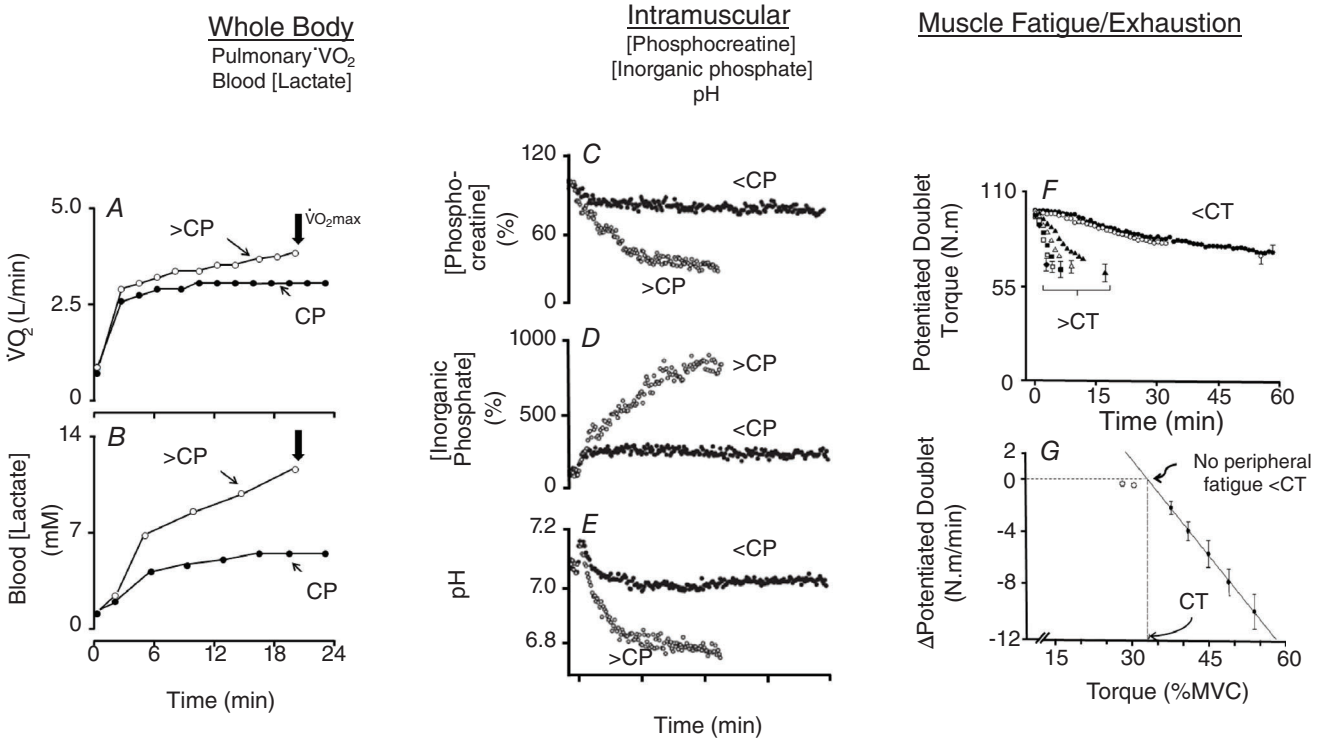


Figure 13. Compendium of physiological responses for critical power (CP) and critical torque (CT)
CP and CT denote the metabolic threshold above which each of the following variables changes inexorably until exhaustion is manifested: *A*, oxygen uptake ($\dot{V}_{O_2}$); *B*, blood [La^-]; *C*, muscle [phosphocreatine]; *D*, [inorganic phosphate]; *E*, pH; *F*, potentiated muscle torque (i.e. progressive fatigue); *G*, delta potentiated doublet torque. *A* and *B* from (Poole & Jones,2012); *C*–*E* from (Jones *et al*. 2008); *F* and *G* redrawn with permission from (Burnley *et al*. 2012).

of pulmonary (ventilation and gas exchange, $\dot{V}_{O_2}$), blood acid–base and intramuscular (P_i, H^+, ADP, PCr) profiles as well as muscle fatigue characteristics (i.e. critical torque (CT)). Specifically, in all instances, during <CP exercise each variable can be stabilized and is associated with the ability to sustain exercise. In marked contrast, during >CP exercise no stabilization is feasible and the perturbation of each variable increases to the point of exhaustion. Crucially, the continuous depletion of the energy equivalent parameter W' (which is related to, but not synonymous with, 'anaerobic work capacity') substantiates that CP constitutes, at least in Wasserman *et al.*'s terminology, a threshold for reliance on glycolysis with net La^- accumulation contributing to the sustained energetics of exercise (Naimark *et al.* 1964; Wasserman & McIlroy, 1964). However, as was the case for the original AT theory, no compelling evidence has been presented for the muscle becoming 'anaerobic' even above CP; see previous section on 'O_2 and the anaerobic threshold' for background.

Historical importance of thresholds to humankind and shaping the modern world

Many of the athletic events that constitute today's modern Olympics are based upon physical abilities that were once critical for the survival of our species, *Homo sapiens*, or shaping the modern world through exploration, warfare and conquest (Fig. 14). Across all of these endeavours it is evident that the CP (or CS) rather than the AT, as considered by Wasserman et al. (Naimark *et al.* 1964; Wasserman & McIlroy, 1964), denotes the pertinent 'threshold' (Poole *et al.* 2016; Craig *et al.* 2018). A few selected exemplars throughout history are as follows:

- Our ancestors' hunting prowess over ungulate prey such as antelope, deer and bison, tens of millennia ago, fuelled our migration 'out of Africa'. By sustaining running speeds (i.e. heavy intensity, <CS) faster than their prey's trot-gallop transition (Bramble & Lieberman, 2004; Lieberman & Bramble, 2007) early members of the genus *Homo* could compromise that prey's thermoregulatory and metabolic stability leading to exhaustion.
- Modern reconstructions using trained rowers indicate that 75% (full crew of 170 men) and 83% (54 resting) $\dot{V}_{O_2max}$ was required to row an Athenian *trieresis* (modern: triremes, three-tiered warships) at five knots (Rossiter & Whipp, 2012) (Fig. 14). This is consistent with sustained, intermittent exercise (at a 2:1 duty ratio), at metabolic rates in the heavy intensity domain (i.e. <CP) for well-trained men. A small fleet of such ships sustaining this for >24 h famously defeated Persian invaders in the battle of Salamis (Morrison *et al.* 2000): a victory that conserved the political and economic conditions necessary for Athenian society to

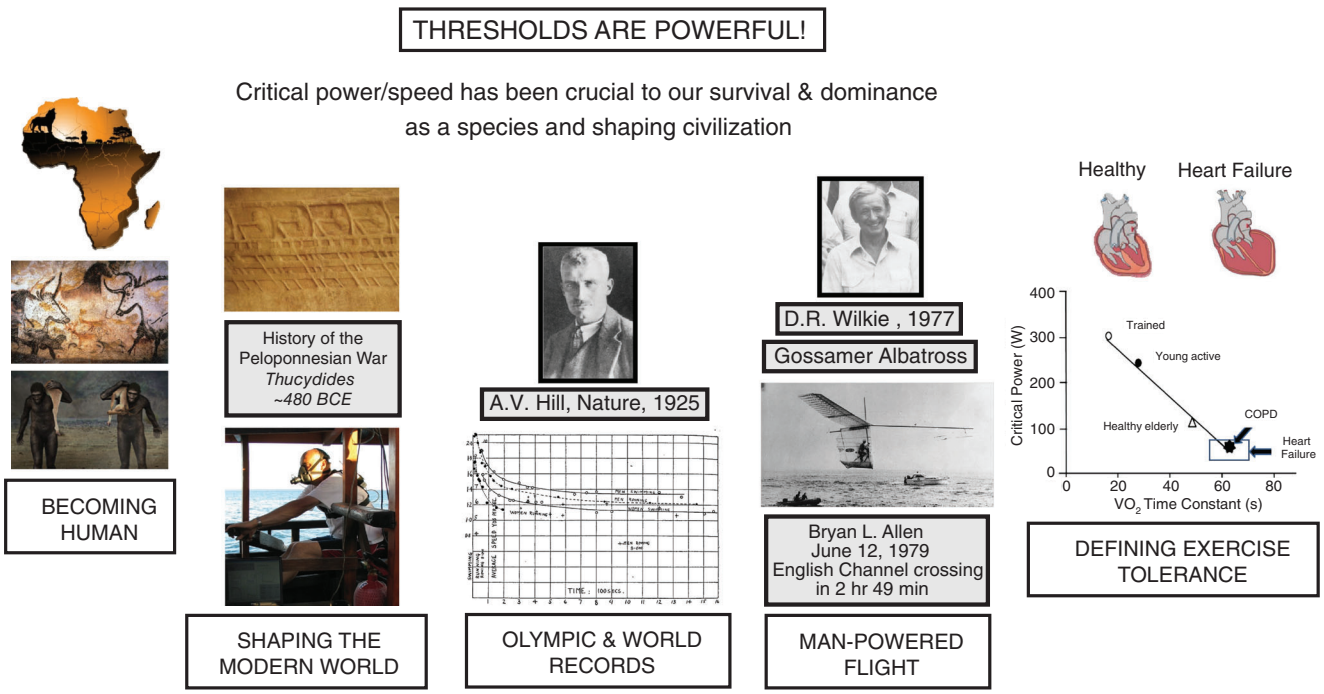


Figure 14. Practical exemplars of how thresholds, most notably the critical power/speed, have helped shape the survival of humankind as a species and, throughout history down to the modern world, defined a great empire, crafted Olympic and world records, enabled man-powered flight and determined exercise tolerance across the age-span in health and in disease
See text for more information and salient references.

make its contributions to art, literature, philosophy and democracy.

