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COMPARING OF THE EFFECTS OF PHYSICAL AND MENTAL FATIGUE IN ATHLETES AND ACTIVE INDIVIDUALS: A PSYCHOPHYSIOLOGICAL APPROACH

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ABSTRACT

Fatigue is a complex and multidimensional phenomenon that arises from the interaction between physiological, cognitive, and motivational processes, influencing performance and perception of effort. Although physical and mental fatigue are often considered separate entities, they share common psychophysiological mechanisms. Understanding these interactions is essential for interpreting the regulation of effort and well-being in athletes and active individuals.

Two of the studies compared the effects of physical and mental fatigue using a psychophysiological approach, evaluating physiological, cognitive, and perceptual responses in different populations. Elite cyclists and active non-competitive subjects were involved in time to exhaustion test (TTE) at 95% of the respiratory compensation point (RCP) in three conditions: (1) mental fatigue (MF) induced by a prolonged cognitive task (Psychomotor Vigilance Task, PVT); (2) locomotor muscle fatigue (LMF) induced by 100 drop jumps; (3) control condition involving viewing a neutral documentary.

The results show distinct but converging effects of the two kinds of fatigue on endurance performance. In athletes, greater self-regulation capacity reduces the impact of MF and LMF, while in active non-competitive subjects, LMF has a greater impact on performance. In both cases, the reduction in exhaustion time follows an increase in perceived exertion, confirming the central role of the perceptual domain.

The eSPORT project, developed in continuity with these studies, has shown that gaming (Play) generates a significant increase in mental load and perceived physical load compared to spectating (Watch), demonstrating that physical fatigue can also emerge in cognitively intense tasks.

Overall, these results outline fatigue as a complex and dynamic psychophysiological experience, regulated by common central mechanisms and not limited to the physical or mental dimension alone.

In addition, the thesis presents the experimental protocol "Veillonella atypica FB0054." This, exploring the interaction between probiotic supplementation, microbiome and fatigue, will be the next step in this line of research.

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STUDY 1

Tullio Senatore: study concept and design; recruits participants; acquisition of data; analysis and interpretation of data; drafting the manuscript.

Samuele Marcora: critical revision of the manuscript; obtain funding; study supervision.

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Tullio Senatore: study concept and design; acquisition of data; analysis and interpretation of data; drafting the manuscript

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PROTOCOL 1

Tullio Senatore: study concept and design

Daniela Pinto: critical revision of the protocol; obtain supplements; obtain funding.

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CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION TO FATIGUE

"I was in Rome at the end of March. Having heard that the quail passage had already begun, I went to the sea beach to see if these birds, arriving from Africa, showed any phenomenon of fatigue." (Mosso, 1911). With this word began the book *La fatica* written by Angelo Mosso in 1911. From that moment the phenomenon of fatigue started to be studied and deepened, although it is fair to point out that we are not yet in a position to be able to know all aspects related to it, and we may not be able to in the short term. The concept of fatigue is broad and intersects with different aspects of human life. "Fatigue is a universal, everyday phenomenon and ranks third on the list of the most reported problems in the general practitioner's office" (Lamberts, 1991). As might be expected, there is no single definition of fatigue. It can be described as an overwhelming sense of fatigue at rest, exhaustion with activity, lack of energy that precludes daily activities, inertia or lack of stamina, and loss of vigor (Davies and Walsh, 2010). From a scientific perspective, this lack of a univocal definition reflects the intrinsic complexity of fatigue and the coexistence of multiple underlying mechanisms. Fatigue is now recognised as a multidimensional phenomenon, in which both physiological and cognitive components converge (Bestwick-Stevenson et al., 2022). In the scientific field, it is proposed to define it as a perceived symptom that limits physical and mental function through the interaction of two domains: performance fatigue (objective decline in performance) and perceived fatigue (increase in subjective effort) (Enoka & Duchateau, 2016). This integrative perspective goes beyond traditional dichotomies (e.g., 'physical fatigue' vs. 'mental fatigue') and emphasises that fatigue arises from the dynamic interaction of multiple psychophysiological factors, rather than from a single isolated peripheral cause (Enoka & Duchateau, 2016; Bestwick-Stevenson et al., 2022). In line with this unified view, recent theoretical models attribute a central role to neurocognitive mechanisms in the regulation of effort. For example, Marcora's psychobiological model conceives endurance performance as a decision-making process based on the comparison between perceived effort and motivation, postulating that exhaustion occurs when perceived effort reaches the maximum tolerable level relative to remaining motivation (Marcora et al., 2009; Pageaux & Lepers, 2016). Similarly, the 'central governor' theory proposes that the brain acts as an anticipatory regulator of exercise, using the sensation of fatigue as an emotional signal to prevent damage and preserve the body's homeostasis (Noakes, 2012). Experimental evidence confirms that both mental fatigue (induced by prolonged cognitive tasks) and local physical fatigue produce convergent effects on performance: both lead to a reduction in endurance capacity accompanied by a significant increase in the perception of effort, in the absence of marked alterations in basic physiological parameters (Marcora et al., 2009; Van Cutsem et al., 2017). In other words, the performance limit does not necessarily coincide with absolute peripheral muscle exhaustion, but is modulated by central fatigue regulation mechanisms: it is the high perception of effort that causes the athlete to stop exercising, acting as a protective strategy managed by the central nervous system (Noakes, 2012).

Physical and Mental Fatigue: Psychophysiological States, Task Specificity Motivational Factors, Boredom role and Circadian Regulation

The concept of fatigue represents one of the most discussed and complex themes in the field of motor and physiological sciences. The fundamental questions that have guided and continue to guide research are: how is fatigue identified? How do its manifestations differ? And what elements allow a researcher, coach, or athletic trainer to hypothesize which state of fatigue predominates in a given individual?

Initially, fatigue was almost exclusively conceived as a physiological phenomenon, resulting from a reduction in energy availability or the accumulation of metabolites in the muscle. It was argued that the limit to which muscles can be pushed while breathing air is determined not so much by the exhaustion of the muscles themselves, but by the discomfort (cardiac and cerebral) resulting from the increased concentration of hydrogen ions in the blood or its imperfect oxygen saturation during the rapid passage through the lungs (Hill, Long and Lupton, 1924). Later observations suggested that the impairment of muscle functions could explain the reduction in performance, but also that the lack of excitation or activation could prevail over the lack of energy supply (Edwards, 1981).

A further advancement in understanding led to the distinction between fatigue and its perception. In this phase of research, the subjective sensation of fatigue was predominantly interpreted as a peripheral consequence: fatigued muscles were thought to send afferent signals to the central nervous system (CNS), which in turn generated the conscious experience of fatigue (Amann, 2016). According to this perspective, the perception of fatigue largely reflects the magnitude of peripheral physiological disturbance. This interpretation remains influential, and in applied and field contexts the idea that fatigue corresponds primarily to muscle resource depletion or to an objective physiological inability to sustain effort is still widespread.

At the same time, other lines of research have emphasized that the relationship between peripheral impairment and subjective exhaustion is not necessarily direct or linear. Several experiments have highlighted the psychophysiological nature of fatigue, indicating that central and cognitive processes may substantially contribute to the emergence of the sensation of effort and to exercise intolerance (Marcora, 2009; Marcora and Staiano, 2010; de Morree and Marcora, 2015). In one influential study, participants reached volitional exhaustion at a constant workload, yet were able to produce higher power output immediately afterwards when motivational conditions were modified (Marcora and Staiano, 2010). These observations suggest that peripheral limitations alone may not fully account for task disengagement in all circumstances.

On the basis of such findings, the psychobiological model of fatigue was proposed, conceptualizing performance as the outcome of an interaction between physiological processes and psychological variables such as motivation, attention, emotional state, and tolerance to discomfort (Marcora, 2009). Within this framework, fatigue is considered a multidimensional phenomenon emerging from the dynamic integration of peripheral mechanisms (e.g., glycogen depletion, metabolite accumulation, ionic disturbances) and central mechanisms (e.g., changes in cortical motor drive and perception of effort) (Allen, Lamb and Westerblad, 2008; Enoka and Duchateau, 2016), consistent with the two-domain distinction between performance fatigability and perceived fatigability (Fig. 1).

Contemporary literature therefore reflects the coexistence of partially divergent interpretations. Traditional physiological models continue to attribute a central role to peripheral disturbances and afferent feedback in shaping fatigue and limiting performance. Conversely, psychobiological and cognitive–motivational approaches assign greater relevance to centrally generated processes, particularly to the perception of effort, in determining exercise tolerance. Rather than being mutually exclusive, these perspectives have progressively converged toward integrative frameworks in which fatigue is understood as the result of interactions between peripheral signals and central regulatory processes.

Following this integrative interpretation, a mechanism of particular interest is corollary discharge (Fig. 2), defined as a copy of the central motor command transmitted to sensory areas during voluntary movement. This signal allows the CNS to monitor and predict the intensity of the required effort. According to corollary discharge theory, the perception of fatigue depends primarily on internally generated signals that encode the magnitude of the motor command. Experimental evidence shows that during muscle fatigue there is an increase in central motor drive accompanied by a proportional increase in perceived exertion (McCloskey et al., 1974; de Morree and Marcora, 2014).

Additional neurophysiological support for this interpretation was provided by de Morree, Klein and Marcora (2012), who demonstrated that the amplitude of movement-related cortical potentials, an index of central motor command, closely tracks changes in perceived exertion during dynamic exercise performed under different load and fatigue conditions. In the conceptual framework adopted in this thesis, this mechanism contributes specifically to the domain of perceived fatigability, complementing peripheral determinants of performance fatigability as schematically illustrated in Fig. 1. Importantly, perceived effort increased in parallel with cortical motor activity even when peripheral mechanical output was experimentally manipulated, indicating that subjective effort reflects the magnitude of the efferent motor signal rather than peripheral feedback alone. This finding provides direct experimental support for the role of corollary discharge as a neural substrate of effort perception.

Further converging evidence comes from studies in which afferent feedback from peripheral organs was substantially altered. Individuals with cardiac transplantation, who lack normal afferent cardiac innervation, report levels of perceived exertion comparable to healthy controls during graded exercise. Similarly, experiments performed under epidural anesthesia, which markedly reduces sensory input from the working muscles, show preserved or only minimally altered ratings of perceived exertion despite profound changes in muscle contractile capacity (Fernandes et al., 1990; Kjaer et al., 1999; Smith et al., 2003). These observations suggest that afferent feedback is not a necessary prerequisite for the emergence of effort perception and further support the dominant contribution of centrally generated motor command signals.

At the same time, other investigations have documented that afferent feedback from skeletal and respiratory muscles and from the cardiovascular system increases during prolonged or intense exercise and may influence motor output and exercise regulation (Amann, 2018) (Fig. 3). Accordingly, current models increasingly describe perception of fatigue as arising from the interaction between centrally generated motor command signals and peripheral sensory information, without implying a strict one-to-one causal relationship between either component and the subjective experience of effort (de Morree and Marcora, 2014). This integrative view helps explain why fatigue may increase in the absence of overt physiological failure and why substantial mechanical power can still be generated immediately after voluntary termination of exercise (Marcora, 2010).

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Overall, the evolution of fatigue research illustrates a progressive shift from early metabolite-based explanations to afferent-feedback models and, more recently, to psychophysiological frameworks that conceptualize fatigue as a regulated perceptual state. This progression does not replace earlier interpretations but incorporates them into broader models, providing a theoretical basis for investigating how different sources of fatigue, including physical and mental demands, may converge on common central mechanisms involved in effort perception and performance regulation.

Muscle homeostasis clearly plays a key role in determining the limits of contraction, but the willingness to continue exercise depends on mental and motivational processes. This integrated view has important implications for both research and practical applications. For researchers, it implies the need to develop experimental models that simultaneously consider the physiological and cognitive determinants of fatigue. For coaches and trainers, it means that the observation of a decline in performance cannot be automatically attributed to a single mechanism: the assessment must also include motivational state, psychological stress, sleep quality, and other psychosocial variables. From a practical perspective, this framework encourages coaches to move beyond the exclusive monitoring of external load (e.g., training volume, intensity, power output) and to systematically integrate indicators of internal load and perceived effort, such as ratings of perceived exertion (RPE), mood state questionnaires, and subjective measures of mental fatigue. Such tools allow practitioners to identify early signs of excessive psychophysiological strain even when objective performance metrics remain stable. Training design may therefore benefit from the deliberate management of both physical and cognitive stressors. For example, highly demanding tactical meetings, prolonged video analysis, or academic/work-related obligations may be scheduled away from key high-intensity training sessions or competitions to reduce cumulative mental fatigue. Similarly, during periods of congested competition calendars or heavy training loads, reducing unnecessary cognitive demands may help preserve effort tolerance and decision-making capacity. Moreover, this integrated perspective supports the adoption of recovery strategies that target not only muscular restoration but also psychological recovery, including sleep optimization, stress-management techniques, and interventions aimed at sustaining motivation and attentional resources. Coaches may also consider periodizing cognitive load in parallel with physical load, particularly in elite settings characterized by frequent travel, media exposure, and complex decision-making requirements. Finally, recognizing the central role of perception and motivation highlights the importance of communication style, goal setting, and feedback strategies, which can directly influence effort tolerance and how athletes interpret and respond to fatigue signals during both training and competition.

It also highlights the need to distinguish between “fatigue” and “perception of fatigue.” The former can be understood as the complex of processes that limit performance, while the latter refers to the conscious representation that the athlete constructs of these processes. The dissociation between these two levels represents one of the most fascinating and still partially unexplored aspects of the phenomenon.

Traditionally, the distinction between physical and mental fatigue depended on the type of task performed: a physical task should induce muscle or physiological fatigue, while a cognitive task should generate mental fatigue. However, recent literature shows that this distinction is reductive, and the phenomenon is much more complex. Mental fatigue has been shown to impair performance after cognitively demanding tasks, which may be explained by the accumulation of mental fatigue (Boksem and Tops, 2008; Marcora et al., 2009), or alternatively by decreased arousal, boredom, or reduced motivation (Hopstaken et al., 2015). Cognitive tasks can also produce measurable physiological responses such as increased heart rate or hormonal variations, typical of physical fatigue.

Similarly, physical tasks can induce mental fatigue because of the cognitive load required (strategies, attention, feedback). An athlete subjected to intense training may experience reduced performance not only from peripheral or neuromuscular mechanisms but also from the need for sustained concentration, which increases cognitive load. Therefore, reduced performance can stem from both physiological and cognitive factors.

Several theoretical models attempt to explain the reduction in performance observed after cognitive or physical tasks. The Ego Depletion Model suggests that self-control and cognitive effort deplete a limited resource, compromising subsequent performance (Baumeister et al., 1998), although its validity has been questioned (Carter et al., 2015). The Resource-Control Theory proposes that fatigue reflects reduced motivation to exert control rather than true depletion (Inzlicht and Schmeichel, 2012). Motivational models have emphasized the increasing cost of maintaining engagement (Hockey, 2013), while arousal studies show that low activation can produce similar performance declines (Yerkes and Dodson, 1908; Boksem and Tops, 2008).

It is therefore evident that fatigue cannot be rigidly linked to the type of task. It should instead be interpreted as a multidimensional construct, emerging from the interaction of physiological, cognitive, motivational, and emotional factors.

Circadian rhythms further modulate perceived fatigue, which tends to increase at night and in the early morning, while decreasing during daytime hours (Caldwell, 2018). Other findings suggest an increase in perceived fatigue throughout the day, with lower levels in the morning and higher levels before bedtime. Perceived fatigue scores have been shown to correlate with time of day and activity levels across the week (Micklewright et al., 2017). Sleep plays a central role, as even partial deprivation leads to rapid accumulation of fatigue and cognitive decline, particularly in attention and task performance (Goel, 2024). Chronotype must also be considered, since evening types tend to perform worse in the morning compared to morning types (Marchesano, 2025).

Cognitive functions such as attention, working memory, and executive control undergo both homeostatic variations related to wakefulness and circadian variations related to time of day. These functions are lowest during the night and early morning, improve around noon, and peak in the afternoon and evening, though individual factors such as chronotype, age, medication, or sleep history can significantly alter this profile (Valdes, 2019).

In conclusion, fatigue should be understood not only as a complex phenomenon involving both mental and physical components, but also as dynamically modulated by biological rhythms and contextual conditions. Perceived fatigue assumes distinct characteristics depending on time of day, wakefulness, activity context, and individual traits, all of which are essential for understanding its impact on cognition.

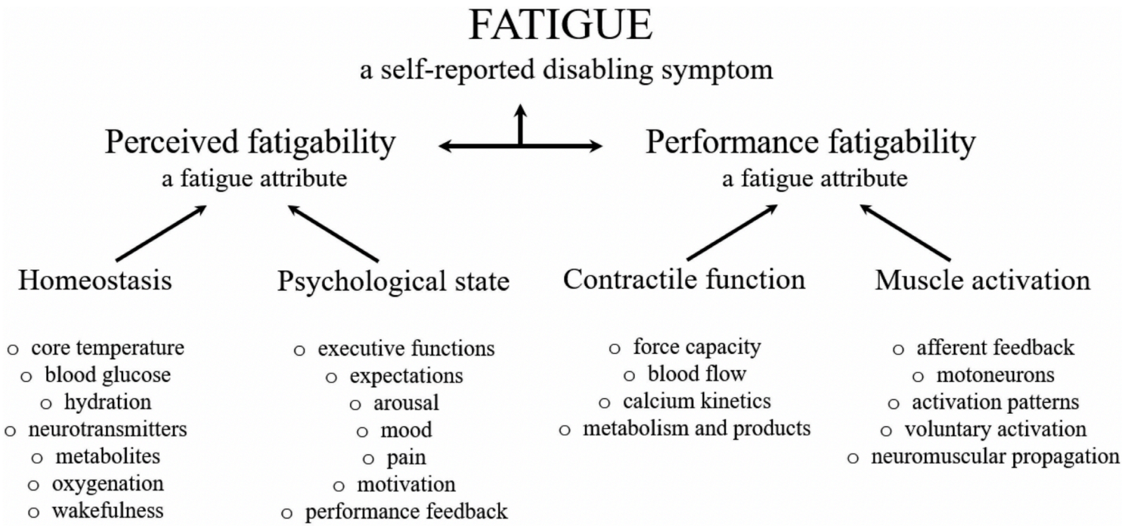


Fig 1.

Major factors contributing to the two domains of fatigue: perceived and performance fatigability. Extracted from Beretta-Piccoli, M., Cescon, C., & D'Antona, G. (2020). Evaluation of performance fatigability through surface EMG in health and muscle disease: state of the art. *Arab Journal of Basic and Applied Sciences*, *28*(1), 21–40.

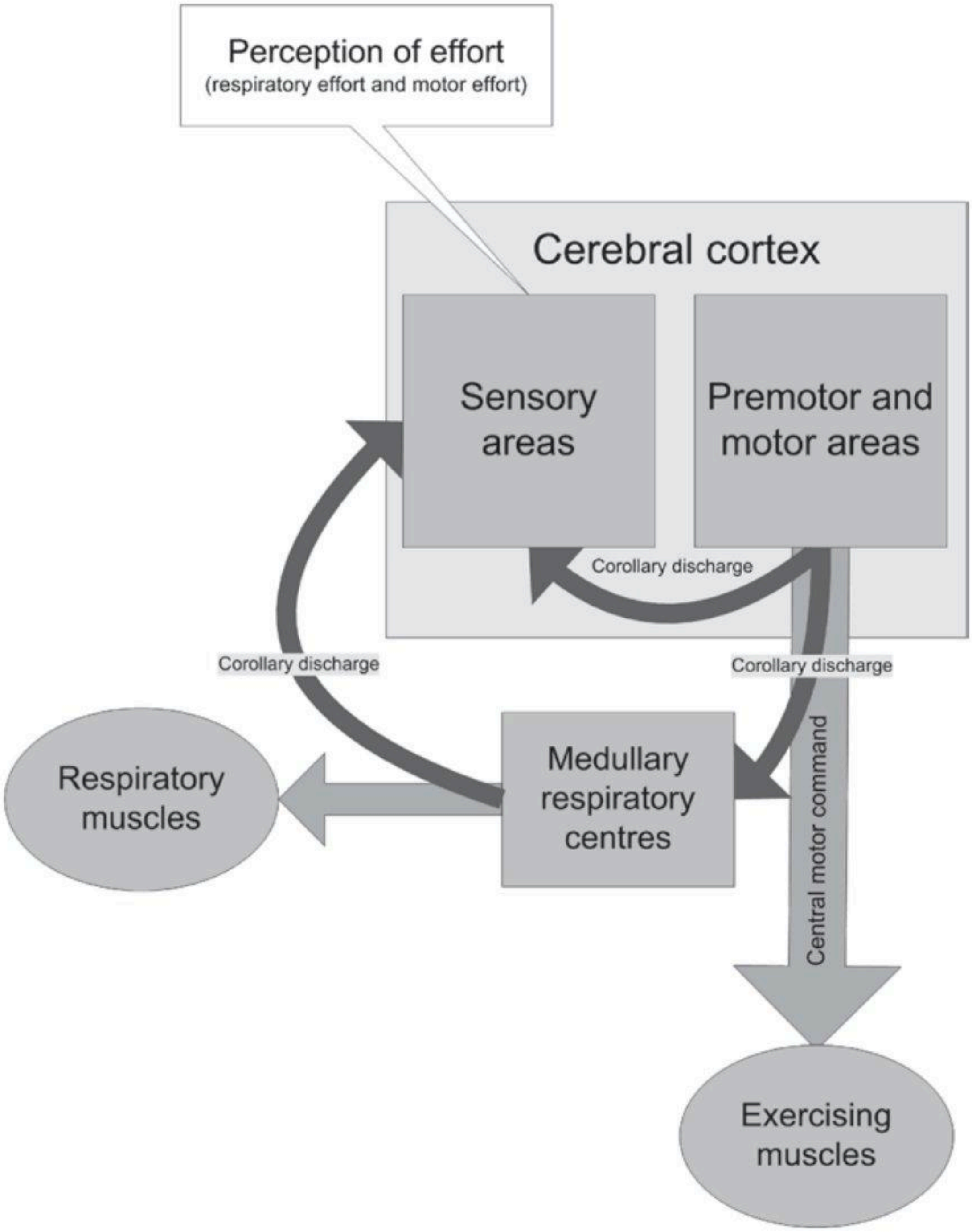


Fig 2. Schematic representation of the neurophysiological pathways underlying the corollary discharge model of perception of effort. Extracted from: de Morree, H., Marcora, S. (2015). Psychobiology of Perceived Effort During Physical Tasks. In: Gendolla, G., Tops, M., Koole, S. (eds) Handbook of Biobehavioral Approaches to Self-Regulation. Springer, New York, NY.

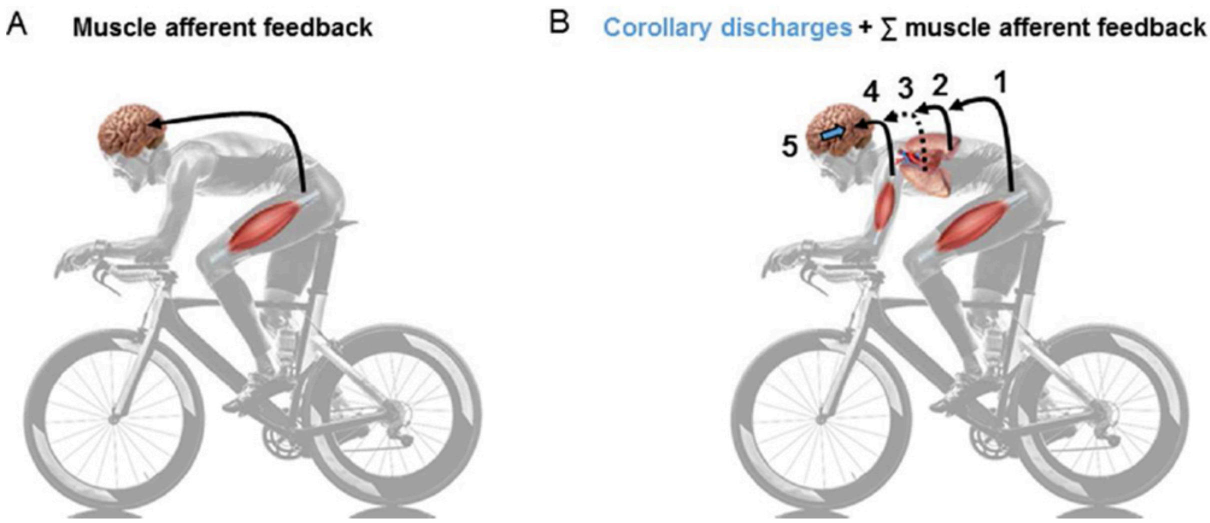


Fig. 3 Schematic representation of neural signals that limit endurance performance. On the left (A) is the classic afferent feedback from the muscles: the brain receives signals from fatigued muscles (black line) and can reduce motor activation. On the right (B) is the integrated model: the brain sends motor commands to the muscles and simultaneously sends corollary discharges (blue arrow, 5) to the sensory areas. These afferent copies, together with the sum of afferent feedback from locomotor muscles (1), respiratory muscles (2), internal organs (3), and other muscles (4), are integrated by the CNS to regulate the central motor drive and determine perceived fatigue. Extracted from: Hureau, T. J., Romer, L. M., & Amann, M. (2018). The 'sensory tolerance limit': A hypothetical construct determining exercise performance?. *European journal of sport science*, *18*(1), 13–24.

The Boredom role

Boredom is a common and pervasive experience that influences individuals' thoughts, emotions, motivations, and actions; however, its definition, functions, and correlates are still not fully understood (Raffaelli et al., 2018). It may arise from multiple sources and manifest in different forms. Boredom is commonly conceptualized as an affective response signaling the perception that a situation or activity lacks stimulation and meaning, thereby indicating the need to invest effort to cope with or resolve this condition (Bench & Lench, 2019). Although similar experiential features may be observed in other affective states such as anger, sadness, or frustration, it is important to distinguish these experiences from boredom, which is specifically characterized by feelings of insufficient stimulation and by the perception that the ongoing activity is meaningless (van Tilburg & Igou, 2012).

In light of empirical evidence indicating that boredom, disengagement, and mental fatigue constitute distinct psychological constructs, characterized by different cognitive, affective, and motivational profiles and by non-overlapping effects on performance (van Tilburg & Igou, 2012; Bench & Lench, 2013; Westgate & Wilson, 2018), it can therefore be inferred that their clear conceptual and operational discrimination represents not only a theoretical requirement, but also a relevant methodological condition for the accurate interpretation of experimental data and for the development of targeted and effective intervention strategies in sport and rehabilitation contexts.

A significant limitation of the current literature is the relatively limited attention devoted to boredom compared to mental fatigue, partly due to the lack of coherent operational definitions and to the predominance of studies focusing on high cognitive load (Raffaelli et al., 2018). Although boredom may impair performance in a manner similar to mental fatigue, conceptual evidence suggests that the two phenomena arise from different psychological mechanisms—for instance, prolonged and monotonous tasks may induce boredom or underarousal rather than fatigue resulting from active cognitive load—with distinct implications for prevention, identification, and intervention (Schampheleer et al., 2025). For these reasons, it is essential to further investigate the distinction between boredom and mental fatigue, despite their strong interconnection making a strict theoretical and empirical demarcation challenging (Pattyn et al., 2018).

A concrete example is provided by the widespread use of the Psychomotor Vigilance Task (PVT), which is extensively employed in studies of mental fatigue. Even in the absence of high cognitive demands, this task requires sustained attention, vigilance, and continuous responsiveness. In some cases, observed decrements in reaction time have been shown to co-occur with task characteristics such as monotony and low external stimulation, suggesting that performance deterioration may not be attributable exclusively to mental fatigue, but also to boredom induced by the repetitive and weakly stimulating nature of the task (Pattyn et al., 2008; Alikonis et al., 2002).

From a methodological perspective, these considerations raise concerns regarding the widespread practice of using emotionally neutral documentary viewing as a control condition in experimental research. Although this approach is commonly adopted, the potential contribution of boredom is rarely considered as a confounding factor. Consequently, performance decrements observed under such conditions may be at risk of being misattributed to cognitive or physiological mechanisms, while the effects of monotony and insufficient stimulation remain unaccounted for.

Accordingly, the present thesis aims to systematically examine the "boredom role" as a theoretically and methodologically relevant variable in the study of mental fatigue and performance. Rather than treating boredom as a secondary or epiphenomenal experience, this work seeks to evaluate its contribution to performance changes and its interaction with task characteristics and attentional demands. A clearer understanding of these mechanisms is expected to refine both theoretical models of fatigue-related performance decline and the methodological design of experimental paradigms, with implications for applied domains such as sport science and rehabilitation.

The Psychobiotic role of the Microbiome

The first published study on the gut-brain axis dates back to the 1980s (Banks, 1980), although the quote attributed to Hippocrates of Kos “All disease begins in the gut” shows how the centrality of this organ was already known more than 2,000 years ago. Beyond the historical excursus, studies and research on the gut-brain axis have increased exponentially in the last decade. The peak of interest coincided with the entry of one of the key regulators of the gut-brain axis: the trillions of microbes in the gut, the microbiota[1] (Cryan, 2019). Studies on animals and humans have shown that the central nervous system and the microbiota are in continuous dialogue (Cryan et al., 2019; Sharon et al., 2016; Morais et al., 2021; Osadchiy et al., 2019; Zhu et al., 2017). Thus, the concept of gut-brain axis has been extended to

[1] The gut microbiota is a diverse ecosystem consisting of bacteria, archaea, viruses, protists and fungal communities (mycobiome) living in the human gut. Microbiome refers to the collection of genomes from all microorganisms in a particular environment (Jager, 2019)

microbiota-gut-brain (Round, 2009; De Palma, 2014). It is now clear that the microbiota is a crucial determinant of human health and disease and a key regulator of host physiology (Cryan, 2019). However, given the magnitude of physiological action and the number of strains belonging to various bacterial genera (greater than the number of cells in the human body), we are still far from being able to explain every possible mechanism related to the microbiota. Moreover, it remains complicated to define a "healthy microbiota"; the considerable interindividual differences in microbiota composition make a one-size-fits-all approach difficult (Cryan, 2019). While this represents an opportunity, as it could be the conduit for effective personalized medicine approaches in the future (Zmora, 2018), it should be kept in mind that this will always result in large heterogeneity in outcomes and thus often discordant results. The growing trend of interest and research towards the microbiome will likely continue or even increase in the near future. To confirm this, it should be noted that to date at least 105 diseases and disorders, substantially different in nature and etiology, have been associated with alterations in the gastrointestinal, respiratory, oral, skin, and urinary/vaginal microbiota (Rojo, 2017).

The effectiveness of probiotic supplementation in promoting and maintaining optimal health status has been recognized by the scientific community for only a few years (Di Dio, 2022). Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host (Hill, 2014). Intake of probiotics has demonstrated anti-cancer, anti-obesity, anti-diabetic, and anti-inflammatory activities (Dong, 2021). It has also been found that probiotic supplementation can affect the brain and central nervous system (Kerry, 2018). Furthermore, altering the microbiota with beneficial bacteria (probiotics) or prebiotics can lead to changes in mood as well (Dong, 2021; Mayer, 2015). In this regard, the term "psychobiotics" refers to interventions targeting the microbiota, such as beneficial bacteria (probiotics) or support for such bacteria (e.g., prebiotics), that can influence bacteria-brain relationships by producing psychological effects through direct or indirect effects on the central nervous system (Cryan, 2019).

The findings on the complex pathways involved in the microbiome-gut-brain axis are mostly the result of studies in germ-free (GF) animals, along with animals whose microbiome is altered by exposure to probiotics, prebiotics, pathogenic bacterial infections, antibiotics, and fecal microbiota transplantation (Butler, 2019). It is not certain that current knowledge is sufficient to unequivocally explain the mechanisms of transmission of communicative signals, but they seem to include neural, endocrine, immune, and metabolic pathways (Dinan, 2017). The vagus nerve is reported to be one of the main pathways of neural communication between gut microbes and centrally mediated behavioral effects (Bravo, 2011). In addition, more than 90% of the body's serotonin (5-HT) is synthesized in the gut. The metabolic pathway involves the conversion of tryptophan to 5-hydroxytryptophan (a short-lived product quickly converted to serotonin) by the enzyme tryptophan hydroxylase (TPH) (Berger, 2009). The microbiome drives increased transcription of the limiting enzyme TPH1. High levels of TPH allow increased serotonin synthesis in enterochromaffin cells (endocrine cells that specialize in 5-HT production), resulting in increased peripheral levels of 5-HT (Butler, 2019). Moreover, several probiotic genera have been associated with the specific production of neurotransmitters (e.g., Lactobacillus spp. and Bifidobacterium spp. produce GABA; Bacillus spp. produces dopamine) (Berger, 2009). However, the amount produced is limited and it seems unlikely that these molecules are able to cross the blood-brain barrier (BBB) (Roshchina, 2010). Short-chain fatty acids such as butyrate, propionate, and acetate are also produced by the gut microbiome and are likely to act as epigenetic modulators (Stilling, 2014). On the other hand, the immune communication pathway is carried by cytokines (El Aidy, 2014). These, under normal physiological situations, have little chance of passing the BBB; however, it is increasingly believed that they are able to influence brain areas, such as the hypothalamus, in which the BBB is deficient (Dinan, 2017). Another mechanism is the activation of the hypothalamic-pituitary-adrenal (HPA) axis by cytokines interleukin IL-1 and IL-6, causing cortisol release (Dinan, 2017). The activation of the HPA axis by the microbiome is not mediated solely by the immune pathway. In fact, it also appears to be influenced by the probable ability of the microbiome to alter central gene expression in the amygdala, hippocampus, and prefrontal cortex, regions known to play a role in learning, memory, mood, and anxiety (Butler, 2019). Psychobiotic supplementation could therefore play a crucial role in preserving correct gut-brain communication. It seems increasingly clear that in several pathological conditions, psychological or physical stress is able to significantly dysregulate the HPA axis and consequently the brain-gut-microbiota axis, for example in irritable bowel syndrome (Dinan, 2006).