- A century ago, British physiologist and Nobel laureate Archibald Vivian Hill, studying the energetics of isolated frog muscles and human performance, determined that aerobic and anaerobic energy sources were coordinated to power muscle contractile performance (Hill, 1910; Hill *et al.* 1924*a*). Hill's speed–time curves for extant athletic World records yielded the familiar hyperbolic decrease in speed or velocity when plotted against event duration (Figs 11 and 14) (Hill, 1925). This relationship, with obviously different parameter (CS, D') values accounting for improved athletic performance, is evident for contemporary world records for runners, swimmers, rowers and cyclists (Bassett, 2002; Jones *et al.* 2010; Poole *et al.* 2016; Jones & Vanhatalo, 2017).
- The work of Monod & Scherrer (1965) introduced above was critical to Dawson & Wilkie (1977)'s calculation of the power–mass ratio necessary for the 22.5 mile man-powered flight of the Gossamer Albatross across the English Channel by Bryan L. Allen in 1979 (Fig. 14) (see also Wilkie, 1960).

Today, it is widely acknowledged that, for individuals performing almost any form of physical activity, relating the elevated blood [La^-] response to $\dot{V}_{O_2}$ is an excellent predictor of performance and certainly superior to $\dot{V}_{O_2max}$ (rev. Jacobs, 1986). But, as CP/CS approximates more closely the exercise intensity used in competitive events (Jones & Vanhatalo, 2017) and discriminates between heavy and very heavy/severe-intensity exercise, this measurement has substantial utility and relates tightly to exercise performance *per se*, as expected. For instance, CP explains more than 80% of the performance variance among cyclists over 17 and 40 km time trials (Smith *et al.* 1999; see also Black *et al.* 2014; Jones & Vanhatalo, 2017). The significant correlation between CP and $\dot{V}_{O_2}$ kinetics across the performance spectrum from athletes to pulmonary and cardiac patients (Fig. 14) (Rossiter, 2011) highlights the strong interplay between metabolic control and exercise tolerance (Black *et al.* 2017; Jones & Vanhatalo, 2017; Burnley & Jones, 2018; Goulding *et al.* 2019).

Conclusions of this section

(1) Thresholds represent fundamental 'tipping points' that can teach us about physiological control mechanisms.
(2) Problems of definition combined with a wide array of forcing functions and measurement techniques have created general confusion.
(3) Critical power/speed likely represents the threshold above which there is a continuous obligatory glycolytic contribution with net La^- accumulation to bioenergetics – though there is no evidence that the muscle itself becomes 'anaerobic' or dysoxic.
(4) At present, when it is possible for an individual to undertake high-intensity exhausting exercise, critical power/speed offers, perhaps, the greatest *potential* to predict athletic performance, clinically assess healthy/patient physiological function and monitor training efficacy.

Overall summary and conclusions

The AT concept, as proposed 50 years ago by Karlman Wasserman and colleagues (Naimark *et al.* 1964; Wasserman & McIlroy, 1964), considered muscle anoxia/hypoxia as the progenitor of the increased muscle and blood [La^-], termed 'Owles's point' in 1930 (Owles, 1930). In turn, the H^+ associated with La^- accumulation, alters blood acid–base status and augments CO_2 evolution driving a non-linear ventilatory response detectable, most conveniently, breath-by-breath during a progressive exercise test (e.g. see Fig. 9*C* for averaged minute responses). This was a bold but largely unsubstantiated schema. However, in keeping with Francis Bacon's edict that 'truth emerges more readily from error than from confusion' (Bacon, 1869), the AT inspired intense scientific attention that led to an unprecedented spate of discoveries in muscle metabolic control, La^- metabolism, blood homeostasis and the control of breathing. Today no direct evidence has been found to support that exercising muscle becomes anaerobic or dysoxic at the LT, or indeed at any $\dot{V}_{O_2}$ up to $\dot{V}_{O_2max}$. In fact, there is much evidence to the contrary (e.g. Jöbsis & Stainsby, 1968; Connett *et al.* 1985; Richardson *et al.* 1995, 1998; Honig *et al.* 1997). Moreover, it is now recognized that elevated blood [La^-] indicates that the rate of La^- production or appearance (R_a) has exceeded that of its disposal or disappearance (R_d). Far from being the 'dead-end' metabolite considered by Huckabee (1958), La^- is now known to constitute an important energetic substrate via oxidation in multiple tissues including contracting skeletal muscles and heart (Gertz *et al.* 1988; Bergman *et al.* 1999*b*) as well as serving as a gluconeogenic precursor (Bergman *et al.* 2000). Rather than being blamed for fatigue-inducing or otherwise pernicious properties, compelling evidence supports that La^- production is crucial to myocyte energetics in aerobic tissues (Connett *et al.* 1985) and serves as a fundamental strain response mitigating stress with its targeted supplementation opposing physiological or life-threatening challenges to homeostasis (Brooks, 2018; Ferguson *et al.* 2018). Whereas the potential therapeutic application of La^- in the clinical environments of cancer treatment and brain trauma recovery, for example, is still

in its infancy, without Wasserman's bold AT concept such possibilities may not have been recognized or at least not as soon.

Across the spectrum of work rates from unloaded cycling to the maximum achievable on the rapid (reaching exhaustion ∼10–12 min) progressive ramp or its equivalent on the motor-driven treadmill, for example, and depending on the precise measurements made, at least two points of discontinuity (i.e. thresholds) are detectable. For young healthy, but not highly trained individuals, at ∼50% $\dot{V}_{O_2max}$ blood [La^-] begins to increase above resting baseline values. At present, this point is best identified directly by blood sampling and [La^-] measurement or non-invasively as the gas exchange threshold or GET by measurement of the upward inflection of $\dot{V}_{CO_2}$ *versus* $\dot{V}_{O_2}$ breath-by-breath (i.e. V-slope with corroboration of isocapnic buffering from other gas exchange variables) (Beaver *et al*. 1986) rather than the ventilatory profile *per se* (Wasserman & McIlroy, 1964; Wasserman *et al*. 1973; Caiozzo *et al*. 1982). Crucially, although the $\dot{V}_{O_2}$ (but not the power output) at LT/GET does discriminate the boundary between the moderate (no increase in [La^-] or $\dot{V}_{O_2}$ slow component) and heavy (increased [La^-], $\dot{V}_{O_2}$ slow component, decreased work efficiency) exercise intensity domains, it does not identify the $\dot{V}_{O_2}$ above which there is an obligatory non-oxidative energetic contribution to the exercise energetics. Rather, it is the $\dot{V}_{O_2}$ corresponding to the heavy–very heavy/severe intensity boundary, typically ∼70% $\dot{V}_{O_2max}$ (or higher in trained/elite athletes), that separates exercise for which blood [La^-] and $\dot{V}_{O_2}$ can be stabilized (i.e. heavy) from that demanding a sustained non-oxidative contribution that drives $\dot{V}_{O_2}$ to $\dot{V}_{O_2max}$ (i.e. very heavy/severe). This heavy–very heavy/severe boundary has garnered a panoply of names dependent, in part, on its method of determination including AT, AT_2, lactate turnpoint, respiratory compensation threshold or point (RCT/RCP), and maximal La^- steady-state (MLSS). Irrespective of the precise measurement, be it blood gas, metabolite or gas exchange, or methodology (test type, ramp or square wave, etc.) it is proposed that the underlying physiological mechanisms discriminating heavy and thus sustainable exercise from very heavy/severe exercise are encompassed within the CP (or CS) concept (Jones *et al*. 2010, 2019; Poole *et al*. 2016; Craig *et al*. 2018). Of extant thresholds, CP/CS may thus best represent the scientific intent of Dr Wasserman's AT concept and offer the greatest potential to predict athletic performance, assess healthy/patient physiological function and monitor training efficacy.