A growing body of evidence indicates that the gut microbiota plays a modulatory role in subjective fatigue and the perception of energy availability (Álvarez-Herms et al., 2025). Although no specific bacterial taxon has been unequivocally identified as a direct biomarker of fatigue, studies in animals and humans highlight consistent functional patterns, suggesting that microbial metabolic activity and inflammatory signaling are central mediators (Boolani et al., 2022).

A key mechanism involves microbial production of short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, derived from the fermentation of indigestible carbohydrates. SCFAs contribute significantly to the host's energy balance and are rapidly absorbed in the colon, with a fraction entering the systemic circulation and reaching peripheral tissues, including skeletal muscle. There, SCFAs interact with specific G protein-coupled receptors (GPR41 and GPR43) (Brown et al., 2003) and influence substrate oxidation, mitochondrial efficiency, and ATP resynthesis, processes that are fundamental for sustaining prolonged physical effort and delaying the onset of fatigue (Sales et al. 2022).

Exercise-associated microbiota profiles are enriched in butyrate-producing and anti-inflammatory taxa such as *Faecalibacterium prausnitzii*, *Roseburia hominis*, *Akkermansia muciniphila*, and members of the genus *Coprococcus (*Anhe et al., 2015; Li et al., 2015). These bacteria support intestinal barrier integrity (L'Huillier et al., 2019), reduce endotoxin translocation (Roberts et al., 2016), and downregulate systemic inflammatory tone. Since low-grade chronic inflammation is a recognized contributor to both central and peripheral fatigue, this microbial configuration provides a plausible biological link between gut and fatigue perception (Donati Zeppa et al., 2019). Conversely, exercise-induced physiological stress can disrupt gut homeostasis, increasing intestinal permeability and circulating lipopolysaccharides, thereby activating immune pathways and the hypothalamic–pituitary–adrenal axis (Clark and Mach, 2016). Such responses are associated with mood disturbances, impaired motivation, and heightened fatigue sensations. Experimental evidence further shows that stress-related exercise alters the abundance of taxa involved in mucus degradation and immune modulation, reinforcing the connection between dysbiosis, immune activation, and fatigue-related symptomatology (Clark and Mach, 2016).

Interventional studies provide additional support for this framework. Probiotic supplementation has been shown to increase time to exhaustion during endurance exercise in the heat while significantly reducing circulating lipopolysaccharide concentrations, indicating improved gut barrier function and lower microbial-derived inflammatory load (Shing et al., 2013). These effects occurred even in the absence of major changes in circulating cytokines, suggesting that gut integrity alone may meaningfully influence physiological strain and fatigue development during exercise (Shing et al., 2013).

Collectively, these findings support the conceptualization of fatigue as a multifactorial phenomenon partly shaped by microbiota-dependent regulation of energy harvest, metabolic efficiency, immune activity, and neuroendocrine stress signaling. A microbiota optimized for SCFA production and low inflammatory output may therefore contribute to enhanced perceived energy and fatigue resistance, whereas dysbiotic and pro-inflammatory profiles may predispose individuals to persistent tiredness and reduced physical performance. Although a full discussion is beyond our scope here, let us briefly mention the data on myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) that point to a relationship between fatigue and the microbiome that warrants further study. Several studies have observed a decrease in butyrate-producing bacteria in ME/CFS, including taxa such as *Ruminococcus, Faecalibacterium prausnitzii, and Eubacterium rectale* (Xiong et al., 2023; Nagy-Szakal et al., 2017; Guo et al., 2023; Lupo et al., 2021). Moreover, the abundance of these species is negatively correlated with the severity of fatigue in ME/CFS patients (Wang et al., 2024). In parallel, these patients show a significant decrease in energy-relevant metabolites of microbial origin, such as acetate (Armstrong et al., 2016). There are also preliminary indications that probiotic supplementation may improve post-exercise fatigue in this population (Ranisavljev et al., 2024).

We emphasize that these latter findings relate to ME/CFS and therefore should not be generalized to healthy individuals or athletes. However, in illustrating the psychobiotic perspective we propose here, it is important to recognize these emerging interactions between the microbiome and fatigue. In this context, our planned study on supplementation with *Veillonella atypica FB0054* will further explore this relationship, which remains a relatively unexplored area.

HYPOTHESIS

Study 1

Perceived exertion is considered a primary determinant of time-to-exhaustion performance in elite cyclists, regardless of whether it originates from locomotor muscle fatigue or mental fatigue. If the experimental protocol successfully induces mental fatigue and leads to an increase in perceived exertion comparable to that induced by locomotor muscle fatigue, a similar reduction in performance is expected. Therefore, no significant differences in performance are anticipated between the two fatigue conditions when perceived exertion is matched.

Study 2

Perceived exertion is a primary determinant of endurance performance, regardless of whether it arises from physical or mental fatigue. No significant differences are expected between the two experimental conditions; however, both performances are anticipated to be impaired compared with the control condition.

Study 3

Actively engaging in a medium-to-long session of a first-person shooter (FPS) video game increases perceived fatigue, alters mood states, and reduces reaction time and attentional performance to a greater extent than merely watching the same game for the same duration, due to the additional effect of mental fatigue induced by active participation.

Protocol 1

Supplementation with Veillonella atypica FB0054 for a period of four weeks could result in improved endurance performance. This positive effect would be explainable through an increase in the ability of the microbiota to utilize lactate produced during exercise by converting it into propionate. This mechanism would promote faster clearance of lactate and improved energy performance. In parallel, it is plausible that supplementation could contribute to a modulation of the composition of the microbiome, with a reduction in the index of dysbiosis and an enrichment of metabolic pathways associated with short-chain fatty acids. These changes could not only impact performance but also improve quality of life and support some aspects of cognitive function, making the effect relevant not only to athletes but also to the health of a general population.

THESIS FORMAT

This thesis is presented in the form of a collection of three independent studies, each written in the style of a scientific article. Despite their methodological differences, all studies are unified by a common research theme that has guided the doctoral project: the multifaceted nature of fatigue and its perception in different contexts of human performance.

Each study follows the conventional structure of a peer-reviewed article, comprising an introduction, a detailed description of materials and methods, the presentation of results, and a discussion that contextualizes the findings within the broader scientific literature. The contribution of co-authors and collaborators is explicitly acknowledged in the acknowledgments. In addition it will be presented in this thesis, a research protocol was also developed during the phd program.

The first two studies are presented as separate investigations, even though they are based on nearly identical methodological protocols. This choice reflects the deliberate aim of testing the same hypothesis across two distinct populations, thereby allowing for the exploration of potential differences in responses and the generalizability of the findings.

Study three was conducted during an international research stay at the Capital University of Physical Education and Sport (CUPES) in Beijing. These collaborations provided the opportunity to broaden the scope of the thesis by integrating cross-cultural perspectives and additional methodological approaches, further enriching the overall contribution of the project.

The protocol 1 was formally submitted to the Independent Ethics Committee for Non-Pharmacological Clinical Trials and received a positive evaluation. However, due to logistic issues related to the procurement of the probiotic preparation that was to be tested, the study has not yet commenced. Despite this delay, the trial is expected to be conducted in the near future, representing a natural continuation and expansion of the concepts presented and discussed in this thesis. In particular, it will build upon the psychophysiological framework of fatigue explored here, opening new perspectives on the role of the gut microbiome and probiotic interventions in endurance performance and fatigue management.

CHAPTER 2
LOCOMOTOR VS. MENTAL FATIGUE IN ELITE CYCLISTS

TULLIO SENATORE, SAMUELE MARCORA

ABSTRACT

This study compared the effects of mental fatigue (MF) and locomotor muscle fatigue (LMF) on endurance performance, perceived exertion, and physiological responses in well-trained and elite cyclists. Ten cyclists (performance levels 4–5; 21.8 ± 2.7 yrs; 8 males: VO_2max 68.4 ± 2.5 $ml \cdot kg^{-1} \cdot min^{-1}$, PPO 438.8 ± 50.1 W; 2 females: VO_2max 58.7 ± 5.4 $ml \cdot kg^{-1} \cdot min^{-1}$, PPO 335.0 ± 14.1 W) completed an incremental ramp test to determine respiratory compensation point (RCP), VO_2max, and peak power output (PPO).

In three subsequent randomized visits, participants performed a time-to-exhaustion test (TTE) at 95% RCP under: (1) MF induced by a 1-h psychomotor vigilance task (PVT), (2) LMF induced by 100 drop jumps, and (3) a control condition involving documentary viewing. Heart rate (HR), VO_2, ventilation, blood lactate concentration, and rating of perceived exertion (RPE) were measured during exercise. Manipulation checks included knee-extensor maximal voluntary contraction, PVT performance, and perceived physical and mental fatigability. Data were analyzed using repeated-measures ANOVA ($p < 0.05$) and the individual isotime method.

MF impaired PVT performance ($p < 0.001$), whereas LMF increased perceived physical fatigability ($p < 0.001$). TTE did not differ between conditions (Control 989 ± 387 s, MF 1019 ± 434 s, LMF 973 ± 457 s; $p = 0.876$). RPE and HR at isotime tended to be higher in LMF but were not significant.

Elite cyclists appear resistant to performance impairments induced by MF and LMF. Importantly, boredom did not differ between MF and control conditions, suggesting that the monotony of passive documentary viewing may elicit psychological effects comparable to those induced by prolonged and repetitive cognitive tasks such as the PVT. This indicates that commonly used passive control conditions may not be psychologically neutral and could partially reproduce key features of mental fatigue.

INTRODUCTION

Over the past 15 years, research has clearly demonstrated that mental fatigue—a state induced by prolonged cognitive activity—can significantly impair endurance performance. A seminal study showed that a demanding cognitive task induced mental fatigue and reduced subsequent cycling time-to-exhaustion by ~15% compared with a control condition (Marcora et al., 2009). Importantly, this decline occurred without any alteration in cardiorespiratory or muscular function: heart rate, oxygen uptake, and other physiological responses during exercise remained unchanged (Marcora et al., 2009). Instead, mentally fatigued athletes reported higher perceived exertion throughout the exercise and reached their tolerable maximum effort earlier, leading to premature termination compared to the non-fatigued state. This pivotal finding suggested that mental fatigue impairs endurance via an increased perception of effort rather than through direct muscular or cardiovascular limitations (Marcora et al., 2009).

The relationship between mental fatigue and endurance performance has since become a central theme in sports science. Several studies have investigated the influence of prior cognitive exertion on whole-body endurance performance (MacMahon et al., 2014; Pageaux et al., 2014; Smith et al., 2015, 2016). Collectively, these studies highlight the deleterious impact of mental fatigue on endurance, although elite athletes appear to demonstrate greater tolerance to this type of impairment (Martin et al., 2016). This effect has been observed not only in cycling (Marcora et al., 2009; Martin et al., 2016) but also in running (MacMahon et al., 2014; Pageaux et al., 2014), including both intermittent (Smith et al., 2015) and incremental protocols (Smith et al., 2016).

The effects of locomotor muscle fatigue—fatigue arising from prior physical exertion—on endurance performance have long been established. Intense or prolonged muscular activity leaves the working muscles fatigued, which typically reduces subsequent endurance capacity, and this has been confirmed in multiple experimental models. Bangsbo et al. (1996) and Nordsborg et al. (2003) demonstrated that fatigue induced by repeated arm-cranking exercise impairs the endurance of the lower limbs. Using the same protocol (4 × 1 min arm cranking at ~140 W), they observed a 26% (Bangsbo et al., 1996) and 32% (Nordsborg et al., 2003) reduction in quadriceps (during knee extension(KE)) isotonic contraction time-to-exhaustion at ~60 W. Similarly, Pageaux and Lepers (2016) reported that repeated exhaustive arm exercise can decrease subsequent leg endurance time by ~25–30%. Heavy eccentric exercise, producing muscle soreness and fiber damage, is also well known to impair next-day endurance. Unlike mental fatigue, physical fatigue directly reduces muscle function, causing a measurable drop in maximal force or power output (Pageaux and Lepers, 2016). The gold standard for identifying such reductions is the maximal voluntary contraction (MVC) (Gandevia, 2001). These impairments may originate either from incomplete neural drive to the working muscles—commonly termed central fatigue (Gandevia, 2001)—or from impairments at or beyond the neuromuscular junction that alter contractile properties, defined as peripheral fatigue (Allen et al., 2008).

Nevertheless, as with mental fatigue, perceived effort again plays a critical role. Marcora et al. (2008) demonstrated that prior fatigue of the knee extensors increased RPE during a subsequent cycling task at 80% of maximal aerobic power. Likewise, Amann et al. (2013) showed that fatigue of the knee extensors not directly involved in a subsequent endurance task still elevated perceived exertion during contralateral isotonic knee extensions at 85% of maximal aerobic power. Thus, although locomotor fatigue originates from neuromuscular impairments, its limiting effects on performance are once again closely tied to the brain's perception of effort. This reinforces the notion that endurance performance is ultimately constrained by the brain: the point at which perceived effort reaches its intolerable maximum leads the athlete to disengage, even if physiological reserves remain (Marcora et al., 2009).

A striking convergence emerges: both mental and physical fatigue impair endurance by elevating perceived effort. An important review has emphasized that, despite their distinct origins, the common denominator in both cases is the heightened sense of how hard the exercise feels . This framework is encompassed by the psychobiological model, which posits that endurance limits are governed not solely by muscular failure, but by perceived effort and motivation. Both mental and physical fatigue intensify the conscious sensation of effort, effectively draining the athlete's willpower or drive to continue. The brain thus initiates disengagement ("I cannot go on") even when muscular capacity is not fully exhausted (Marcora et al., 2009). This shared mechanism may account for the convergence of different fatigue modalities on similar performance outcomes. Accordingly, our hypothesis is that performance is determined not by the origin of fatigue (mental vs. physical), but by the magnitude of perceived effort it elicits. This hypothesis was specifically examined in a cohort of elite cyclists.

Much of the early work on mental fatigue relied on recreational or moderately trained samples (Pageaux et al., 2013 and 2015; Budini et al., 2014; Duncan et al., 2015). More recent evidence suggests that elite endurance athletes may respond differently, displaying greater resilience to mental fatigue. Martin et al. (2016) provided direct comparison between professional road cyclists and recreational cyclists. Recreational athletes exhibited clear impairments under mental fatigue, while elite professionals showed no such decrement in time-trial performance. Interestingly, elite cyclists also outperformed recreational participants in the cognitive tasks themselves, suggesting stronger inhibitory control and greater resistance to mental fatigue—traits that may be partly innate and partly acquired through years of training (Martin et al., 2016).

Elite athletes also display heightened resistance to physical fatigue. Observational analyses of professional stage races reveal that power output does not systematically decline across consecutive days; rather, variations in wattage depend on stage characteristics (Vogt et al., 2006). Although inducing physical fatigue in laboratory settings with elite cyclists poses practical and ethical challenges, evidence from Ducrocq et al. (2016) indicates that excitation–contraction coupling is preserved after fatiguing exercise in elite athletes compared to moderately trained athletes.

To our knowledge, the present study is the first to directly contrast these two fatigue paradigms in a within-subject design using elite cyclists. A recent investigation by Mortimer et al. (2024) did explore mental, physical, and combined fatigue, but in a mixed group of trained participants performing a general exercise protocol. Our study was designed to bridge this gap by applying both a mental fatigue and a locomotor muscle fatigue intervention prior to cycling time-to-exhaustion trials in elite cyclists. One of the aims of the present study is to determine whether the proposed mental fatigue protocol, which differs from that used by Martin et al. (2016) (i.e., 60-min psychomotor vigilance task vs. 30-min Stroop task), is capable of inducing mental fatigue also in elite athletes. If the first objective were achieved another aim was to determine whether endurance performance is more compromised by one type of fatigue than the other, or whether the effects are comparable. The central hypothesis underlying this work is that perceived effort is the determining factor in any fatigue-induced decline in performance. Put simply, we suspect that it is not the origin of fatigue (mental vs. physical), but rather the level of effort perceived by the athlete that dictates the outcome. If true, then mental and physical fatigue of equivalent intensity should lead to similar impairments in endurance performance and comparable psychophysiological responses, since both elevate perceived effort to a similar extent.

MATERIALS AND METHODS

Participants

Ten performance level 4 and three level 5 (Decroix 2016; De Pauw 2013) (21.8 ± 2.7 yrs, 8 Male 68.4 ± 2.5 VO_2Max and 438.8 ± 50.1 PPO, 2 Female 58.7 ± 5.4 VO_2Max and 335.0 ± 14.1 PPO) were recruited and volunteered to take part in this study[2]. The sample size was calculated based on the previous study that explored the effect of locomotor muscle fatigue or mental fatigue (Amann and Dempsey 2008; de Morree and Marcora 2013; Deley et al., 2006; Martin et al., 2016; Pageaux et al., 2013 and 2015; Budini et al., 2014; Duncan et al., 2015). The inclusion criteria were being in the Performance Level 4 and 5. The experimental protocol was conformed to the standards set by the Declaration of Helsinki.