Notwithstanding a variety of concerns, unlike CP/CS or $\dot{V}_{O_2max}$, measurements of GET or LT possess the singular advantage of being effort-independent, a benefit that cannot be overemphasized in clinical medicine as opposed to athletics. Moreover, though requiring more extensive and expensive equipment as well as specialist data interpretation, GET is non-invasive. In addition, GET/LT is highly responsive to clinical conditions that manipulate O_2 delivery including therapeutic interventions such as cardiac resynchronization therapy, and exercise training in chronic heart and/or lung disease. This lower threshold also has utility for predicting athletic performance, informing optimal nutritional strategies for ultra-endurance athletes, assessing integrative systemic function to identify pathological dysfunction, and gauging a patient's exercise load for surgical pre- or re-habilitation. Finally, GET/LT informs decision-making for high-risk surgical/transplant patients and serves to estimate life expectancy in heart failure patients.

With these valuable practical attributes to the GET/LT phenomenon across the fields of physiology, medicine and athletics it is indeed unfortunate that its mechanistically and scientifically inappropriate sobriquet, 'anaerobic threshold', faces continued widespread misunderstanding and sometimes misuse. It is the authors' intent that this review serves to focus attention on the enormous power of threshold concepts in exercise physiology and clinical medicine. Among many other attributes, when interpreted correctly, thresholds can act as a springboard to enhance our mechanistic understanding of metabolic and respiratory control, exercise energetics, integrative physiology and the processes of fatigue and intolerance in health and disease.

References

Agostoni P, Bianchi M, Moraschi A, Palermo P, Cattadori G, La Gioia R, Bussotti M & Wasserman K (2005). Work-rate affects cardiopulmonary exercise test results in heart failure. *Eur J Heart Fail* **7**, 498–504.

Agostoni P, Corra U, Cattadori G, Veglia F, Battaia E, La Gioia R, Scardovi AB, Emdin M, Metra M, Sinagra G, Limongelli G, Raimondo R, Re F, Guazzi M, Belardinelli R, Parati G, Magri D, Fiorentini C, Cicoira M, Salvioni E, Giovannardi M, Mezzani A, Scrutinio D, Di Lenarda A, Mantegazza V, Ricci R, Apostolo A, Iorio A, Paolillo S, Palermo P, Contini M, Vassanelli C, Passino C, Piepoli MF & Group MSR (2013). Prognostic value of indeterminable anaerobic threshold in heart failure. *Circ Heart Fail* **6**, 977–987.

Agostoni P, Valentini M, Magri D, Revera M, Caldara G, Gregorini F, Bilo G, Styczkiewicz K, Savia G & Parati G (2008). Disappearance of isocapnic buffering period during increasing work rate exercise at high altitude. *Eur J Cardiovasc Prev Rehabil* **15**, 354–358.

Ahmed K, Tunaru S, Tang C, Muller M, Gille A, Sassmann A, Hanson J & Offermanns S (2010). An autocrine lactate loop mediates insulin-dependent inhibition of lipolysis through GPR81. *Cell Metab* **11**, 311–319.

Alberton CL, Andrade LS, Pinheiro RB & Pinto SS (2019). Anaerobic threshold in a water-based exercise: agreement between heart rate deflection point and lactate threshold methods. *J Strength Cond Res*, doi: 10.1519/JSC.0000000000003161.

Auricchio A, Stellbrink C, Sack S, Block M, Vogt J, Bakker P, Huth C, Schondube F, Wolfhard U, Bocker D, Krahnefeld O, Kirkels H; Pacing Therapies in Congestive Heart Failure (PATH-CHF) Study Group (2002). Long-term clinical effect of hemodynamically optimized cardiac resynchronization therapy in patients with heart failure and ventricular conduction delay. *J Am Coll Cardiol* **39**, 2026–2033.

Bacon F (1869). *Novum Organum. The Works of Francis Bacon*, Vol. VIII. Houghton Mifflin, New York.

Bakeeva LE, Chentsov Yu S & Skulachev VP (1978). Mitochondrial framework (reticulum mitochondriale) in rat diaphragm muscle. *Biochim Biophys Acta* **501**, 349–369.

Bang O (1936). The lactate content of the blood during and after muscular exercise in man. *Skand Archiv Physiol* **74**, 51–82.

Barker T, Poole DC, Noble ML & Barstow TJ (2006). Human critical power-oxygen uptake relationship at different pedalling frequencies. *Exp Physiol* **91**, 621–632.

Barnard RJ & Holloszy JO (2003). The metabolic systems: aerobic metabolism and substrate untilization in exercising skeletal muscle. In *Exercise Physiology People and Ideas*, ed. Tipton CE, pp. 292–321. Oxford University Press, New York.

Barnett JA (2003). A history of research on yeasts 5: The fermentation pathway. *Yeast* **20**, 509–543.

Bassett DR Jr (2002). Scientific contributions of A. V. Hill: exercise physiology pioneer. *J Appl Physiol* **93**, 1567–1582.

Beaver WL, Wasserman K & Whipp BJ (1986). A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* **60**, 2020–2027.

Benninga HA (1990). *A History of Lactic Acid Making: A Chapter in the History of Biotechnology*. Kluwer Academic Publishers, Boston, MA.

Bergman BC, Butterfield GE, Wolfel EE, Lopaschuk GD, Casazza GA, Horning MA & Brooks GA (1999*a*). Muscle net glucose uptake and glucose kinetics after endurance training in men. *Am J Physiol* **277**, E81–E92.

Bergman BC, Horning MA, Casazza GA, Wolfel EE, Butterfield GE & Brooks GA (2000). Endurance training increases gluconeogenesis during rest and exercise in men. *Am J Physiol Endocrinol Metab* **278**, E244–E251.

Bergman BC, Tsvetkova T, Lowes B & Wolfel EE (2009). Myocardial glucose and lactate metabolism during rest and atrial pacing in humans. *J Physiol* **587**, 2087–2099.

Bergman BC, Wolfel EE, Butterfield GE, Lopaschuk GD, Casazza GA, Horning MA & Brooks GA (1999*b*). Active muscle and whole body lactate kinetics after endurance training in men. *J Appl Physiol* **87**, 1684–1696.

Billat VL, Mouisel E, Roblot N & Melki J (2005). Inter- and intrastrain variation in mouse critical running speed. *J Appl Physiol* **98**, 1258–1263.

Black MI, Durant J, Jones AM & Vanhatalo A (2014). Critical power derived from a 3-min all-out test predicts 16.1-km road time-trial performance. *Eur J Sport Sci* **14**, 217–223.

Black MI, Jones AM, Blackwell JR, Bailey SJ, Wylie LJ, McDonagh ST, Thompson C, Kelly J, Sumners P, Mileva KN, Bowtell JL & Vanhatalo A (2017). Muscle metabolic and neuromuscular determinants of fatigue during cycling in different exercise intensity domains. *J Appl Physiol* **122**, 446–459.

Bowen TS, Cannon DT, Begg G, Baliga V, Witte KK & Rossiter HB (2012). A novel cardiopulmonary exercise test protocol and criterion to determine maximal oxygen uptake in chronic heart failure. *J Appl Physiol* **113**, 451–458.

Bramble DM & Lieberman DE (2004). Endurance running and the evolution of *Homo*. *Nature* **432**, 345–352.

Bricker DK, Taylor EB, Schell JC, Orsak T, Boutron A, Chen YC, Cox JE, Cardon CM, Van Vranken JG, Dephoure N, Redin C, Boudina S, Gygi SP, Brivet M, Thummel CS & Rutter J (2012). A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, *Drosophila*, and humans. *Science* **337**, 96–100.

Briggs H (1920). Physical exertion, fitness and breathing. *J Physiol* **54**, 292–318.

Brooks GA (1985). Anaerobic threshold: review of the concept and directions for future research. *Med Sci Sports Exerc* **17**, 22–34.

Brooks GA (1986). The lactate shuttle during exercise and recovery. *Med Sci Sports Exerc* **18**, 360–368.

Brooks GA (2000). Intra- and extra-cellular lactate shuttles. *Med Sci Sports Exerc* **32**, 790–799.

Brooks GA (2002). Lactate shuttles in nature. *Biochem Soc Trans* **30**, 258–264.

Brooks GA (2007). Lactate: link between glycolytic and oxidative metabolism. *Sports Med* **37**, 341–343.

Brooks GA (2009). Cell-cell and intracellular lactate shuttles. *J Physiol* **587**, 5591–5600.

Brooks GA (2016). Energy flux, lactate shuttling, mitochondrial dynamics, and hypoxia. *Adv Exp Med Biol* **903**, 439–455.

Brooks GA (2018). The science and translation of lactate shuttle theory. *Cell Metab* **27**, 757–785.

Brooks GA (2020*a*). Lactate as a fulcrum of metabolism. *Redox Biol* **35**, 101454.