Procedures

All cyclists visited the laboratory on four occasions. A schematic overview of the protocol can be found in Figure 4. The first visit was a familiarization and physiological profile detection visit. During this visit, participants signed an informed consent form, a Physical Activity Readiness Questionnaire (PAR-Q), and anthropometric measures were taken. A Ramp exercise test (Warm-up for 3 minutes at 100W, the first step was set at 125W and later 5 W increments every 12 seconds) was then performed until exhaustion (or when cadence was less than 70 rpm for more than 5 s, despite strong verbal encouragement) on Cyclus2 Ergometer (RBM elektronik-automation GmbH; Leipzig, Germany) to measure VO_2peak (highest 30 s average VO_2 during the test) (Mauger 2012) , Respiratory compensation point (RCP) (The point where both the ratio VE/VO_2 and VE/VCO_2 increase) (Wasserman 1987) and peak power output (Ppeak). Ppeak was estimated as the Watt at the end of the trial. During the test, VO_2 was measured breath-by-breath using a computerized metabolic gas analysis system (Quark CPET; COSMED, Rome, Italy). All anthropometric measurements were recorded and added to the OMNIA software. Before the incremental exercise test, we fitted the personal bike of the cyclist on the ergometer. Familiarization also included the anchor to Borg scale: standard instructions were given on how to assess their perceived

[2] Another two participants have been recruited but have not yet finished testing. The goal is to have randomized and counterbalanced interventions

exertion (i.e., conscious feeling of how heavy and strenuous a physical task is) on Borg's Perceived Exertion Rating Scale (RPE) (6-20) (Borg 1998). During the warm-up the low anchor point (point 7) was established using standard procedures. Similarly, close to the task failure the high point was anchored (point 19) (Noble 1996). After a 30 min of rest, subjects perform in a time to exhaustion habituation at 95% of RCP without reaching the exhaustion.
After a minimum of 48 h, subjects returned to the laboratory for the second time. For all participants, intervention order was randomly allocated by a web-based computer program (www.randomization.com). The visit started with a 10 min of standard warmup (100W at cadence range 70-100 rpm). After this, baseline unilateral knee extensor strength was assessed by measuring force during 90 degrees isometric maximal voluntary contraction (MVC) of the dominant knee extensor muscles. Participants were instructed to grip the handles of the leg extension machine and push three times at sub-maximal force (25%, 50%, 75%). After this warm-up, three trials of six s 100% MVC were recorded.The force produced in each isometric contraction was monitored using a wireless dynamometer (K-Pull, Kinvent Physio, Montpellier, 3400, France). Between all trials, 2-min rest periods were observed. During the maximal trials, strong verbal encouragement was given. Before starting the intervention participants give a subjective valuation of mental and physical fatigue based on Fatigue and Fatigability scale (Schnelle 2012). Before and after intervention we assess the state of boredom with a short form of Multidimensional scale of boredom state (MSBS-SF) (Donati et al., 2021; Spoto et al., 2021) and record an objective measurement of mental fatigue with a 5-min PVT (Spoto et al., 2021).
Based on the Fatigue and Fatigability scale a post intervention valuation of mental and physical fatigability was recorded. Moreover, a Physical and Mental session RPE scale (Nigro et al., 2023) was administered to participants.
After completing the standard warmup, post intervention knee extensor strength was assessed with three maximal trials. 2-min after MVC, Motivation related to the time-to-exhaustion tests was measured using the success motivation and intrinsic motivation scales (Matthews 2001). All subjects perform the TTE at intensity relative to 50% of RCP as warm up for 5 min, consecutively the intensity has been brought to 95% of RCP until exhaustion or when the cadence was lower than 70 rpm for at least 5 s despite verbal encouragement. For all visits verbal encouragement was offered by an investigator blinded with respect to intervention. Lactate measurements were taken with a portable blood lactate analyser, Lactate Scout 4 (LS, SensLab GmbH, Germany) before, every 5 min during the test and at task

failure. During the TTE a rate of perceived exertion based on 6-20 Borg Scale was recorded every 2 min.

Psychomotor vigilance task (PVT)

The PVT is a simple visual reaction time test first used as a research tool by Dinges and Powell (1985) (Dinges 1985). We used the Nasa-validated application to perform a 1-hour task (Arsintescu 2017). In this test, a millisecond counter appears in the center of the screen until the participant presses on the side of his dominant hand of the screen, stopping the counter to display the response time (RT). If participants pressed before or within the first 150 ms of the timer display, a "false start" message was displayed on the screen. When the response was delayed by 500 ms, we considered it as a lapse of attention. The time interval between the disappearance of the RT or false start message and the appearance of the next stimulus varied randomly from 2 to 10 s. Approximately 600 stimuli were displayed during the 60-minute treatment period (Since the interval between stimuli is random and varies between 2 and 10 seconds, there may be differences of more than 50 stimuli between two tests of equal duration).

100-Drop Jump protocol (DJ)

This eccentric fatigue protocol does not induce respiratory muscle fatigue because of the very low aerobic demands. Participants dropped 100 times from a 50 cm platform to 90° knee angle before jumping upward as far as possible. Subsequent jumps were separated by 20 s of rest. This eccentric fatigue protocol took 34 min in total (Skurvydas 2000). In this study, unlike the original study, the platform height was changed from 40 cm to 50 cm. The subject stepped on a 50-cm high platform with legs in which muscle contraction force was not tested.

Control (C)

A passive control treatment was used as a control (Marcora 2009) and consisted of watching one of four different documentaries: "How the universe work— *Hunt for the Universe's Origin"* (Pioneer Production, 2010-); "Secrets of the world's super skyscrapers— *Episode*

#1.1" (Lambent Production, 2018); "Prehistoric Planet— *Forests"* (BBC Studios Natural History Unit, 2022-); "Mega factories— *Speed rail*" (Hoff Production, 2006-2012). To increase the participants' engagement, they were given the opportunity to choose which documentary to watch. Documentaries on these topics were chosen as they were able to maintain a neutral mood and stable HR (Silvestrini 2007) and for a duration of about 50 minutes. During the treatments, one of the investigators monitors proper compliance with the intervention.

Physiological and Psychological Measurements

At the familiarization visit, all the psychological and physiological measures that would be collected were explained. We collect for all visit times the HR (bpm). During the endurance test, tidal volume (liters), breathing frequency (breaths/min), ventilation (l/min), VO_2 (l/min), and carbon dioxide production (VCO_2 ; l/min) were measured breath-by-breath.

Multidimensional scale of boredom state (MSBS-SF)

A short validated version of the Multidimensional scale of boredom state (Hunter 2015) was used to assess the level of boredom pre and post each intervention. The scale was proposed in the Italian version selecting the same eight items from the Italian version of the original MSBS (Craparo 2017) , as already made by Donati et al., 2021. The scale contains eight Likert-type items on a 7-points scale from 1 (strongly disagree) to 7 (strongly agree). The result was analyzed as the sum of the score. (Appendix A)

Fatigue/Fatigability scale

Before the intervention, participants were asked to rate their current level of Mental and Muscle tiredness (e.g., "How tired are you right now?") on a 7-point scale from 7 (extremely tired) to 1 (extremely energetic). Participants were asked when they finished the intervention, "Are you more or less at the same energy level as when you started the intervention?" A score of 4 was assigned if there was no change in perceived energy level. If participants reported that they had less energy, they were asked whether they were extremely, somewhat or a little more tired. If they reported having more energy, they were asked if they were extremely, somewhat, or a little more energetic. This branching question format was tested to be less confusing than asking for a rating across the full 7-point scale of energy change (Schnelle et al., 2012) . (Appendix B)

Success and Interest Motivation

This questionnaire was developed by Matthews et al. in 2001, based on the Dundee Stress State Questionnaire (DSSQ). Although the DSSQ can be used to assess motivation, the scales in question focus specifically on aspects of motivation related to success and interest in task performance. Both dimensions contribute to Task Engagement, but Success Motivation is also linked to negative emotions and self-beliefs.

The scale consists of 15 Likert-type items rated on a 5-point scale ranging from 0 (not at all) to 4 (extremely). Seven items assess Success Motivation, and another seven measure Interest Motivation, while the final item evaluates a general state of motivation. Some items were reverse-scored, and the results were analyzed as the sum of the scores.

Statistical analysis

All data are reported as means ± standard deviation (SD). A one-way repeated-measures ANOVA was used to evaluate the effects of different experimental conditions (mental fatigue, muscle fatigue, and control). For variables measured both before and after each intervention, a two-way repeated-measures ANOVA (condition × time) was applied.

An additional two-way ANOVA (condition × iso-time) was used to analyze the physiological variables and perceived exertion recorded during the exhaustion test (TTE). Because the exhaustion trials had different durations, the comparison between trials was performed using the individual iso-time technique (Nicolò et al., 2019). Comparison points were selected at the beginning and peak of each trial. Midpoints were determined at 20%, 40%, 60%, 80%, and 100% of the duration of the trial with the worst performance among the three sustained by the athlete, and then applied to all trials.

The level of statistical significance was set at $p < .05$.

RESULTS

Time to Exhaustion (TTE) and Perceived Exertion

No significant differences were observed between conditions in time to exhaustion (TTE) ($p = 0.876$). Mean TTE was 989 ± 387 s in the control condition (C), 1019 ± 434 s in the mental fatigue condition (MF), and 973 ± 457 s in the locomotor muscle fatigue condition (LMF) (Figure 5).

Perceived exertion during TTE showed a trend toward differences between conditions at isotime comparison ($p = 0.08$; $\eta^2_p = 0.244$), with higher values in the LMF condition (C = 15.8 ± 1.04; MF = 15.9 ± 1.15; LMF = 16.6 ± 1.16). However, no significant time × condition interaction was found ($p = 0.874$) (Figure 6). Peak ratings of perceived exertion (RPE) at the end of exercise did not differ significantly between conditions ($p = 0.144$). (Figure 7)

Effects of Conditions on Physiological and Psychological Responses

No significant differences were observed in physiological parameters between conditions or in isotime × condition interactions. A trend was found for heart rate (HR) across conditions ($p = 0.090$; $\eta^2_p = 0.214$) (Figure 8).

Other physiological parameters showed no significant differences between conditions at isotime analysis: minute ventilation ($\dot{V}E$, $p = 0.163$), oxygen uptake ($\dot{V}O_2$, $p = 0.747$), carbon dioxide production ($\dot{V}CO_2$, $p = 0.314$), respiratory quotient (RQ, $p = 0.304$), and respiratory frequency (Rf, $p = 0.192$). Similarly, comparisons of peak values recorded in the last 30 s of each trial revealed no differences between conditions, despite varying trial durations: peak HR ($p = 0.360$), peak $\dot{V}E$ ($p = 0.944$), peak $\dot{V}O_2$ ($p = 0.651$), peak $\dot{V}CO_2$ ($p = 0.192$), peak RQ ($p = 0.278$), and peak Rf ($p = 0.350$).

Blood lactate levels did not differ significantly between conditions, either before or after the test ($p = 0.949$) (Figure 9).

Manipulation Check

Maximal Voluntary Contraction (MVC)

MVC comparisons revealed a significant time effect for both peak force ($p = 0.027$; $\eta^2_p = 0.437$) and the 6-s isometric contraction average ($p = 0.039$; $\eta^2_p = 0.394$), with a 5% reduction in force over time. No differences were found between conditions (Peak: $p = 0.883$; Average: $p = 0.973$) or in time × condition interactions (Peak: $p = 0.120$; Average: $p = 0.130$) (Figure 10).

Psychomotor Vigilance Task (PVT)

Response speed (RS) in the PVT showed significant changes over time ($p = 0.001$; $\eta^2_p = 0.697$), between conditions ($p = 0.002$; $\eta^2_p = 0.488$), and in the time × condition interaction ($p < .001$; $\eta^2_p = 0.623$) (Figure 11). Pre- to post-condition RS differed significantly in the MF condition ($p < .001$), with a trend in the control condition ($p = 0.057$), while the LMF condition had no effect ($p = 0.165$). Between-condition comparisons revealed significant differences between LMF and MF ($p < .001$) and between MF and C ($p = 0.044$). Post hoc analysis of the time × condition interaction showed significant differences in post-condition RS between MF and LMF ($p < .001$) and between MF and C ($p = 0.007$). Additionally, pre-condition RS differed significantly between MF and LMF ($p = 0.022$). Lapses of attention (>500 ms) were influenced by condition ($p = 0.046$) but not by the time × condition interaction ($p = 0.146$). The MF condition had significantly more lapses than LMF and C. No differences were found in false starts. (Figure 12)

Perceptual Measures

Boredom

Boredom scores differed significantly over time ($p = 0.001$; $\eta^2_p = 0.632$) and between conditions ($p = 0.003$; $\eta^2_p = 0.518$) but not in the time × condition interaction ($p = 0.543$)

(Figure 13). Both C and MF conditions had significantly higher boredom scores than LMF ($p = 0.004$ and $p = 0.012$, respectively), with no difference between MF and C ($p = 0.413$).

Mental and Physical Fatigability

Mental fatigability scores were slightly above neutral (4 = Neither more energetic nor tired) in all groups (C = 4.6 ± 0.7; MF = 4.75 ± 1.09; LMF = 4.2 ± 0.63), with no differences between conditions ($p = 0.289$). (Figure 14)

Physical fatigue differed significantly between conditions (C = 4.0 ± 0.67; MF = 4.05 ± 0.69; LMF = 5.5 ± 0.53) ($p < .001$; $\eta^2_p = 0.785$). Post hoc tests indicated higher physical fatigue in LMF compared to both C ($p < .001$) and MF ($p < .001$), with no difference between C and MF ($p = 0.823$). (Figure 15)

DISCUSSION

The present study investigated the comparative effects of mental fatigue (MF) and locomotor muscle fatigue (LMF) on endurance performance in elite cyclists. No significant differences were observed in time to exhaustion (TTE) across conditions, despite clear manipulations of both mental and physical fatigue, confirming the hypothesis that one type of fatigue does not reduce endurance performance more than the other. The fact that neither condition differed from the control condition also deserves further examination, which will be addressed later. While endurance performance remained stable, important perceptual and cognitive changes were evident: MF impaired psychomotor vigilance and increased attentional lapses, whereas LMF elevated physical fatigability. Importantly, physiological variables—including heart rate (HR), oxygen uptake (VO_2), ventilation, and blood lactate—remained unaffected by condition. This aligns with earlier work suggesting that endurance decrements under fatigue are not mediated by alterations in cardiorespiratory function but rather by perceptual factors (Marcora et al., 2009; Pageaux and Lepers, 2016). Likewise, motivation remained consistently high across all conditions, indicating that elite cyclists are able to preserve their task engagement even under challenging mental or physical stressors.

Marcora et al. (2009) reported a ~15% reduction in time-to-exhaustion (TTE) following prolonged cognitive activity in recreational athletes. Similarly, Pageaux et al. (2014) and

Smith et al. (2015) demonstrated significant impairments in endurance running and intermittent exercise, respectively, following mental fatigue. The discrepancy with our findings is likely attributable to the elite status of our participants. Supporting this interpretation, Martin et al. (2016) reported that professional cyclists do not exhibit performance decrements in time-trial tasks following mental fatigue induction, despite increased perceived exertion, in contrast to sub-elite athletes. Elite performers maintained mean power output and pacing strategy, suggesting that mental fatigue exerts a weaker influence on behavioral regulation in this population. This resistance has been attributed to more efficient executive control, superior attentional regulation, and enhanced inhibitory capacity, likely developed through prolonged exposure to high training loads and competitive demands. Such neurocognitive adaptations may underpin the greater resilience to mental fatigue observed in elite athletes and help explain the discrepancy with our findings.

Although several studies have demonstrated that prior locomotor fatigue increases perceived exertion during subsequent cycling (Marcora et al., 2008; Amann et al., 2013), no significant differences in rating of perceived exertion (RPE) were observed in the condition × iso-time interaction in the present study. A trend was noted, however, toward higher RPE in the locomotor fatigue (LMF) condition compared to the other groups. The absence of statistical significance, combined with the very small magnitude of difference, suggests that the higher ratings may reflect sensations of lower limb soreness rather than an actual increase in perceived exertion.