Brooks GA (2020*b*). The tortuous path of lactate shuttle discovery: From cinders and boards to the lab and ICU. *J Sport Health Sci* **9**, 446–460.

Brooks GA, Butterfield GE, Wolfe RR, Groves BM, Mazzeo RS, Sutton JR, Wolfel EE & Reeves JT (1991). Decreased reliance on lactate during exercise after acclimatization to 4,300 m. *J Appl Physiol* **71**, 333–341.

Brooks GA, Fahey TD & Baldwin KM (2019). *Exercise Physiology: Human Bioenergetics and its Applications*, vol. **1**. Kindle Direct Publishing.

Brooks GA & Gladden LB (2003). The metabolic systems: anaerobic metabolism (glycolytic and phosphagen). In *Exercise Physiology People and Ideas*, ed. Tipton CE, pp. 322–360. Oxford University Press, New York.

Brooks GA & Martin NA (2014). Cerebral metabolism following traumatic brain injury: new discoveries with implications for treatment. *Front Neurosci* **8**, 408.

Broxterman RM, Ade CJ, Poole DC, Harms CA & Barstow TJ (2013). A single test for the determination of parameters of the speed-time relationship for running. *Respir Physiol Neurobiol* **185**, 380–385.

Broxterman RM, Craig JC & Richardson RS (2018). The respiratory compensation point and the deoxygenation break point are not valid surrogates for critical power and maximum lactate steady state. *Med Sci Sports Exerc* **50**, 2379–2382.

Buchfuhrer MJ, Hansen JE, Robinson TE, Sue DY, Wasserman K & Whipp BJ (1983). Optimizing the exercise protocol for cardiopulmonary assessment. *J Appl Physiol Respir Environ Exerc Physiol* **55**, 1558–1564.

Buckler KJ, Vaughan-Jones RD, Peers C, Lagadic-Gossmann D & Nye PC (1991). Effects of extracellular pH, PCO_2 and HCO_3^- on intracellular pH in isolated type-I cells of the neonatal rat carotid body. *J Physiol* **444**, 703–721.

Burnley M (2009). Estimation of critical torque using intermittent isometric maximal voluntary contractions of the quadriceps in humans. *J Appl Physiol* **106**, 975–983.

Burnley M & Jones AM (2018). Power-duration relationship: Physiology, fatigue, and the limits of human performance. *Eur J Sport Sci* **18**, 1–12.

Burnley M, Vanhatalo A & Jones AM (2012). Distinct profiles of neuromuscular fatigue during muscle contractions below and above the critical torque in humans. *J Appl Physiol* **113**, 215–223.

Caiozzo VJ, Davis JA, Ellis JF, Azus JL, Vandagriff R, Prietto CA & McMaster WC (1982). A comparison of gas exchange indices used to detect the anaerobic threshold. *J Appl Physiol Respir Environ Exerc Physiol* **53**, 1184–1189.

Casaburi R, Barstow TJ, Robinson T & Wasserman K (1989). Influence of work rate on ventilatory and gas exchange kinetics. *J Appl Physiol* **67**, 547–555.

Casaburi R, Patessio A, Ioli F, Zanaboni S, Donner CF & Wasserman K (1991). Reductions in exercise lactic acidosis and ventilation as a result of exercise training in patients with obstructive lung disease. *Am Rev Respir Dis* **143**, 9–18.

Chance B, Cohen P, Jöbsis F & Schoener B (1962). Intracellular oxidation-reduction states in vivo. *Science* **137**, 499–508.

Chance B & Williams GR (1956). The respiratory chain and oxidative phosphorylation. *Adv Enzymol Relat Subj Biochem* **17**, 65–134.

Chicharro JL, Perez M, Carvajal A, Bandres F & Lucia A (1999). The salivary amylase, lactate and electromyographic response to exercise. *Jpn J Physiol* **49**, 551–554.

Clanton TL (2019). Managing the power grid: how myoglobin can regulate PO_2 and energy distribution in skeletal muscle. *J Appl Physiol* **126**, 787–790.

Clanton TL, Hogan MC & Gladden LB (2013). Regulation of cellular gas exchange, oxygen sensing, and metabolic control. *Compr Physiol* **3**, 1135–1190.

Colson M, Baglin J, Bolsin S & Grocott MP (2012). Cardiopulmonary exercise testing predicts 5 yr survival after major surgery. *Br J Anaesth* **109**, 735–741.

Conconi F, Ferrari M, Ziglio PG, Droghetti P & Codeca L (1980). [Determination of the anaerobic threshold by a noninvasive field in man]. *Boll Soc Ital Biol Sper* **56**, 2504–2510.

Connett RJ, Gayeski TE & Honig CR (1983). Lactate production in a pure red muscle in absence of anoxia: mechanisms and significance. *Adv Exp Med Biol* **159**, 327–335.

Connett RJ, Gayeski TE & Honig CR (1984). Lactate accumulation in fully aerobic, working, dog gracilis muscle. *Am J Physiol* **246**, H120–H128.

Connett RJ, Gayeski TE & Honig CR (1985). Energy sources in fully aerobic rest-work transitions: a new role for glycolysis. *Am J Physiol* **248**, H922–H929.

Connett RJ, Gayeski TE & Honig CR (1986). Lactate efflux is unrelated to intracellular PO_2 in a working red muscle in situ. *J Appl Physiol* **61**, 402–408.

Connett RJ, Honig CR, Gayeski TE & Brooks GA (1990). Defining hypoxia: a systems view of VO_2, glycolysis, energetics, and intracellular PO_2. *J Appl Physiol* **68**, 833–842.

Copp SW, Hirai DM, Musch TI & Poole DC (2010). Critical speed in the rat: implications for hindlimb muscle blood flow distribution and fibre recruitment. *J Physiol* **588**, 5077–5087.

Coquart JB, Mucci P, L'Hermette M, Chamari K, Tourny C & Garcin M (2017). Correlation of gas exchange threshold and first muscle oxyhemoglobin inflection point with time-to-exhaustion during heavy-intensity exercise. *J Sports Med Phys Fitness* **57**, 171–178.

Craig JC, Vanhatalo A, Burnley M, Jones AM & Poole DC (2018). Critical power: Possibly the most important fatigue threshold in exercise physiology. In *Muscle and Exercise Physiology*, ed. Zoladz J, pp. 159–181. Academic Press, London.

Crisafulli A, Tocco F, Pittau G, Caria M, Lorrai L, Melis F & Concu A (2006). Detection of lactate threshold by including haemodynamic and oxygen extraction data. *Physiol Meas* **27**, 85–97.

Davies CT, Few J, Foster KG & Sargeant AJ (1974). Plasma catecholamine concentration during dynamic exercise involving different muscle groups. *Eur J Appl Physiol Occup Physiol* **32**, 195–206.

Davis JA (1985). Anaerobic threshold: Review of the concept and directions for future research. *Med Sci Sports Exerc* **17**, 6–21.

Davis JA, Whipp BJ, Lamarra N, Huntsman DJ, Frank MH & Wasserman K (1982). Effect of ramp slope on determination of aerobic parameters from the ramp exercise test. *Med Sci Sports Exerc* **14**, 339–343.

Dawson MJ & Wilkie DR (1977). Theoretical and practical considerations in harnessing manpower. In *Proceedings of the Royal Aeronaut Society: Symposium on* Manpowered Flight.

Donovan CM & Brooks GA (1983). Endurance training affects lactate clearance, not lactate production. *Am J Physiol* **244**, E83–E92.

Dubouchaud H, Butterfield GE, Wolfel EE, Bergman BC & Brooks GA (2000). Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am J Physiol Endocrinol Metab* **278**, E571–E579.

Emhoff CA, Messonnier LA, Horning MA, Fattor JA, Carlson TJ & Brooks GA (2013*a*). Direct and indirect lactate oxidation in trained and untrained men. *J Appl Physiol* **115**, 829–838.

Emhoff CA, Messonnier LA, Horning MA, Fattor JA, Carlson TJ & Brooks GA (2013*b*). Gluconeogenesis and hepatic glycogenolysis during exercise at the lactate threshold. *J Appl Physiol* **114**, 297–306.

Faude O, Kindermann W & Meyer T (2009). Lactate threshold concepts: how valid are they? *Sports Med* **39**, 469–490.

Ferguson BS, Rogatzki MJ, Goodwin ML, Kane DA, Rightmire Z & Gladden LB (2018). Lactate metabolism: historical context, prior misinterpretations, and current understanding. *Eur J Appl Physiol* **118**, 691–728.

Fletcher WM & Hopkins FG (1907). Lactic acid in amphibian muscle. *J Physiol* **35**, 247–309.

Fukuba Y, Miura A, Endo M, Kan A, Yanagawa K & Whipp BJ (2003). The curvature constant parameter of the power-duration curve for varied-power exercise. *Med Sci Sports Exerc* **35**, 1413–1418.

Fukuba Y & Whipp BJ (1999). A metabolic limit on the ability to make up for lost time in endurance events. *J Appl Physiol* **87**, 853–861.

Full RJ (1986). Locomotion without lungs: energetics and performance of a lungless salamander. *Am J Physiol* **251**, R775–R780.

Galán-Rioja MA, Gonzalez-Mohino F, Poole DC & Gonzalez-Ravé JM (2020). Relative proximity of critical power and metabolic/ventilatory thresholds: Systematic review and meta-analysis. *Sports Med* **50**, 1771–1783.

Garcia-Alvarez M, Marik P & Bellomo R (2014). Sepsis-associated hyperlactatemia. *Crit Care* **18**, 503.

Gerich JE, Meyer C, Woerle HJ & Stumvoll M (2001). Renal gluconeogenesis: its importance in human glucose homeostasis. *Diabetes Care* **24**, 382–391.

Gertz EW, Wisneski JA, Neese R, Bristow JD, Searle GL & Hanlon JT (1981). Myocardial lactate metabolism: evidence of lactate release during net chemical extraction in man. *Circulation* **63**, 1273–1279.

Gertz EW, Wisneski JA, Stanley WC & Neese RA (1988). Myocardial substrate utilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments. *J Clin Invest* **82**, 2017–2025.

Gitt AK, Wasserman K, Kilkowski C, Kleemann T, Kilkowski A, Bangert M, Schneider S, Schwarz A & Senges J (2002). Exercise anaerobic threshold and ventilatory efficiency identify heart failure patients for high risk of early death. *Circulation* **106**, 3079–3084.

Gladden LB (1991). Net lactate uptake during progressive steady-level contractions in canine skeletal muscle. *J Appl Physiol* **71**, 514–520.

Gladden LB (1996). Lactate transport and exchange. In Handbook of Physiology, *Section 12,* Exercise: Regulation and Integration of Multiple Systems, ed. Rowell LB & Shepherd TJ, pp. 614–648. Oxford University Press, New York.

Gladden LB (2004*a*). Lactate metabolism during exercise. In *Principles of Exercise Biochemistry*, ed. Poortmans JR, pp. 152–196. Karger, Basel.

Gladden LB (2004*b*). Lactate metabolism: a new paradigm for the third millennium. *J Physiol* **558**, 5–30.

Gladden LB (2008). 200th anniversary of lactate research in muscle. *Exerc Sport Sci Rev* **36**, 109–115.

Gladden LB, Crawford RE & Webster MJ (1992). Effect of blood flow on net lactate uptake during steady-level contractions in canine skeletal muscle. *J Appl Physiol* **72**, 1826–1830.

Gladden LB, Crawford RE & Webster MJ (1994). Effect of lactate concentration and metabolic rate on net lactate uptake by canine skeletal muscle. *Am J Physiol* **266**, R1095–R1101.

Glancy B, Hartnell LM, Malide D, Yu ZX, Combs CA, Connelly PS, Subramaniam S & Balaban RS (2015). Mitochondrial reticulum for cellular energy distribution in muscle. *Nature* **523**, 617–620.

Glancy B, Kane DA, Kavazis AN, Goodwin ML, Willis WT & Gladden LB (2020). Mitochondrial lactate metabolism: history and implications for exercise and disease. *J Physiol*, doi: 10.1113/JP278930.

Glenn TC, Martin NA, Horning MA, McArthur DL, Hovda DA, Vespa P & Brooks GA (2015). Lactate: brain fuel in human traumatic brain injury: a comparison with normal healthy control subjects. *J Neurotrauma* **32**, 820–832.

Gnaiger E & Kuznetsov AV (2002). Mitochondrial respiration at low levels of oxygen and cytochrome c. *Biochem Soc Trans* **30**, 252–258.

Gnaiger E, Steinlechner-Maran R, Mendez G, Eberl T & Margreiter R (1995). Control of mitochondrial and cellular respiration by oxygen. *J Bioenerg Biomembr* **27**, 583–596.

Gohil K & Brooks GA (2012). Exercise tames the wild side of the Myc network: a hypothesis. *Am J Physiol Endocrinol Metab* **303**, E18–E30.

Goodyear SJ, Yow H, Saedon M, Shakespeare J, Hill CE, Watson D, Marshall C, Mahmood A, Higman D & Imray CH (2013). Risk stratification by pre-operative cardiopulmonary exercise testing improves outcomes following elective abdominal aortic aneurysm surgery: a cohort study. *Perioper Med* **2**, 10.

Goulding RP, Roche DM & Marwood S (2019). Hyperoxia speeds pulmonary oxygen uptake kinetics and increases critical power during supine cycling. *Exp Physiol* **104**, 1061–1073.

Grassi B, Gladden LB, Samaja M, Stary CM & Hogan MC (1998). Faster adjustment of O_2 delivery does not affect VO_2 on-kinetics in isolated in situ canine muscle. *J Appl Physiol* **85**, 1394–1403.

Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK & Wagner PD (1996). Muscle O_2 uptake kinetics in humans: implications for metabolic control. *J Appl Physiol* **80**, 988–998.

Hagberg JM, Coyle EF, Carroll JE, Miller JM, Martin WH & Brooke MH (1982). Exercise hyperventilation in patients with McArdle's disease. *J Appl Physiol Respir Environ Exerc Physiol* **52**, 991–994.

Hashimoto T & Brooks GA (2008). Mitochondrial lactate oxidation complex and an adaptive role for lactate production. *Med Sci Sports Exerc* **40**, 486–494.

Hashimoto T, Hussien R & Brooks GA (2006). Colocalization of MCT1, CD147, and LDH in mitochondrial inner membrane of L6 muscle cells: evidence of a mitochondrial lactate oxidation complex. *Am J Physiol Endocrinol Metab* **290**, E1237–E1244.

Hashimoto T, Hussien R, Cho HS, Kaufer D & Brooks GA (2008). Evidence for the mitochondrial lactate oxidation complex in rat neurons: demonstration of an essential component of brain lactate shuttles. *PLoS One* **3**, e2915.

Henson LC, Poole DC & Whipp BJ (1989). Fitness as a determinant of oxygen uptake response to constant-load exercise. *Eur J Appl Physiol Occup Physiol* **59**, 21–28.

Herzig S, Raemy E, Montessuit S, Veuthey JL, Zamboni N, Westermann B, Kunji ER & Martinou JC (2012). Identification and functional expression of the mitochondrial pyruvate carrier. *Science* **337**, 93–96.

Hill AV (1910). The heat produced in contracture and muscular tone. *J Physiol* **40**, 389–403.

Hill AV (1925). The physiological basis of athletic records. *Nature* **116**, 544–548.

Hill AV, Long CN & Lupton H (1924*a*). The effect of fatigue on the relation between work and speed, in contraction of human arm muscles. *J Physiol* **58**, 334–337.

Hill AV, Long CNH & Lupton H (1924*b*). Muscular exercise, lactic acid, and the supply and utilisation of oxygen. Parts I–III. *Proc R Soc Lond B* **96**, 438–475.

Hill AV, Long CNH & Lupton H (1924*c*). Muscular exercise, lactic acid, and the supply and utilisation of oxygen. Parts IV–VI. *Proc R Soc Lond B* **97**, 84–138.

Hill AV, Long CNH & Lupton H (1924*d*). Muscular exercise, lactic acid and the supply and utilisation of oxygen. Parts VII–VIII. *Proc R Soc Lond B* **97**, 155–176.

Hill DW (1993). The critical power concept. A review. *Sports Med* **16**, 237–254.

Hill DW (2004). The relationship between power and time to fatigue in cycle ergometer exercise. *Int J Sports Med* **25**, 357–361.

Hill DW, Alain C & Kennedy MD (2003). Modeling the relationship between velocity and time to fatigue in rowing. *Med Sci Sports Exerc* **35**, 2098–2105.

Hill DW, Poole DC & Smith JC (2002). The relationship between power and the time to achieve VO_{2max}. *Med Sci Sports Exerc* **34**, 709–714.

Hofmann P, Bunc V, Leitner H, Pokan R & Gaisl G (1994). Heart rate threshold related to lactate turn point and steady-state exercise on a cycle ergometer. *Eur J Appl Physiol Occup Physiol* **69**, 132–139.

Hogan MC, Cox RH & Welch HG (1983). Lactate accumulation during incremental exercise with varied inspired oxygen fractions. *J Appl Physiol Respir Environ Exerc Physiol* **55**, 1134–1140.

Hollmann W (2001). 42 years ago—Development of the concepts of ventilatory and lactate threshold. *Sports Med* **31**, 315–320.

Honig CR, Gayeski TE & Groebe K (1997). Myoglobin and oxygen gradients. In *The Lung: Scientific Foundations*, ed. Crystal RG, West JB, Weibel ER & Barnes PJ, pp. 1925–1933. Raven Press, New York.