Physiological variables measured during or pre/post TTE—including HR, VO_2, ventilation, and blood lactate—did not differ significantly across conditions. A trend toward elevated HR in the LMF condition was observed but did not reach statistical significance. Lactate concentrations remained similar across conditions, consistent with prior findings that perceived exertion, or fatigability in this case, is not tightly linked to lactate accumulation (Marcora et al., 2009). Despite small, nonsignificant trends, all trials terminated with nearly identical exertional ratings (19.9 ± 0.1), supporting the psychobiological model of endurance which posits that exercise termination is governed by perceived exertion and motivation rather than cardiorespiratory or metabolic failure afferent feedback from fatigued locomotor muscles (Marcora 2010 and 2008; Pageaux et al., 2013)

Maximal voluntary contraction (MVC) exhibited a significant main effect of time, with ~5% reductions in peak and average force across trials. However, no differences were observed between conditions. Notably, the largest pre/post reduction was observed in the LMF condition, although it did not reach statistical significance compared with the other groups, whose reductions were minimal. Importantly, the origins of these declines may differ: in the LMF condition, the reductions are likely attributable to both neuromuscular and perceptual factors (Iannetta et al., 2012). It is possible that, under both MF and control conditions, athletes experienced relatively low arousal states that may have limited corticospinal excitability during post-intervention MVC testing. There is evidence that corticospinal excitability can be modulated by affective dimensions such as arousal, with higher arousal associated with increased motor-evoked potentials (MEP), suggesting arousal-dependent changes in motor system responsiveness (Mashiki et al. 2025). It should be emphasized that the overall reductions in force were very small, and the time effect became apparent only when considering all three trials together; individually, pre/post comparisons did not reveal significant differences. The lack of a clear condition effect may reflect the elite cyclists' ability to recover neuromuscular output rapidly or maintain performance even after the execution of 100 drop jumps at maximal effort.

The psychomotor vigilance task (PVT) revealed clear cognitive impairments under MF: response speed declined, lapses of attention increased, and post-condition vigilance worsened compared to both control and LMF conditions. These results confirm that MF impairs attentional performance, in agreement with Dinges and Powell (1985) and more recent sport-specific studies (Smith et al., 2016). Notably, endurance performance was not impaired despite attentional decrements, suggesting a dissociation between mental fatigue and physical endurance in elite athletes. Martin et al. (2016) proposed that professional cyclists preserve performance by engaging stronger attentional control and motivational resources, buffering the effects of mental fatigue. Our results support this interpretation.

From a methodological perspective, the continuous within-session protocol we employed was relatively innovative. However, it should be noted that this approach was applied exclusively to the mental fatigue condition. In this case, instead of conducting separate pre- and post-intervention assessments, a single uninterrupted testing session was administered and later segmented into pre- and post-intervention phases (i.e., the first and last five minutes). This approach streamlined the procedure, enhanced ecological validity, and allowed the detection of within-session changes. By contrast, in the other experimental conditions, pre- and post-intervention cognitive performance was assessed using two distinct 5-min testing sessions conducted before and after the intervention.

Physical fatigability scores were markedly higher in the LMF condition, confirming the successful induction of muscle fatigue. By contrast, mental fatigability scores were slightly above neutral across conditions, without significant differences. These findings highlight that

MF induced objective mental fatigue, but elite athletes were able to manage it effectively and did not consciously perceive it as such. Conversely, in the LMF condition, participants reported greater physical fatigue, despite generating force levels not statistically different from the other conditions.

Boredom scores were significantly higher in both MF and control than in LMF, reflecting the monotonous nature of the cognitive and passive interventions compared with the physically engaging protocol. These findings raise important considerations: boredom may be a critical factor in sport performance. In this study, boredom may have contributed to the lack of differences between fatigue protocols and control. Indeed, participants already reported during pilot testing that watching a documentary was perceived as boring. To mitigate this, we selected four emotionally neutral documentaries with distinct themes, increasing the likelihood of aligning with participants' interests. Nonetheless, the results underscore the need to address what we term the *"Documentary dilemma."* For elite cyclists in particular, passive activities such as documentary viewing may be inherently disengaging, compromising the validity of the control condition. Future research should seek alternative methods to provide neutral yet engaging control conditions, such as low-arousal interactive tasks (e.g., simple visuomotor games), self-selected neutral media content, or standardized virtual environments that maintain attentional engagement without inducing cognitive fatigue. Another option may be to individualize control activities based on participants' preferences while controlling for emotional valence and cognitive load. Importantly, the present study's explicit identification and quantification of boredom represents a methodological strength. By systematically assessing boredom and acknowledging its potential confounding role, this work contributes to a more nuanced understanding of how psychological states interact with fatigue protocols and performance outcomes. Rather than assuming that passive control conditions are psychologically neutral, our findings highlight the necessity of empirically verifying this assumption, particularly in elite athlete populations who may be especially sensitive to under-stimulation.

The strengths of this study include its within-subject design, which minimizes inter-individual variability, and the rare use of elite cyclists under controlled fatigue paradigms. Additionally, the integration of physiological, neuromuscular, perceptual, and cognitive measures provides a multidimensional assessment of fatigue.

However, several limitations must be acknowledged. The small sample size (n = 10) reduces statistical power and may obscure subtle effects. The laboratory setting, while controlled, lacks ecological validity relative to real-world racing. Furthermore, the interventions reflect acute manipulations, whereas competition involves cumulative, multifactorial fatigue. Importantly, neither the MF nor LMF protocols produced sufficiently strong effects: LMF did not elicit a significant reduction in MVC, and MF did not increase perceived mental fatigue. Thus, determining the optimal laboratory "dose" of fatigue for elite athletes remains a critical challenge.

From a practical point of view, these results suggest that elite cyclists are able to "push through" both mental and muscle fatigue without a noticeable drop in endurance performance when riding at high, but still submaximal, intensity (95% of RCP). In other words, even when they feel mentally tired or their muscles are locally fatigued, their ability to sustain effort remains largely unchanged — a key factor for real racing situations. Nonetheless, perceptual and cognitive alterations should not be underestimated. Attentional lapses under MF could impair decision-making and tactical execution during competition. Future research should consider implementing protocols that assess performance both pre- and post-fatiguing intervention, thereby reducing the confounding impact of day-to-day variability (e.g., "bad days") on the final results. In addition, incorporating neurocognitive measures (e.g., EEG, fNIRS, fMRI) could help elucidate the central mechanisms underlying resilience in elite athletes. Expanding cohorts to include larger samples and more female professionals is also critical to broaden generalizability.

CONCLUSION

In conclusion, this study demonstrates that elite cyclists maintain endurance performance despite experimental manipulations of both MF and LMF. Although endurance capacity was preserved, MF selectively impaired vigilance and attention, whereas LMF heightened perceived physical fatigue. These findings reinforce the psychobiological model of endurance by emphasizing the central role of perceived effort and motivation and highlight the distinctive resilience of elite athletes to fatigue-related performance decrements. Moreover, the monotonous nature of a documentary-viewing control condition may constitute an important confounding factor, particularly among elite athletes.

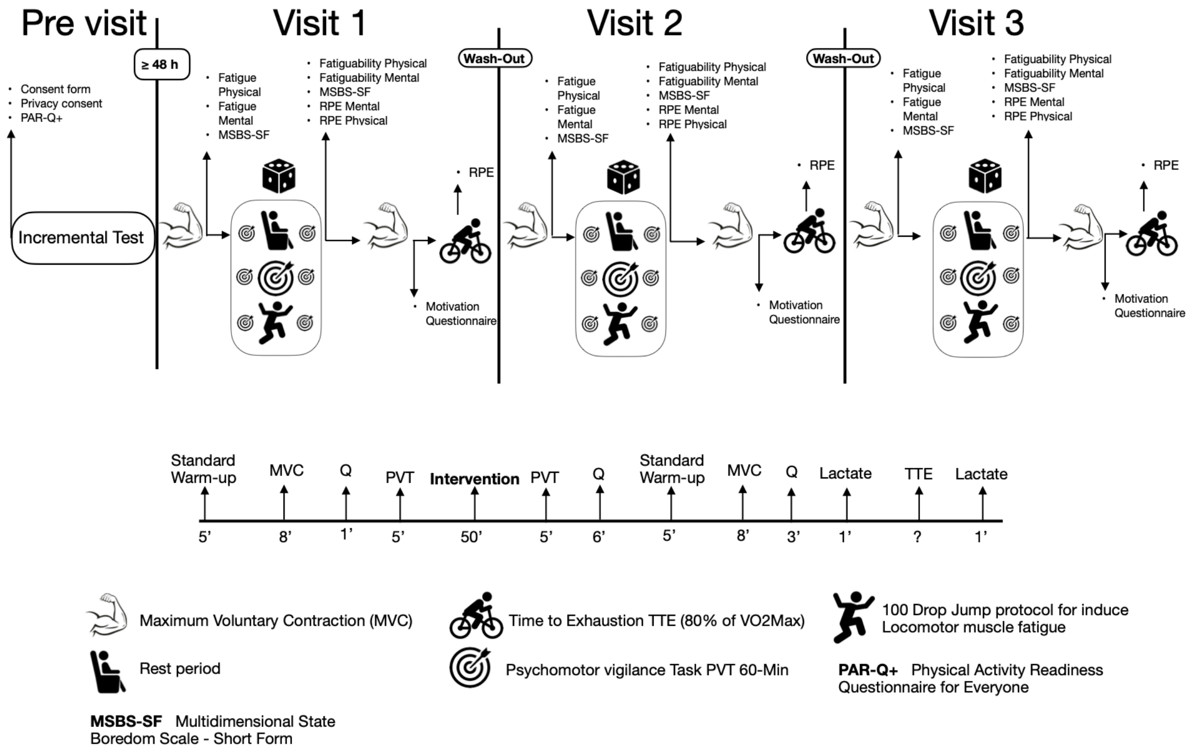


Fig 4. Schematic of the experimental protocol

The diagram illustrates the sequence of laboratory visits and the procedures followed by the elite cyclists. It shows the division into phases (familiarization, mental fatigue intervention, locomotor fatigue intervention, control) and the tests performed (MVC, PVT, TTE, physiological and psychological measures). This scheme provides a clear overview of the experimental design.

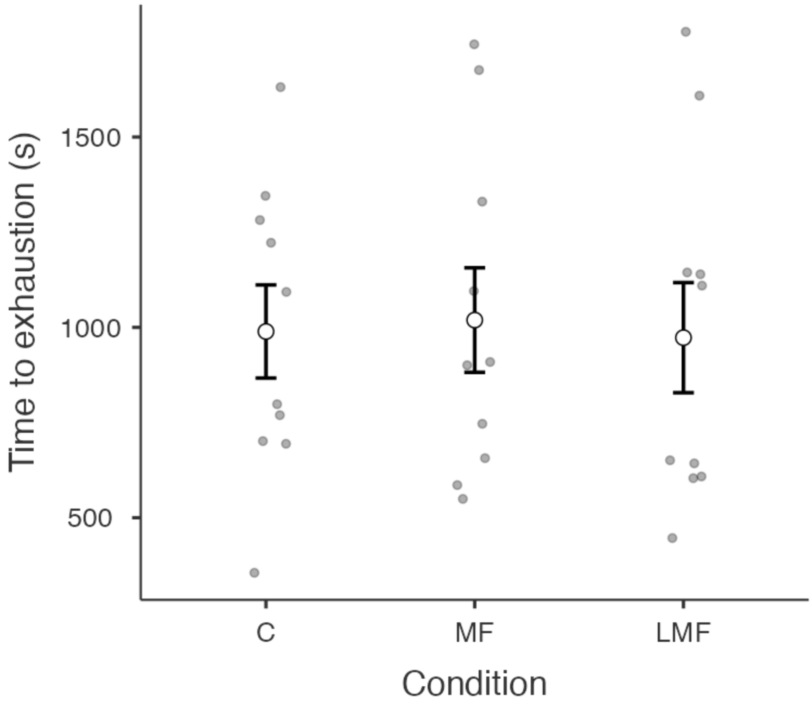


Fig.5 Time to Exhaustion (TTE) across interventions

The graph reports the mean time to exhaustion values in the three conditions: control (C), mental fatigue (MF), and locomotor muscle fatigue (LMF).

Condition	Mean (s)	DS	95% Confidence Interval	
			Lower	Upper
Control (C)	989	386	712	1266
Mental Fatigue (MF)	1019	433	709	1330
Locomotor muscle fatigue (LMF)	973	458	646	1300

Tab. 1

The table shows the **Estimated Marginal Means by Condition** with their corresponding **Standard Errors (SE)** and **95% Confidence Intervals (CI)**.

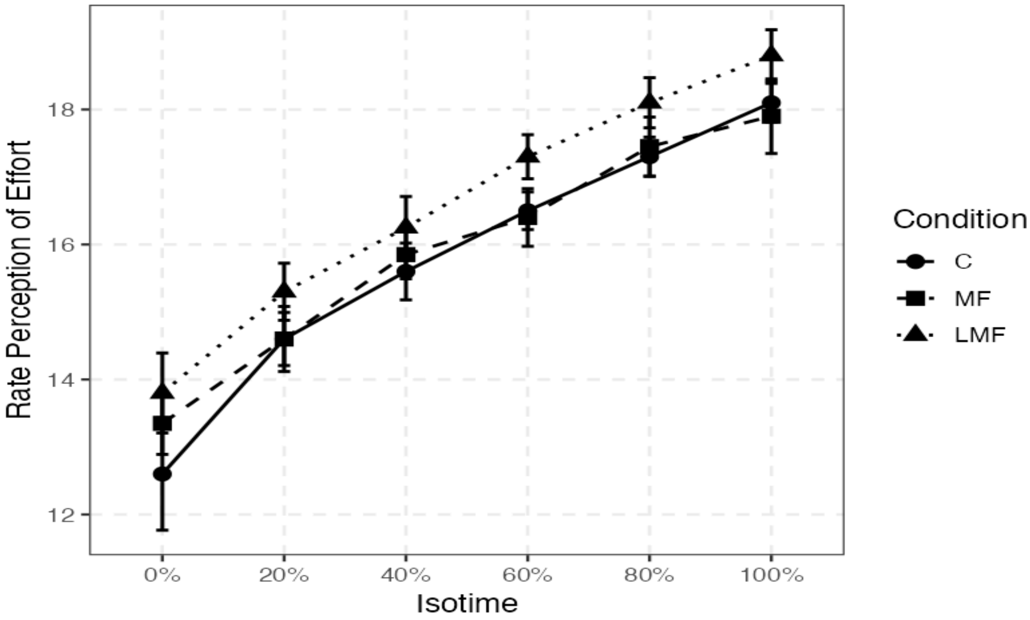


Fig.6 Perceived exertion (RPE) at iso-time

The graph shows the evolution of RPE during the time-to-exhaustion tests.

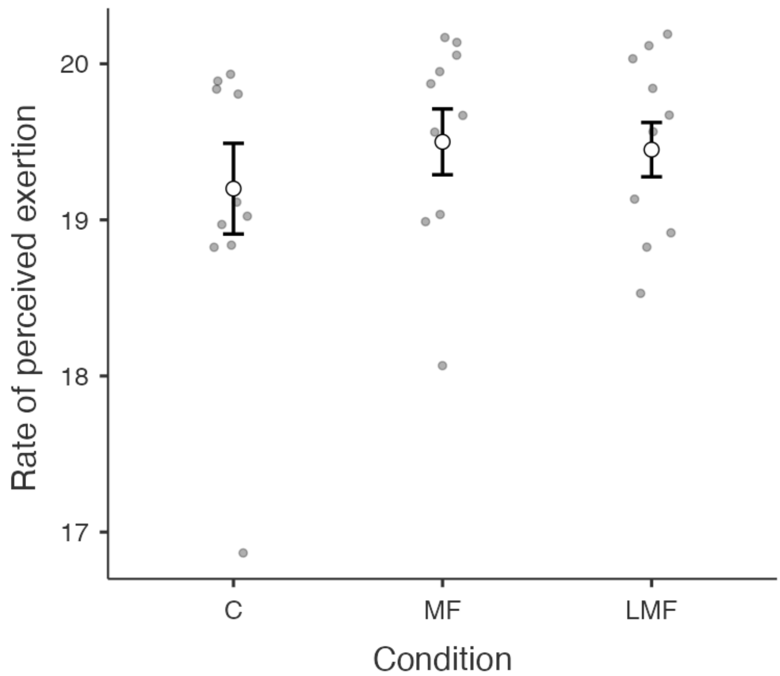


Fig 7. Peak RPE at task failure

This figure displays the maximum RPE values reached at the end of exercise.

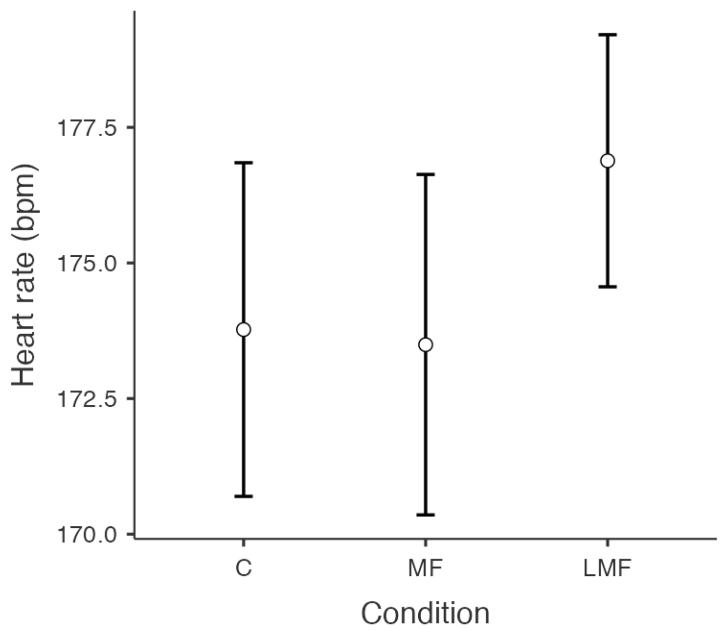


Fig 8 Heart rate (HR) at iso-time

The graph presents mean HR values recorded across the different interventions.