Hoque R, Farooq A, Ghani A, Gorelick F & Mehal WZ (2014). Lactate reduces liver and pancreatic injury in Toll-like receptor- and inflammasome-mediated inflammation via GPR81-mediated suppression of innate immunity. *Gastroenterology* **146**, 1763–1774.

Huckabee WE (1958). Relationships of pyruvate and lactate during anaerobic metabolism. I. Effects of infusion of pyruvate or glucose and of hyperventilation. *J Clin Invest* **37**, 244–254.

Hughes EF, Turner SC & Brooks GA (1982). Effects of glycogen depletion and pedaling speed on "anaerobic threshold". *J Appl Physiol Respir Environ Exerc Physiol* **52**, 1598–1607.

Hughson RL, Orok CJ & Staudt LE (1984). A high velocity treadmill running test to assess endurance running potential. *Int J Sports Med* **05**, 23–25.

Jacobs I (1986). Blood lactate. Implications for training and sports performance. *Sports Med* **3**, 10–25.

Jamnick NA, Botella J, Pyne DB & Bishop DJ (2018). Manipulating graded exercise test variables affects the validity of the lactate threshold and VO_{2peak}. *PLoS One* **13**, e0199794.

Jöbsis FF & Stainsby WN (1968). Oxidation of NADH during contractions of circulated mammalian skeletal muscle. *Respir Physiol* **4**, 292–300.

Johnson JA & Fusaro RM (1972). The role of the skin in carbohydrate metabolism. *Adv Metab Disord* **60**, 1–55.

Jones AM, Burnley M, Black MI, Poole DC & Vanhatalo A (2019). Response to considerations regarding maximal lactate steady state determination before redefining the gold-standard. *Physiol Rep* **7**, e14292.

Jones AM, Burnley M & Vanhatalo A (2018). Aerobic exercise performance. In *Kinanthropometry and Exercise Physiology*, ed. Norton KI & Eston RG, pp. 318–352. Routledge, London.

Jones AM & Poole DC (2008). Physiological demands of endurance exercise. In *Olympic Textbook of Science in Sport*, ed. Maughan RJ, pp. 43–55. Blackwell, Chichester, UK.

Jones AM & Vanhatalo A (2017). The 'critical power' concept: Applications to sports performance with a focus on intermittent high-intensity exercise. *Sports Med* **47**, 65–78.

Jones AM, Vanhatalo A, Burnley M, Morton RH & Poole DC (2010). Critical power: implications for determination of VO_{2max} and exercise tolerance. *Med Sci Sports Exerc* **42**, 1876–1890.

Jones AM, Wilkerson DP, DiMenna F, Fulford J & Poole DC (2008). Muscle metabolic responses to exercise above and below the "critical power" assessed using ^{31}P-MRS. *Am J Physiol Regul Integr Comp Physiol* **294**, R585–R593.

Jurimae J, von Duvillard SP, Maestu J, Cicchella A, Purge P, Ruosi S, Jurimae T & Hamra J (2007). Aerobic-anaerobic transition intensity measured via EMG signals in athletes with different physical activity patterns. *Eur J Appl Physiol* **101**, 341–346.

Keir DA, Fontana FY, Robertson TC, Murias JM, Paterson DH, Kowalchuk JM & Pogliaghi S (2015). Exercise intensity thresholds: Identifying the boundaries of sustainable performance. *Med Sci Sports Exerc* **47**, 1932–1940.

Keir DA, Pogliaghi S & Murias JM (2018). The respiratory compensation point and the deoxygenation break point are valid surrogates for critical power and maximum lactate steady state. *Med Sci Sports Exerc* **50**, 2375–2378.

Kelley KM, Hamann JJ, Navarre C & Gladden LB (2002). Lactate metabolism in resting and contracting canine skeletal muscle with elevated lactate concentration. *J Appl Physiol* **93**, 865–872.

Kiilavuori K, Sovijarvi A, Naveri H, Ikonen T & Leinonen H (1996). Effect of physical training on exercise capacity and gas exchange in patients with chronic heart failure. *Chest* **110**, 985–991.

Kindig CA, Richardson TE & Poole DC (2002). Skeletal muscle capillary hemodynamics from rest to contractions: implications for oxygen transfer. *J Appl Physiol* **92**, 2513–2520.

Kirkwood SP, Munn EA & Brooks GA (1986). Mitochondrial reticulum in limb skeletal muscle. *Am J Physiol* **251**, C395–C402.

Kirkwood SP, Packer L & Brooks GA (1987). Effects of endurance training on a mitochondrial reticulum in limb skeletal muscle. *Arch Biochem Biophys* **255**, 80–88.

Kjaer M, Kiens B, Hargreaves M & Richter EA (1991). Influence of active muscle mass on glucose homeostasis during exercise in humans. *J Appl Physiol* **71**, 552–557.

Koike A & Wasserman K (1992). Effect of acute reduction in oxygen transport on parameters of aerobic function during exercise. *Ann Acad Med Singapore* **21**, 14–22.

Kompanje EJ, Jansen TC, van der Hoven B & Bakker J (2007). The first demonstration of lactic acid in human blood in shock by Johann Joseph Scherer (1814-1869) in January 1843. *Intensive Care Med* **33**, 1967–1971.

Krebs HA (1972). The Pasteur effect and the relations between respiration and fermentation. *Essays Biochem* **8**, 1–34.

Krogh A & Lindhard J (1913). The regulation of respiration and circulation during the initial stages of muscular work. *J Physiol* **47**, 112–136.

Lai CW, Minto G, Challand CP, Hosie KB, Sneyd JR, Creanor S & Struthers RA (2013). Patients' inability to perform a preoperative cardiopulmonary exercise test or demonstrate an anaerobic threshold is associated with inferior outcomes after major colorectal surgery. *Br J Anaesth* **111**, 607–611.

Lauderdale MA & Hinchcliff KW (1999). Hyperbolic relationship between time-to-fatigue and workload. *Equine Vet J Suppl*, 586–590.

Lieberman DE & Bramble DM (2007). The evolution of marathon running: capabilities in humans. *Sports Med* **37**, 288–290.

Marik P & Bellomo R (2016). A rational approach to fluid therapy in sepsis. *Br J Anaesth* **116**, 339–349.

Martinez-Outschoorn UE, Prisco M, Ertel A, Tsirigos A, Lin Z, Pavlides S, Wang C, Flomenberg N, Knudsen ES, Howell A, Pestell RG, Sotgia F & Lisanti MP (2011). Ketones and lactate increase cancer cell "stemness," driving recurrence, metastasis and poor clinical outcome in breast cancer: achieving personalized medicine via metabolo-genomics. *Cell Cycle* **10**, 1271–1286.

Mazzeo RS, Brooks GA, Schoeller DA & Budinger TF (1986). Disposal of blood [1-^{13}C]lactate in humans during rest and exercise. *J Appl Physiol* **60**, 232–241.

Messonnier LA, Emhoff CA, Fattor JA, Horning MA, Carlson TJ & Brooks GA (2013). Lactate kinetics at the lactate threshold in trained and untrained men. *J Appl Physiol* **114**, 1593–1602.

Meyer C, Dostou JM, Welle SL & Gerich JE (2002*a*). Role of human liver, kidney, and skeletal muscle in postprandial glucose homeostasis. *Am J Physiol Endocrinol Metab* **282**, E419–E427.

Meyer C, Stumvoll M, Dostou J, Welle S, Haymond M & Gerich J (2002*b*). Renal substrate exchange and gluconeogenesis in normal postabsorptive humans. *Am J Physiol Endocrinol Metab* **282**, E428–E434.

Meyerhof O (1930*a*). The chemistry of muscular contraction. *Lancet North Am Ed* **216**, 1415–1422.

Meyerhof O (1930*b*). [Die chemischen Vorgänge im Zusammenhang mit der Wärmebildung]. In *Die chemischen Vorgänge im Muskel und ihr Zusammenhang mit Arbeitsleistung und Wärmebildung*. Springer, Berlin.

Mole PA, Chung Y, Tran TK, Sailasuta N, Hurd R & Jue T (1999). Myoglobin desaturation with exercise intensity in human gastrocnemius muscle. *Am J Physiol* **277**, R173–R180.

Monod H & Scherrer J (1965). The work capacity of a synergic muscular group. *Ergonomics* **8**, 329–338.

Morrison JS, Coates JF & Rankov NB (2000). *The Athenian Trireme: The History and Reconstruction of an Ancient Greek Warship*. Cambridge University Press, Cambridge, UK.

Murgatroyd SR, Wylde LA, Cannon DT, Ward SA & Rossiter HB (2014). A 'ramp-sprint' protocol to characterise indices of aerobic function and exercise intensity domains in a single laboratory test. *Eur J Appl Physiol* **114**, 1863–1874.

Naimark A, Wasserman K & McIlroy MB (1964). Continuous measurement of ventilatory exchange ratio during exercise. *J Appl Physiol* **19**, 644–652.

Neder JA, Jones PW, Nery LE & Whipp BJ (2000*a*). Determinants of the exercise endurance capacity in patients with chronic obstructive pulmonary disease. The power-duration relationship. *Am J Respir Crit Care Med* **162**, 497–504.

Neder JA, Jones PW, Nery LE & Whipp BJ (2000*b*). The effect of age on the power/duration relationship and the intensity-domain limits in sedentary men. *Eur J Appl Physiol* **82**, 326–332.

Neder JA, Nery LE, Castelo A, Andreoni S, Lerario MC, Sachs A, Silva AC & Whipp BJ (1999). Prediction of metabolic and cardiopulmonary responses to maximum cycle ergometry: a randomised study. *Eur Respir J* **14**, 1304–1313.

Older P, Hall A & Hader R (1999). Cardiopulmonary exercise testing as a screening test for perioperative management of major surgery in the elderly. *Chest* **116**, 355–362.

Older PO & Levett DZH (2017). Cardiopulmonary exercise testing and surgery. *Ann Am Thorac Soc* **14**, S74–S83.

Owles WH (1930). Alterations in the lactic acid content of the blood as a result of light exercise, and associated changes in the CO_2-combining power of the blood and in the alveolar CO_2 pressure. *J Physiol* **69**, 214–237.

Ozcelik O, Ward SA & Whipp BJ (1999). Effect of altered body CO_2 stores on pulmonary gas exchange dynamics during incremental exercise in humans. *Exp Physiol* **84**, 999–1011.

Pagliassotti MJ & Donovan CM (1990). Role of cell type in net lactate removal by skeletal muscle. *Am J Physiol* **258**, E635–E642.

Pasteur L (1861). Expériences et vues nouvelles sur la nature des fermentations. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, Paris* **53**, 1260–1264.

Peinado AB, Rojo JJ, Calderon FJ & Maffulli N (2014). Responses to increasing exercise upon reaching the anaerobic threshold, and their control by the central nervous system. *BMC Sports Sci Med Rehabil* **6**, 17.

Pettitt RW, Jamnick N & Clark IE (2012). 3-min all-out exercise test for running. *Int J Sports Med* **33**, 426–431.

Poole DC (2019). Edward F. Adolph distinguished lecture. Contemporary model of muscle microcirculation: gateway to function and dysfunction. *J Appl Physiol* **127**, 1012–1033.

Poole DC, Burnley M, Vanhatalo A, Rossiter HB & Jones AM (2016). Critical power: An important fatigue threshold in exercise physiology. *Med Sci Sports Exerc* **48**, 2320–2334.

Poole DC & Gaesser GA (1985). Response of ventilatory and lactate thresholds to continuous and interval training. *J Appl Physiol* **58**, 1115–1121.

Poole DC & Jones AM (2012). Oxygen uptake kinetics. *Compr Physiol* **2**, 933–996.

Poole DC, Ward SA, Gardner GW & Whipp BJ (1988). Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics* **31**, 1265–1279.

Poole DC, Ward SA & Whipp BJ (1990). The effects of training on the metabolic and respiratory profile of high-intensity cycle ergometer exercise. *Eur J Appl Physiol Occup Physiol* **59**, 421–429.

Powell KE, King AC, Buchner DM, Campbell WW, DiPietro L, Erickson KI, Hillman CH, Jakicic JM, Janz KF, Katzmarzyk PT, Kraus WE, Macko RF, Marquez DX, McTiernan A, Pate RR, Pescatello LS & Whitt-Glover MC (2019). The scientific foundation for the Physical Activity Guidelines for Americans, 2nd edition. *J Phys Act Health* **16**, 1–11.

Pringle JS & Jones AM (2002). Maximal lactate steady state, critical power and EMG during cycling. *Eur J Appl Physiol* **88**, 214–226.

Racker E (1972). Bioenergetics and the problem of tumor growth. *Am Sci* **60**, 56–63.

Racker E (1974). History of the Pasteur effect and its pathobiology. *Mol Cell Biochem* **5**, 17–23.

Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS & Wagner PD (1995). Myoglobin O_2 desaturation during exercise. Evidence of limited O_2 transport. *J Clin Invest* **96**, 1916–1926.

Richardson RS, Noyszewski EA, Leigh JS & Wagner PD (1998). Lactate efflux from exercising human skeletal muscle: role of intracellular PO_2. *J Appl Physiol* **85**, 627–634.

Rogatzki MJ, Ferguson BS, Goodwin ML & Gladden LB (2015). Lactate is always the end product of glycolysis. *Front Neurosci* **9**, 22.

Rossiter HB. (2011). Exercise: Kinetic considerations for gas exchange. *Compr Physiol* **1**, 203–244.

Rossiter HB & Whipp BJ (2012). Paleo-bioenergetics: clues to the maximum sustainable speed of a trireme under oar. In *Trireme Olympias: The Final Report*, ed. Rankov NB, pp. 165–168. Oxbow Books, Oxford.

Rumsey WL, Schlosser C, Nuutinen EM, Robiolio M & Wilson DF (1990). Cellular energetics and the oxygen dependence of respiration in cardiac myocytes isolated from adult rat. *J Biol Chem* **265**, 15392–15402.

San-Millan I & Brooks GA (2017). Reexamining cancer metabolism: lactate production for carcinogenesis could be the purpose and explanation of the Warburg Effect. *Carcinogenesis* **38**, 119–133.

San-Millan I & Brooks GA (2018). Assessment of metabolic flexibility by means of measuring blood lactate, fat, and carbohydrate oxidation responses to exercise in professional endurance athletes and less-fit individuals. *Sports Med* **48**, 467–479.

San-Millan I, Julian CG, Matarazzo C, Martinez J & Brooks GA (2019). Is lactate an oncometabolite? Evidence supporting a role for lactate in the regulation of transcriptional activity of cancer-related genes in MCF7 breast cancer cells. *Front Oncol* **9**, 1536.

Scheiman J, Luber JM, Chavkin TA, MacDonald T, Tung A, Pham LD, Wibowo MC, Wurth RC, Punthambaker S, Tierney BT, Yang Z, Hattab MW, Avila-Pacheco J, Clish CB, Lessard S, Church GM & Kostic AD (2019). Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. *Nat Med* **25**, 1104–1109.

Selye H & Fortier C (1950). Adaptive reaction to stress. *Psychosom Med* **12**, 149–157.

Semenza GL (2008). Tumor metabolism: cancer cells give and take lactate. *J Clin Invest* **118**, 3835–3837.

Shapiro NI, Howell MD, Talmor D, Nathanson LA, Lisbon A, Wolfe RE & Weiss JW (2005). Serum lactate as a predictor of mortality in emergency department patients with infection. *Ann Emerg Med* **45**, 524–528.

Sietsema K, Stringer WW, Sue DY, Ward SA, Rossiter HB & Porszasz J (2020). *Wasserman & Whipp's Principles of Exercise Testing and Interpretation: Including Pathophysiology and Clinical Applications*. Wolters Kluwer, Philadelphia, PA.

Sjodin B & Jacobs I (1981). Onset of blood lactate accumulation and marathon running performance. *Int J Sports Med* **2**, 23–26.

Smith CG & Jones AM (2001). The relationship between critical velocity, maximal lactate steady-state velocity and lactate turnpoint velocity in runners. *Eur J Appl Physiol* **85**, 19–26.

Smith JC, Dangelmaier BS & Hill DW (1999). Critical power is related to cycling time trial performance. *Int J Sports Med* **20**, 374–378.

Snowden CP, Prentis JM, Anderson HL, Roberts DR, Randles D, Renton M & Manas DM (2010). Submaximal cardiopulmonary exercise testing predicts complications and hospital length of stay in patients undergoing major elective surgery. *Ann Surg* **251**, 535–541.

Somasetia DH, Setiati TE, Sjahrodji AM, Idjradinata PS, Setiabudi D, Roth H, Ichai C, Fontaine E & Leverve XM (2014). Early resuscitation of dengue shock syndrome in children with hyperosmolar sodium-lactate: a randomized single-blind clinical trial of efficacy and safety. *Crit Care* **18**, 466.

Southam CM & Erlich J (1943). Effects of extracts of western red-cedar heartwood on certain decaying fungi in culture. *Phytopathology* **33**, 517–541.

Stanley WC, Gertz EW, Wisneski JA, Morris DL, Neese RA & Brooks GA (1985). Systemic lactate kinetics during graded exercise in man. *Am J Physiol* **249**, E595–E602.

Stanley WC, Gertz EW, Wisneski JA, Neese RA, Morris DL & Brooks GA (1986). Lactate extraction during net lactate release in legs of humans during exercise. *J Appl Physiol* **60**, 1116–1120.