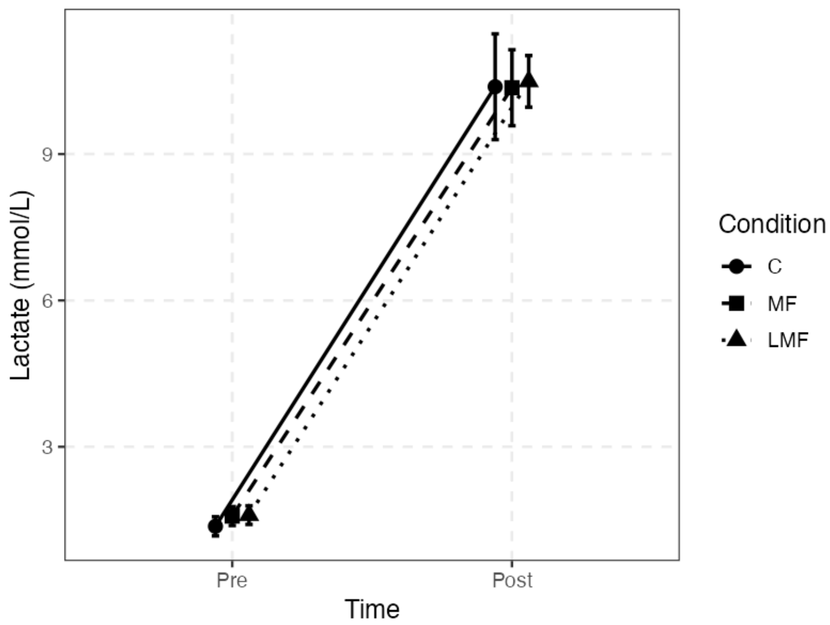
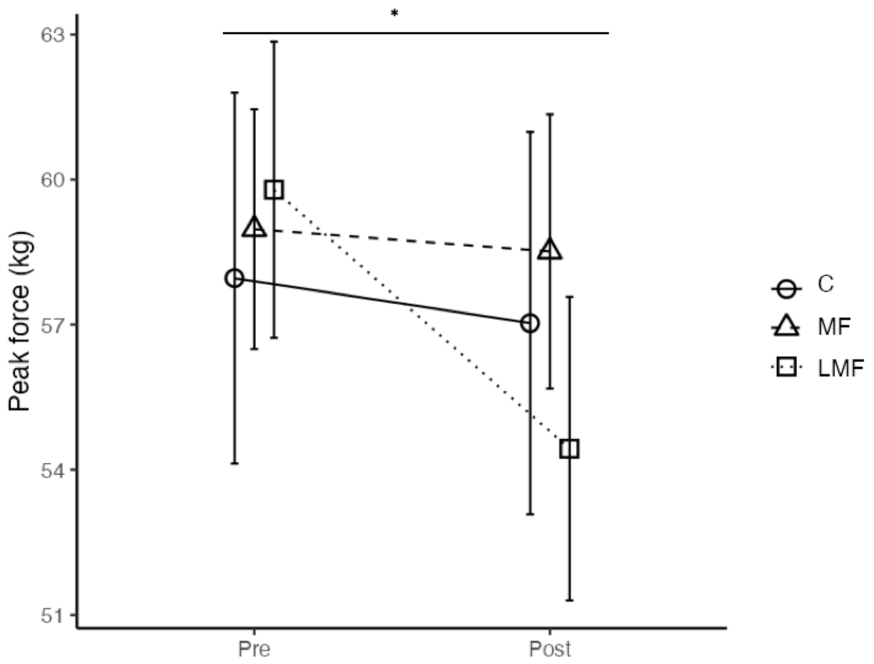


Fig 9. Blood lactate concentration

The graph shows lactate levels before and after exercise in the three conditions.

(A)



(B)

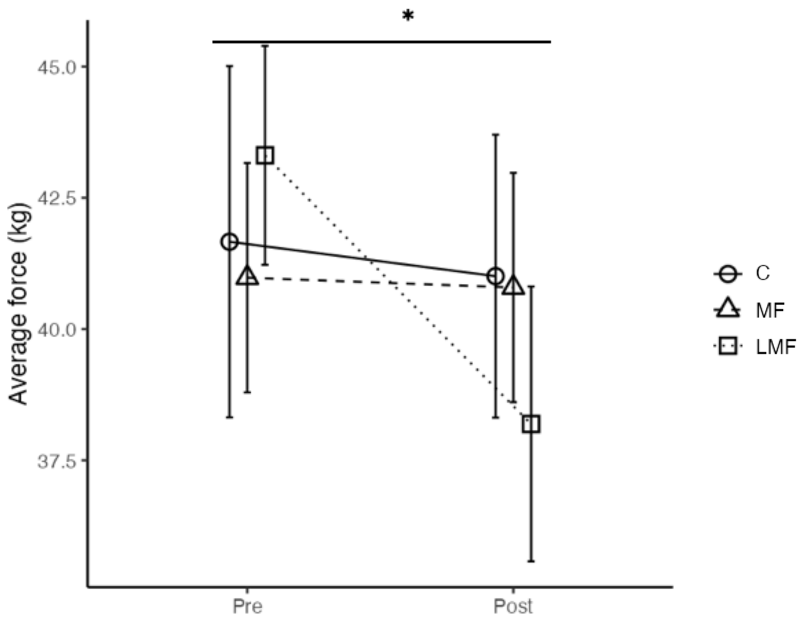


Fig. 10 Maximal Voluntary Contraction (MVC)

(A) Peak force: Panel A reports the peak values of the isometric 6-s contractions of the dominant knee extensors. **(B) Average force:** Panel B shows the mean values of the 6-s isometric contractions.

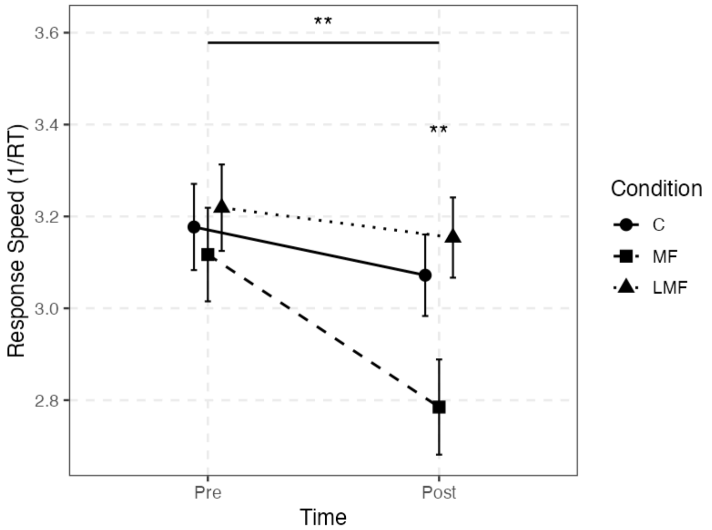


Fig. 11 Response speed in the PVT

The graph illustrates changes in response speed (RS).

Comparison	Time/Condition	Mean ± SD (1/RT)	p-value
C	PRE	3.18 ± 0.30	
	POST	3.07 ± 0.28	0.057
MF	PRE	3.12 ± 0.32	
	POST	2.78 ± 0.33	**< .001*** **
C vs MF	C POST	3.07 ± 0.28	
	MF POST	2.78 ± 0.33	**0.007** **
C vs LMF	C POST	3.07 ± 0.28	
	LMF POST	3.15 ± 0.28	**< .001*** **
MF vs LMF	MF POST	2.78 ± 0.33	
	LMF POST	3.15 ± 0.28	**< .001*** **

Tab. 2

Shows the Mean ± SD values of the average speed (1/Reaction time) to stimuli reaction. estimated marginal means (± SD) for each condition and the results of the post hoc comparisons. * $p < .05$; ** $p < .01$; *** $p < .001$.

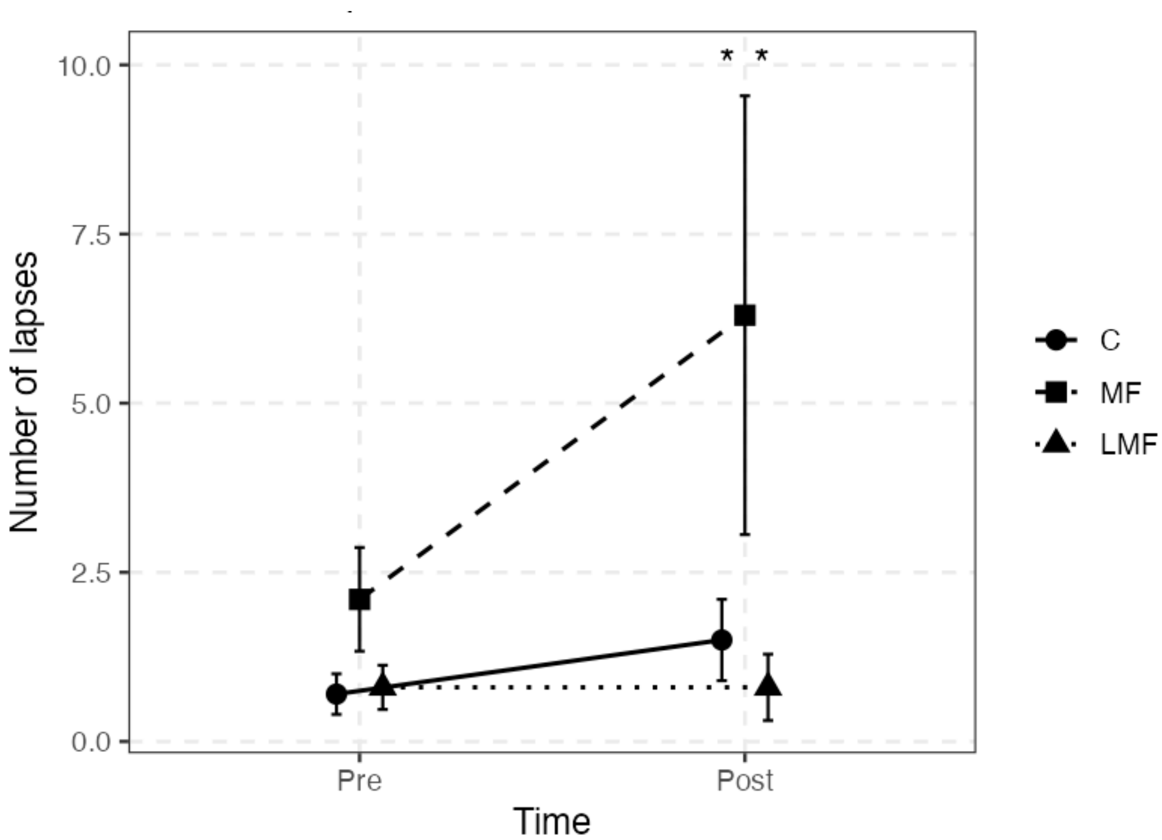


Fig.12 Attention lapses in the PVT

This figure shows the mean number of attention lapses (reaction times > 500 ms) in the Psychomotor Vigilance Task (PVT).

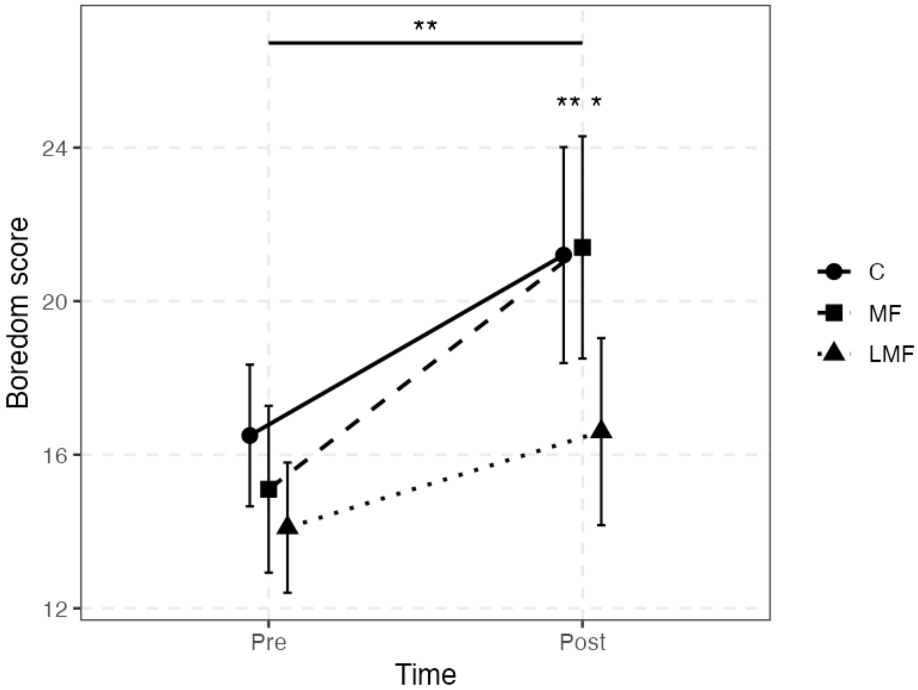


Fig.13 Boredom scores (MSBS-SF)

The graph reports mean boredom scores across time (p = 0.003) and condition (p = 0.001).

Comparison	Time/Condition	Mean ± SD	p-value
C vs MF	C POST	21.2 ± 8.89	
	MF POST	21.4 ± 9.17	0.413
C vs LMF	C POST	21.2 ± 8.89	
	LMF POST	16.6 ± 7.71	**0.004****
MF vs LMF	MF POST	21.4 ± 9.17	
	LMF POST	16.6 ± 7.71	**0.012***

Tab. 3

Show mean ± SD and post hoc comparisons of boredom scores at the Post measurement across conditions. No significant difference emerged between **C** (21.2 ± 8.89) and **MF** (21.4 ± 9.17; p = 0.413). However, both **C** and **MF** reported significantly higher boredom levels compared to **LMF** (16.6 ± 7.71), with differences of p = 0.004 and p = 0.012, respectively. * $p < .05$; ** $p < .01$; *** $p < .001$

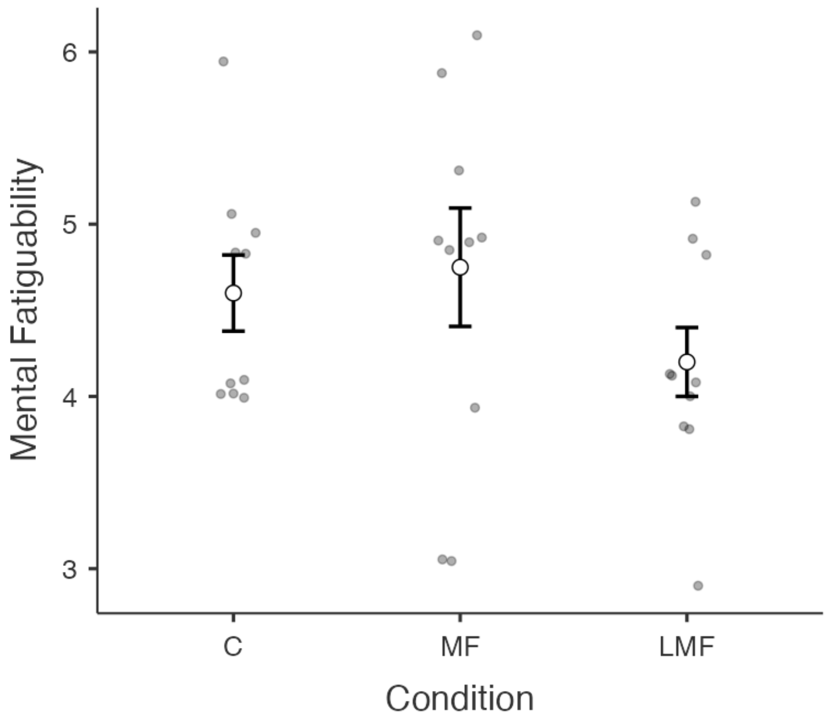


Fig. 14 Mental fatigability

The graph shows average perceived mental fatigue scores, slightly above neutral (4).

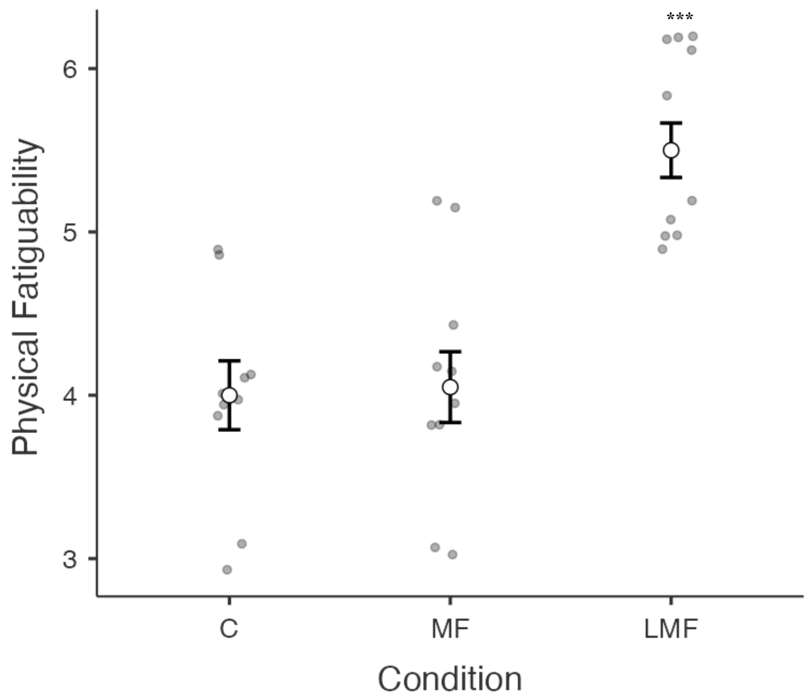


Fig. 15 Physical fatigability

The figure highlights significant differences ($p < .001$) between conditions.

CHAPTER 3
LOCOMOTOR VS. MENTAL FATIGUE IN HEALTHY ADULTS

TULLIO SENATORE, SAMUELE MARCORA

ABSTRACT

This study compared the effects of mental fatigue (MF) and locomotor muscle fatigue (LMF) on endurance performance, perceived exertion, and physiological responses in 18 healthy subjects (26.1 ± 5.1 yrs, 14 Male 45.4 ± 8.7 mL/min/kg VO_2Max, 4 Female 44.2 ± 0.3 mL/min/kg VO_2Max) completed an incremental ramp test to determine respiratory compensation point (RCP), VO_2max, and peak power output (PPO).

In three subsequent randomized visits, participants performed a time-to-exhaustion test (TTE) at 95% RCP under: (1) MF induced by a 1-h psychomotor vigilance task (PVT), (2) LMF induced by 100 drop jumps, and (3) a control condition involving documentary viewing. Heart rate (HR), VO_2, ventilation, blood lactate concentration, and rating of perceived exertion (RPE) were measured during exercise. Manipulation checks included knee-extensor maximal voluntary contraction, PVT performance, and perceived physical and mental fatigability. Data were analyzed using repeated-measures ANOVA ($p < 0.05$) and the individual isotime method.

TTE showed a trend toward condition differences ($p = 0.091$), being reduced by ~10% after MF and ~19% after LMF compared with control. Perceived exertion during exercise differed between conditions ($p = 0.005$), being higher in LMF than in MF and control, whereas peak RPE did not differ ($p = 0.509$). Mean physiological responses (heart rate, ventilation, oxygen uptake, and carbon dioxide production) were higher in LMF than in MF (all $p < 0.05$), while peak values and blood lactate were similar across conditions ($p = 0.814$). Maximal voluntary contraction declined only after LMF (time × condition $p < 0.001$), confirming effective peripheral fatigue. Cognitive performance deteriorated exclusively after MF, with slower response speed and more attentional lapses (both $p < 0.001$). Boredom and mental fatigability were highest following MF ($p < 0.001$), whereas physical fatigability was greatest after LMF ($p < 0.001$).

MF and LMF induced distinct fatigue profiles, affecting primarily cognitive–perceptual and neuromuscular–physiological domains, respectively, while both increased perceived exertion. Despite the absence of significant differences in TTE, convergent physiological, neuromuscular, and perceptual evidence confirmed effective fatigue induction, with LMF showing a greater detrimental impact on endurance performance.

INTRODUCTION

Over the past 15 years, research has consistently demonstrated that mental fatigue (MF)—a psychobiological state induced by prolonged cognitive activity—can significantly impair endurance performance in healthy individuals. A seminal study by Marcora, Staiano, and Manning (2009) demonstrated that exposure to a demanding cognitive task reduced subsequent cycling time-to-exhaustion by approximately 15% compared with a control condition. Notably, this performance decrement occurred without alterations in heart rate, oxygen uptake, or ventilation during exercise, indicating that MF did not measurably affect cardiorespiratory responses to exercise. This suggests that its primary effect was an increase in perceived exertion, rather than the result of direct muscular impairment or physiological cardiorespiratory limitations. Participants consistently reported higher ratings of perceived exertion (RPE) throughout exercise and reached their maximal tolerable effort earlier than in the non-fatigued state, terminating exercise prematurely. This pivotal finding introduced the widely accepted notion that MF compromises endurance by increasing the perception of effort.

Since this initial evidence, multiple studies have confirmed the detrimental impact of MF on whole-body endurance performance across various exercise modalities. Impaired performance following cognitive exertion has been documented in both cycling and running tasks (MacMahon et al., 2014; Pageaux et al., 2014; Smith et al., 2015, 2016). Importantly, physiological variables traditionally associated with endurance performance (e.g., heart rate, blood lactate concentration, oxygen uptake, cardiac output, and maximal aerobic capacity) appear unaffected by MF (Van Cutsem et al., 2017). Similarly, maximal strength, power output, and anaerobic performance do not appear compromised (Van Cutsem et al., 2017). Thus, MF consistently reduces endurance capacity in physically active adults due to elevated perception of effort. Although elite athletes may display greater resilience to MF (Martin et al., 2016), recreationally trained and healthy adults remain susceptible, underscoring the relevance of investigating MF in this population.

In parallel, the effects of locomotor muscle fatigue (LMF)—fatigue arising from prior physical exertion—have long been established. Intense or prolonged muscular activity produces measurable reductions in subsequent performance, largely mediated by impairments in neuromuscular function (Amann and Dempsey, 2013; de Morree and Marcora 2013; Marcora 2010; Marcora et al., 2008; Venhorst et al., 2018). Experimental models, such as repeated arm cranking, have demonstrated that prior localized muscular fatigue can significantly reduce subsequent endurance of other muscle groups (Bangsbo et al., 1996; Nordsborg et al., 2003). Similarly, eccentric resistance exercise protocols reliably produce decrements in muscle strength and endurance, often accompanied by muscle soreness and structural damage (Byrne et al., 2004; Howatson and van Someren, 2008).

Unlike MF, LMF is characterized by reductions in maximal voluntary contraction (MVC) and increased cardiorespiratory strain during subsequent exercise (Marcora, 2008). Although metabolic stress is commonly associated with muscle fatigue during high-intensity exercise (Sargeant, 2007), it has also been demonstrated that exercises inducing muscular fatigue without substantial metabolic stress still result in reduced force production, elevated perceived exertion, and increased cardiorespiratory responses (Marcora, 2008). Consequently, impairments observed under LMF conditions may arise centrally rather than peripherally, as heightened central motor command directed to fatigued locomotor muscles may influence cardiorespiratory regulation during prolonged constant-load exercise (Marcora, 2008; Andrews, 2006; Syabbalo, 1994).

Despite their distinct origins, both MF and LMF share a striking convergence: they impair endurance performance primarily by elevating perceived effort. This supports the psychobiological model of endurance performance, which posits that the decision to terminate exercise is determined not by absolute physiological exhaustion but rather by the interaction between perceived effort and motivation (Brehm and Self, 1989; Marcora et al., 2009; Pageaux and Lepers, 2016). Accordingly, both MF and LMF accelerate the approach to the maximal tolerable effort threshold, leading to premature exercise cessation.

Most previous studies, however, have examined either MF or LMF in isolation. To date, only one investigation has compared them directly within the same group of participants (Mortimer et al., 2024). Findings from that study indicated that a demanding cognitive task induced a state of mental fatigue and impaired both skill and endurance performance. Interestingly, mental fatigue alone was more detrimental than combined fatigue, suggesting that the physical activity manipulation attenuated the negative effects of mental fatigue on performance. Although Mortimer and colleagues employed a relatively large sample, their design relied on four separate groups, each assigned to a distinct condition. As such, the comparison between MF and LMF remained indirect.

Therefore, while the present study shares similar aims, it extends and strengthens this line of research on the multifactorial nature of fatigue. Unlike Mortimer et al., the present work does not assess the combination of MF and LMF but instead directly compares their isolated effects on endurance performance, perceptual responses, neuromuscular function, and cognitive outcomes in healthy adults. Using a within-subject design, participants were exposed to either a prolonged cognitive task inducing MF, a drop-jump protocol inducing LMF, or a control condition (watching a neutral documentary) prior to a cycling time-to-exhaustion trial. We hypothesized that both MF and LMF would impair endurance relative to the control condition, albeit through distinct mechanisms, with increased perception of effort serving as the principal determinant of exercise cessation.

MATERIALS AND METHODS

Participants

18 healthy subjects performance level 1 and 2 (Decroix 2016; De Pauw 2013) (26.1 ± 5.1 yrs, 14 Male 45.4 ± 8.7 mL/min/kg VO_2Max, 4 Female 44.2 ± 0.3 mL/min/kg VO_2Max) were recruited and volunteered to take part in this study. The sample size was calculated based on the previous study that explored the effect of locomotor muscle fatigue or mental fatigue (Amann and Dempsey 2008; de Morree and Marcora 2013; Deley et al., 2006; Martin et al., 2016; Pageaux et al., 2013 and 2015; Budini et al., 2014; Duncan et al., 2015) and so that we could have the order of interventions counterbalanced. The inclusion criteria were: engagement in at least 150–300 minutes of moderate-intensity physical activity or 75–150 minutes of vigorous-intensity physical activity per week, and possession of a valid medical certificate authorizing participation in non-competitive physical activity. Men with a VO_2max > 55 $mL \cdot kg^{-1} \cdot min^{-1}$ and women with a VO_2max > 51.5 $mL \cdot kg^{-1} \cdot min^{-1}$ were excluded, as they did not fall within the predefined performance categories (levels 1 and 2).
The experimental protocol was conformed to the standards set by the Declaration of Helsinki.

Procedures

All subjects visited the laboratory on four occasions. A schematic overview of the protocol can be found in Figure 16. The first visit was a familiarization and physiological profile detection visit. During this visit, participants signed an informed consent form, a Physical Activity Readiness Questionnaire (PAR-Q), and anthropometric measures were taken. A Ramp exercise test (Warm-up for 3 minutes at 50W, the first step was set at 75W and later 1 W increments every 4 seconds) was then performed until exhaustion (or when cadence was less than 60 rpm for more than 5 s, despite strong verbal encouragement) on Cyclus2 Ergometer (RBM elektronik-automation GmbH; Leipzig, Germany) to measure VO_2peak (highest 30 s average VO_2 during the test) (Mauger and Sculthorpe, 2012), Respiratory compensation point (RCP) (The point where both the ratio VE/VO_2 and VE/VCO_2 increase) (Wasserman et al., 1987) and peak power output (Ppeak). Ppeak was estimated as the Watt at the end of the trial. During the test, VO_2 was measured breath-by-breath using a computerized metabolic gas analysis system (Quark CPET; COSMED, Rome, Italy).All anthropometric measurements were recorded and added to the OMNIA software. Before the incremental exercise test, we fitted the personal bike of the cyclist on the ergometer. Familiarization also included the anchor to Borg scale: standard instructions were given on how to assess their perceived exertion (i.e., conscious feeling of how heavy and strenuous a physical task is) on Borg's Perceived Exertion Rating Scale (RPE) (6-20) (Borg 1998). During the warm-up the low anchor point (point 7) was established using standard procedures. Similarly, close to the task failure the high point was anchored (point 19) (Noble and Robertson, 1996). After a 30 min of rest, subjects perform in a time to exhaustion familiarization at 95% of RCP without reaching the exhaustion.

After a minimum of 48 h, subjects returned to the laboratory for the second time. For all participants, intervention order was randomly allocated by a web-based computer program (www.randomization.com). The visit started with a 10 min of standard warmup (100W at cadence range 60-100 rpm). After this, baseline unilateral knee extensor strength was assessed by measuring force during 90 degrees isometric maximal voluntary contraction (MVC) of the dominant knee extensor muscles. Participants were instructed to grip the handles of the leg extension machine and push three times at sub-maximal force (25%, 50%, 75%). After this warm-up, three trials of six s 100% MVC were recorded. The force produced in each isometric contraction was monitored using a wireless dynamometer (K-Pull, Kinvent Physio, Montpellier, 3400, France). Between all trials, 2-min rest periods were observed. During the maximal trials, strong verbal encouragement was given. Before starting the intervention participants give a subjective valuation of mental and physical fatigue based on Fatigue and Fatigability scale (Schnelle et al., 2012). Before and after intervention we assess the state of boredom with a short form of Multidimensional scale of boredom state (MSBS-SF) (Donati et al., 2021; Spoto et al., 2021) and record an objective measurement of mental fatigue with a 5-min PVT (Dinges and Powell, 1985).

Based on the Fatigue and Fatigability scale a post intervention valuation of mental and physical fatigability was recorded. Moreover, a Physical and Mental session RPE scale (Nigro and Marcora, 2023) was administered to participants.

After completing the standard warmup, post intervention knee extensor strength was assessed with three maximal trials. 2-min after MVC, Motivation related to the time-to-exhaustion tests was measured using the success motivation and intrinsic motivation scales (Matthews et al., 2001) . All subjects perform the TTE at intensity relative to 50% of RCP as warm up for 5 min, consecutively the intensity has been brought to 95% of RCP until exhaustion or when the cadence was lower than 60 rpm for at least 5 s despite verbal encouragement. For all visits verbal encouragement was offered by an investigator blinded with respect to intervention. Lactate measurements were taken with a portable blood lactate analyser, Lactate Scout 4 (LS, SensLab GmbH, Germany) before, every 5 min during the test and at task failure. During the TTE a rate of perceived exertion based on 6-20 Borg Scale was recorded every 2 min.

Psychomotor vigilance task (PVT)

The PVT is a simple visual reaction time test first used as a research tool by Dinges and Powell (1985). We used the Nasa-validated application to perform a 1-hour task (Arsintescu et al., 2017). In this test, a millisecond counter appears in the center of the screen until the participant presses on the side of his dominant hand of the screen, stopping the counter to display the response time (RT). If participants pressed before or within the first 150 ms of the timer display, a "false start" message was displayed on the screen. When the response was delayed by 500 ms, we considered it as a lapse of attention. The time interval between the disappearance of the RT or false start message and the appearance of the next stimulus varied randomly from 2 to 10 s. A total of about 600 stimuli were displayed during the 60-minute treatment period.

100-Drop Jump protocol (DJ)

This eccentric fatigue protocol does not induce respiratory muscle fatigue because of the very low aerobic demands. Participants dropped 100 times from a 50 cm platform to 90° knee angle before jumping upward as far as possible. Subsequent jumps were separated by 20 s of rest. This eccentric fatigue protocol took 34 min in total (Skurvydas et al., 2000). In this study, unlike the original study, the platform height was changed from 40 cm to 50 cm. The subject stepped on a 50-cm high platform with legs in which muscle contraction force was not tested.

Control (C)

A passive control treatment was used as a control (Marcora et al., 2009) and consisted of watching one of four different documentaries: "How the universe work— *Hunt for the Universe's Origin"* (Pioneer Production, 2010-); "Secrets of the world's super skyscrapers— *Episode #1.1*" (Lambent Production, 2018); "Prehistoric Planet— *Forests"* (BBC Studios Natural History Unit, 2022-); "Mega factories— *Speed rail*" (Hoff Production, 2006-2012). To increase the participants' engagement, they were given the opportunity to choose which documentary to watch. Documentaries on these topics were chosen as they were able to maintain a neutral mood and stable HR (Silvestrini and Gendolla, 2007) and for a duration of about 50 minutes. During the treatments, one of the investigators monitors proper compliance with the intervention.

Physiological and Psychological Measurements

At the familiarization visit, all the psychological and physiological measures that would be collected were explained. We collect for all visit times the HR (bpm). During the endurance test, tidal volume (liters), breathing frequency (minutes), ventilation (l/min),VO_2 (l/min), and carbon dioxide production (VCO_2) (l/min) were measured breath-by-breath.