Stanley WC, Wisneski JA, Gertz EW, Neese RA & Brooks GA (1988). Glucose and lactate interrelations during moderate-intensity exercise in humans. *Metabolism* **37**, 850–858.

Stringer W, Casaburi R & Wasserman K (1992). Acid-base regulation during exercise and recovery in humans. *J Appl Physiol* **72**, 954–961.

Sue DY, Wasserman K, Moricca RB & Casaburi R (1988). Metabolic acidosis during exercise in patients with chronic obstructive pulmonary disease. Use of the V-slope method for anaerobic threshold determination. *Chest* **94**, 931–938.

Takahashi H, Alves CRR, Stanford KI, Middelbeek RJW, Pasquale N, Ryan RE, Xue R, Sakaguchi M, Lynes MD, So K, Mul JD, Lee MY, Balan E, Pan H, Dreyfuss JM, Hirshman MF, Azhar M, Hannukainen JC, Nuutila P, Kalliokoski KK, Nielsen S, Pedersen BK, Kahn CR, Tseng YH & Goodyear LJ (2019). TGF-β2 is an exercise-induced adipokine that regulates glucose and fatty acid metabolism. *Nat Metab* **1**, 291–303.

Thurber C, Dugas LR, Ocobock C, Carlson B, Speakman JR & Pontzer H (2019). Extreme events reveal an alimentary limit on sustained maximal human energy expenditure. *Sci Adv* **5**, eaaw0341.

Vanhatalo A, Doust JH & Burnley M (2007). Determination of critical power using a 3-min all-out cycling test. *Med Sci Sports Exerc* **39**, 548–555.

Vanhatalo A, Jones AM & Burnley M (2011). Application of critical power in sport. *Int J Sports Physiol Perform* **6**, 128–136.

Von Muralt A (1950). The development of muscle-chemistry, a lesson in neurophysiology. *Biochim Biophys Acta* **4**, 126–129.

Voorn EL, Gerrits KH, Koopman FS, Nollet F & Beelen A (2014). Determining the anaerobic threshold in post-polio syndrome: comparison with current guidelines for training intensity prescription. *Arch Phys Med Rehabil* **95**, 935–940.

Voter WA & Gayeski TE (1995). Determination of myoglobin saturation of frozen specimens using a reflecting cryospectrophotometer. *Am J Physiol* **269**, H1328–H1341.

Wakayoshi K, Yoshida T, Udo M, Harada T, Moritani T, Mutoh Y & Miyashita M (1993). Does critical swimming velocity represent exercise intensity at maximal lactate steady state? *Eur J Appl Physiol Occup Physiol* **66**, 90–95.

Warburg O (1926). [Über den Stoffwechsel der Tumoren]. In *Arbeiten aus dem Kaiser Wilhelm-Institut für Biologie*. Dahlem Julius Springer, Berlin.

Warburg O (1956). On the origin of cancer cells. *Science* **123**, 309–314.

Warburg O, Wind F & Negelein E (1927). The metabolism of tumors in the body. *J Gen Physiol* **8**, 519–530.

Ward SA & Whipp BJ (1992). Influence of body CO_2 stores on ventilatory-metabolic coupling during exercise. In *Control of Breathing and Its Modelling Perspective*, ed. Honda Y, Miyamoto Y, Konno K & Widdicombe JG, pp. 425–431. Plenum Press, New York.

Wasserman K (1984). The anaerobic threshold measurement to evaluate exercise performance. *Am Rev Respir Dis* **129**, S35-S40.

Wasserman K (2002). Exercise gas exchange, breath-by-breath. *Am J Respir Crit Care Med* **165**, 325–326.

Wasserman K, Beaver WL, Davis JA, Pu JZ, Heber D & Whipp BJ (1985). Lactate, pyruvate, and lactate-to-pyruvate ratio during exercise and recovery. *J Appl Physiol* **59**, 935–940.

Wasserman K & McIlroy MB (1964). Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. *Am J Cardiol* **14**, 844–852.

Wasserman K, Van Kessel AL & Burton GG (1967). Interaction of physiological mechanisms during exercise. *J Appl Physiol* **22**, 71–85.

Wasserman K, Whipp BJ, Koyl SN & Beaver WL (1973). Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol* **35**, 236–243.

Welch HG & Stainsby WN (1967). Oxygen debt in contracting dog skeletal muscle in situ. *Respir Physiol* **3**, 229–242.

West MA, Asher R, Browning M, Minto G, Swart M, Richardson K, McGarrity L, Jack S, Grocott MP, Perioperative Exercise T & Training S (2016). Validation of preoperative cardiopulmonary exercise testing-derived variables to predict in-hospital morbidity after major colorectal surgery. *Br J Surg* **103**, 744–752.

Whipp BJ, Davis JA, Torres F & Wasserman K (1981). A test to determine parameters of aerobic function during exercise. *J Appl Physiol Respir Environ Exerc Physiol* **50**, 217–221.

Whipp BJ, Davis JA & Wasserman K (1989). Ventilatory control of the 'isocapnic buffering' region in rapidly-incremental exercise. *Respir Physiol* **76**, 357–367.

Whipp BJ, Lamarra N & Ward SA (1987). Required characteristics of pulmonary gas exchange dynamics for non-invasive determination of the anaerobic threshold. In *Concepts and Formulations in the Control of Breathing*, ed. Benchetrit G, Baconnier P & Demongeot J, pp. 185–200. Manchester University Press, Manchester, UK.

Whipp BJ & Ward SA (1991). The coupling of ventilation to pulmonary gas exchange during exercise. In *Pulmonary Physiology and Pathophysiology of Exercise*, ed. Whipp BJ & Wasserman K, pp. 271–307. Dekker, New York.

Whipp BJ, Ward SA, Lamarra N, Davis JA & Wasserman K (1982). Parameters of ventilatory and gas exchange dynamics during exercise. *J Appl Physiol Respir Environ Exerc Physiol* **52**, 1506–1513.

Whipp BJ & Wasserman K (1986). Effect of anaerobiosis on the kinetics of O_2 uptake during exercise. *Fed Proc* **45**, 2942–2947.

Wilkie DR (1960). Man as a source of mechanical power. *Ergonomics* **3**, 1–8.

Wilson DF, Rumsey WL, Green TJ & Vanderkooi JM (1988). The oxygen dependence of mitochondrial oxidative phosphorylation measured by a new optical method for measuring oxygen concentration. *J Biol Chem* **263**, 2712–2718.

Wolahan SM, Mao HC, Real C, Vespa PM & Glenn TC (2018). Lactate supplementation in severe traumatic brain injured adults by primed constant infusion of sodium L-lactate. *J Neurosci Res* **96**, 688–695.

Zhang D, Tang Z, Huang H, Zhou G, Cui C, Weng Y, Liu W, Kim S, Lee S, Perez-Neut M, Ding J, Czyz D, Hu R, Ye Z, He M, Zheng YG, Shuman HA, Dai L, Ren B, Roeder RG, Becker L & Zhao Y (2019). Metabolic regulation of gene expression by histone lactylation. *Nature* **574**, 575–580.

Zhang YY, Johnson MC, 2nd, Chow N & Wasserman K (1991). Effect of exercise testing protocol on parameters of aerobic function. *Med Sci Sports Exerc* **23**, 625–630.

Zinker BA, Wilson RD & Wasserman DH (1995). Interaction of decreased arterial PO_2 and exercise on carbohydrate metabolism in the dog. *Am J Physiol* **269**, E409-417.

Zoladz JA & Grassi B (2020). A 'fatigue threshold' during incremental exercise was identified (and then forgotten) 100 years ago. *J Physiol* **598**, 2531–2532.

Additional information

Competing interests

For full transparency, G.A.B. (Brooks *et al.* 2019) and H.B.R. (Sietsema *et al.* 2020) are textbook co-authors. None of the authors has any conflict of interest regarding the submission of this article.

Author contributions

All authors contributed to all aspects of this work. All authors approve the final version of this article and are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

No funding was received for this work.

Acknowledgements

We thank Mr Paulo Henrique Caldeira Mesquita for drawing Fig. 2. Finally, we share a deep respect for the overall scientific accomplishments of Drs Karlman Wasserman and Brian Whipp (Sietsema *et al.* 2020) and other thought-provoking scientists (Hollmann, 2001) who explored boundaries of the physiological and metabolic relationships determining human performance in health and disease.

Keywords

cardiopulmonary exercise test, CPET, critical power, critical speed, dysoxia, exercise, gas exchange, gas exchange threshold, gluconeogenesis, glycolysis, hypoxia, isocapnic buffering, lactate, lactate appearance, lactate clearance, lactate disposal, lactate oxidation, lactate signalling, lactate threshold, lactic acid, maximal lactate steady state, oxygen, ventilatory threshold