Multidimensional scale of boredom state (MSBS-SF)

A short validated version of the Multidimensional scale of boredom state (Hunter et al., 2015) was used to assess the level of boredom pre and post each intervention. The scale was proposed in the Italian version selecting the same eight items from the Italian version of the original MSBS (Craparo et al., 2017), as already made by Donati et al., 2021. The scale contains eight Likert-type items on a 7-points scale from 1 (strongly disagree) to 7 (strongly agree). The result was analyzed as the sum of the score. Appendix A

Fatigue/Fatigability scale

Before the intervention, participants were asked to rate their current level of Mental and Muscle tiredness (e.g., "How tired are you right now?") on a 7-point scale from 7 (extremely tired) to 1 (extremely energetic). Participants were asked when they finished the intervention, “Are you more or less at the same energy level as when you started the intervention?” A score of 4 was assigned if there was no change in perceived energy level. If participants reported that they had less energy, they were asked whether they were extremely, somewhat or a little more tired. If they reported having more energy, they were asked if they were extremely, somewhat, or a little more energetic. This branching question format was tested to be less confusing than asking for a rating across the full 7-point scale of energy change (Schnelle et al., 2012). Appendix B

Success and Interest Motivation

This questionnaire was developed by Matthews et al. in 2001, based on the Dundee Stress State Questionnaire (DSSQ) (Matthews et al. 2001). Although the DSSQ can be used to assess motivation, the scales in question focus specifically on aspects of motivation related to success and interest in task performance. Both dimensions contribute to Task Engagement, but Success Motivation is also linked to negative emotions and self-beliefs.

The scale consists of 15 Likert-type items rated on a 5-point scale ranging from 0 (not at all) to 4 (extremely). Seven items assess Success Motivation, and another seven measure Interest Motivation, while the final item evaluates a general state of motivation. Some items were reverse-scored, and the results were analyzed as the sum of the scores.

Statistical analysis

All data are reported as means ± standard deviation (SD). A one-way repeated-measures ANOVA was used to evaluate the effects of different experimental conditions (mental fatigue, muscle fatigue, and control). For variables measured both before and after each intervention, a two-way repeated-measures ANOVA (condition × time) was applied.

An additional two-way ANOVA (condition × iso-time) was used to analyze the physiological variables and perceived exertion recorded during the exhaustion test (TTE). Because the exhaustion trials had different durations, the comparison between trials was performed using

the individual iso-time technique (Nicolo et al.,2019). Comparison points were selected at the beginning and peak of each trial. Midpoints were determined at 20%, 40%, 60%, 80%, and 100% of the duration of the trial with the worst performance among the three sustained by the athlete, and then applied to all trials.
The level of statistical significance was set at $p < .05$.

RESULTS

Time to Exhaustion (TTE) and Rate of Perceived Exertion

A tendency was observed between conditions in time to exhaustion (TTE) ($p = 0.091$). Mean TTE was 985 ± 770 s in the control condition (C), 885 ± 601 s in the mental fatigue condition (MF), and 797 ± 400 s in the locomotor muscle fatigue condition (LMF) (Figure 17). The greatest difference emerged between C and LMF, with a 19.1% reduction in time in the latter condition; the reductions observed in MF compared to C were 10.2%. The two fatiguing conditions also differed from each other, with TTE in LMF 9.9% lower than MF.
Perceived exertion during TTE was statistically different between conditions ($p = 0.005$; $\eta^2_p = 0.265$), and across isotime at isotime comparison ($p < .001$; $\eta^2_p = 0.919$), no difference was found in the interaction condition x isotime ($p = 0.255$). Post hoc analysis shows a significant difference between C vs LMF ($p = 0.009$) and MF vs LMF ($p = 0.031$); Only a tendency was found between C and MF ($p = 0.094$) (Figure 18). The higher average value was in the LMF condition (C = 15.3 ± 1.39; MF = 15.7 ± 1.06; LMF = 16.1 ± 1.01).
Peak ratings of perceived exertion (RPE) at the end of exercise did not differ significantly between conditions ($p = 0.509$).

Effects of Conditions on Physiological and Psychological Responses

All measured physiological parameters showed a significant increase across isotime ($p < .001$). No significant differences, or trend, were observed in physiological parameters in isotime × condition interactions ($p > 0.05$). Statistical differences were found in HR ($p = 0.016$) (figure 19), VE ($p = 0.006$) (figure 20), $\dot{V}CO_2$ ($p = 0.024$) (figure 21), $\dot{V}O_2$ ($p = 0.027$) (figure 22) and Bf ($p = 0.015$) (figure 23) across conditions. RQ ($p = 0.874$) showed no significant differences between conditions.

The average value of HR was higher in LMF, statistically different than MF ($p = 0.009$) and tendentially different than C ($p = 0.088$), no statistical difference in MF vs C.
The VE average was statistically higher in LMF with respect to C ($p = 0.019$) and MF ($p = 0.007$). The average value of $\dot{V}CO_2$ was higher in LMF but was statistically different only with MF ($p = 0.002$). Data show the same results for $\dot{V}O_2$ with the only statistical difference in LMF vs MF ($p < .001$). Bf average values difference in LMF vs MF ($p = 0.007$) and a trend in LMF vs C ($p = 0.058$).
The comparisons of peak values recorded in the last 30 s of each trial revealed no differences between conditions, despite varying trial durations: peak HR ($p = 0.488$), peak $\dot{V}CO_2$ ($p = 0.128$), peak RQ ($p = 0.864$), and peak Bf ($p = 0.808$). However, we found a tendency between condition in peak $\dot{V}O_2$ ($p = 0.096$) and peak $\dot{V}E$ ($p = 0.057$).
Blood lactate levels did not differ significantly between conditions, either before or after the test ($p = 0.814$).

Manipulation Check

Maximal Voluntary Contraction (MVC)

MVC comparisons revealed a significant time effect for both peak force ($p < .001$) and the 6-s isometric contraction average ($p < .001$). We also found significant time × condition interactions (Peak: $p < .001$; Average: $p = 0.006$) (Figure 24 and 25).No differences were found between conditions (Peak: $p = 0.990$; Average: $p = 0.799$). The post hoc analysis shows a non-significant average force reduction in C condition ($p = 0.213$) but a trend in reduction in the peak group under C condition ($p = 0.057$) with 4.3% of force reduction. No differences were found in pre-post analysis of MF condition (Peak: $p = 0.247$; Average: $p = 0.521$). Instead, in LMF condition both peak and average were significantly lower post intervention compared to pre intervention (Peak: $p < .001$; Average: $p < .001$) with a reduction of 16.4% in peak and 15.9% in average. The comparisons between the average force post conditions were significantly only in C post vs LMF post ($p = 0.019$) with a 9.9% of force less in LMF. No difference in the post comparison in the peak force.

Psychomotor Vigilance Task (PVT)

Response speed (RS) in the PVT showed significant changes over time ($p < .001$; $\eta^2_p = 0.666$), between conditions ($p < .001$; $\eta^2_p = 0.503$), and in the time × condition interaction ($p < .001$; $\eta^2_p = 0.545$) (Figure 26). Pre- to post-condition RS differed significantly in the MF condition ($p < .001$) and in the control condition ($p = 0.038$), while the LMF condition had no effect ($p = 0.132$). Post hoc analysis of the time × condition interaction showed significant differences in post-condition RS between MF and LMF ($p < .001$) and between MF and C ($p < .001$). Additionally, pre-condition RS differed significantly between MF and C ($p = 0.022$). Lapses of attention (>500 ms) were influenced by condition ($p < .001$; $\eta^2_p = 0.406$), time ($p = 0.018$; $\eta^2_p = 0.289$) and by the time × condition interaction ($p < .001$; $\eta^2_p = 0.413$) (figure 27). The number of lapse increases significantly only in MF condition ($p = 0.004$), the difference between post conditions shows a number significantly higher in MF compared to C ($p = 0.001$) and LMF ($p < .001$).

False starts show differences between condition ($p = 0.049$; $\eta^2_p = 0.163$) and in time x condition interaction ($p = 0.015$; $\eta^2_p = 0.218$) (figure 28). The number of false starts was less in post C compared to pre C ($p = 0.002$), highlighting significant differences with post MF ($p = 0.019$) and post LMF ($p = 0.002$) . No difference was found for the other interaction.

Perceptual Measures

Boredom

Boredom scores differed significantly over time ($p < .001$; $\eta^2_p = 0.486$), between conditions ($p < .001$; $\eta^2_p = 0.646$) and in the time × condition interaction ($p < .001$; $\eta^2_p = 0.498$) (Figure 29). Both C ($p = 0.025$) and MF ($p < .001$) conditions had significantly higher boredom scores in the post compared to pre, diversely the scores didn't increase in LMF condition. The boredom scores post MF were higher in comparison with other conditions (C: $p < .001$; LMF: $p < .001$).

Mental and Physical Fatigability

Mental fatigability scores were above neutral (4 = Neither more energetic nor tired) in C = 4.47 ± 0.78 and MF = 5.395 ± 0.76, and slightly below in LMF = 3.72 ± 1.13. This difference results statistically significant ($p < .001$; $\eta^2_p = 0.669$) (figure 30). This difference was significant when comparing MF with C ($p < .001$) and LMF ($p < .001$), the difference between C and LMF was significant too ($p = 0.015$).

Physical fatigability differed significantly between conditions (C = 4.0 ± 0.67; MF = 3.61 ± 0.69; LMF = 4.92 ± 0.53) ($p < .001$; $\eta^2_p = 0.296$) (figure 31). Post hoc tests indicated higher physical fatigue in LMF compared to both C ($p = 0.001$) and MF ($p = 0.001$), with no difference between C and MF ($p = 0.168$).

DISCUSSION

The present study aimed to investigate the differential effects of mental fatigue (MF) and locomotor muscle fatigue (LMF), compared with a control condition (watching emotional neutral documentary), on endurance performance in healthy, physically active adults. Beyond endurance capacity, it was crucial to include a broad set of evaluations to gain a multifactorial understanding of these two distinct forms of fatigue. Accordingly, the experimental design combined measures of time to exhaustion (TTE), physiological responses, neuromuscular strength, vigilance, boredom, and perceived fatigability, offering an opportunity to directly contrast the consequences of MF and LMF. As expected, the findings revealed qualitatively different patterns of impairment: MF predominantly affected cognitive and perceptual domains, whereas LMF primarily influenced neuromuscular function and cardiorespiratory responses.

One of the central findings concerns endurance performance. Although the between-condition effect on TTE did not reach conventional significance, a clear trend emerged. The absence of statistical significance is likely attributable to the high inter-individual variability among participants, who, while all healthy and active, presented heterogeneous training backgrounds. The within-subject design mitigated some of this variability, yet differences remained. On average, participants in the LMF condition exhibited the shortest TTE, with a nearly 20% reduction compared with control, while MF induced a smaller (~10%) reduction. In relative terms, LMF reduced TTE by ~9.9% compared with MF. These findings are consistent with previous reports that both mental and locomotor muscle fatigue limit endurance capacity, albeit through distinct rationale (Marcora et al., 2009; Pageaux et al., 2014). Our initial hypothesis that both conditions would reduce TTE increasing RPE without differences between them was only partially supported.

The analysis of perceived exertion (RPE) across the exhaustion tests provides further insight. RPE values were significantly higher in LMF compared with both control and MF, while MF did not differ significantly from control, although a trend toward higher values was observed. This observation aligns with the psychobiological model of endurance performance, which posits that fatigue primarily acts by increasing perceived effort at a given workload, thereby reducing tolerance to prolonged exertion without necessarily altering physiological homeostasis (Marcora et al., 2009). The current findings extend this framework by directly contrasting MF with LMF, demonstrating that only LMF induced both perceptual and physiological perturbations, while MF operated predominantly at the perceptual–cognitive level.

Physiological responses further confirmed this dissociation. Heart rate, ventilation, oxygen uptake, carbon dioxide output, and breathing frequency all showed significantly higher values in LMF compared with MF, whereas no meaningful differences emerged between MF and control. These findings corroborate prior evidence that eccentric exercise and locomotor muscle fatigue increase ventilatory and cardiovascular demands (Marcora et al., 2007; Marcora et al., 2008; Venhorst et al., 2018), while cognitive exertion does not alter metabolic cost but undermines endurance via central mechanisms (Van Cutsem et al., 2017). The absence of condition-by-time interactions in most physiological measures suggests that these differences remained stable throughout the trials, reflecting the distinct physiological burden imposed by LMF.

Neuromuscular outcomes provide direct evidence of the mechanical consequences of LMF. Both peak and mean maximal voluntary contraction (MVC) declined significantly only after the eccentric drop-jump protocol, with reductions exceeding 15%. In contrast, no significant changes were observed in either the control or MF conditions. This result confirms that eccentric contractions are particularly effective in producing structural and functional impairments in skeletal muscle. Previous research has shown that unaccustomed or intense eccentric exercise induces ultrastructural alterations — including widespread Z-line streaming, myofibrillar disruption, sarcolemmal damage, and cytoskeletal disorganization — which compromise excitation–contraction coupling and calcium handling, ultimately leading to reduced force production and impaired force transmission (Proske and Morgan, 2001; Hody et al., 2019; Stožer et al., 2020). These findings align with the broader literature identifying eccentric contractions as the primary mechanism underlying both acute functional impairment and muscle soreness (Byrne et al., 2004; Howatson and van Someren, 2008). The absence of MVC decrements following MF reinforces the interpretation that mental exertion does not impair the contractile apparatus but instead affects performance through perceptual–motivational pathways, even though, in our study, self-reported motivation did not change.

Cognitive performance measures provided additional evidence of MF. Following the mental fatigue protocol, participants displayed markedly slower response speed and a significant increase in attentional lapses compared with both their pre-intervention values and the post-intervention results of the other conditions. This pattern indicates that the MF manipulation effectively induced a broader state of mental fatigue. Interestingly, the number of false starts did not change pre- to post-intervention but decreased after the control condition, resulting in significant differences with the other interventions and contributing to relatively higher cognitive performance in control. Based on Basner and Dinges (2012), false starts and lapses were treated as indices of cognitive performance and combined to derive an overall performance measure. The adoption of the 5-min PVT was supported by Thompson et al. (2022), who showed that this shortened version is a reliable alternative to the standard 10-min protocol. Post-intervention, high cognitive performance (≤5 lapses + false starts) was observed in control and LMF, whereas MF was characterized by lower (>16) or moderate (>5 and ≤16) performance levels.

From a methodological perspective, the continuous PVT protocol adopted in the mental fatigue condition represents a novel application. Specifically, participants completed a single PVT session that was 10 minutes longer than the intervention period (i.e., 50 minutes), and performance during the first and last five minutes was used as the pre- and post-intervention assessment, respectively. This approach allowed us to obtain pre–post measures without interrupting the intervention or administering separate test sessions. Importantly, this procedure was applied only in the mental fatigue condition. In the other experimental conditions, where mental fatigue was not induced, the PVT was administered in the conventional manner as separate pre- and post-intervention assessments.

Perceptual measures provide additional nuance. A significant increase in boredom was observed in C and MF conditions. Post-hoc comparisons showed that post-intervention boredom in the mental fatigue condition was significantly higher than in the control condition, while the control condition also exhibited a significant increase relative to baseline, suggesting that prolonged cognitive engagement carries a higher risk of task disengagement (Pattyn et al., 2018). Importantly, this effect occurred despite no measurable change in motivation, highlighting the partial independence of these constructs. Whereas motivation reflects the willingness to invest effort toward a goal, boredom captures the affective cost of sustained engagement in a task perceived as unrewarding or monotonous (Westgate and Wilson, 2018; Pattyn et al., 2018). In this context, the rise in boredom may represent a specific facet of MF, signaling a heightened tendency toward attentional disengagement without reducing motivational drive.

Self-reported fatigability scores were consistent with these dissociations. Mental fatigability was highest following MF, whereas physical fatigability peaked after LMF, confirming that the fatigue manipulations were effective and domain-specific. Collectively, these results indicate that participants perceived both the mental and physical load of the protocols, which translated into decreased performance through distinct pathways.

From an applied perspective, these findings carry important implications for sports and exercise practice. Mental fatigue is often overlooked in non-competitive settings, yet it may meaningfully impair endurance performance. Coaches and practitioners should consider the impact of daily cognitive and emotional demands on athletes when designing training programs. Conversely, locomotor fatigue clearly produces impairments through neuromuscular and physiological strain. Monitoring perceptual markers such as RPE, boredom, and fatigability scales may help practitioners differentiate between sources of fatigue and tailor interventions accordingly.

Despite the novelty and strengths of this study, some limitations must be acknowledged. The relatively small and gender-imbalanced sample (18 participants, of whom only 4 were women) restricts generalizability. The controlled laboratory setting, while ensuring internal validity, may not fully reflect real-world conditions. Another limitation is the absence of a true baseline endurance test, which would have allowed us to better account for external influences such as "bad day" effects. Finally, the lack of neurophysiological measures (e.g., EEG, NIRS) limits mechanistic interpretation of MF; future research should examine cortical and subcortical correlates of fatigue states.

CONCLUSION

The present study demonstrates distinct patterns of impairment in endurance performance, physiological responses, neuromuscular capacity, and cognitive outcomes produced by MF and LMF. Although the mechanisms differ — cognitive-perceptual versus neuromuscular-physiological — both converge in elevating perceived effort, ultimately impairing endurance performance. These findings underscore the multifaceted nature of fatigue and the challenge of capturing it with a single explanatory model. Although no significant between-condition differences in TTE were observed, the convergent evidence across multiple measures indicates that both manipulations effectively induced fatigue, with LMF exerting a comparatively greater impact on endurance performance.

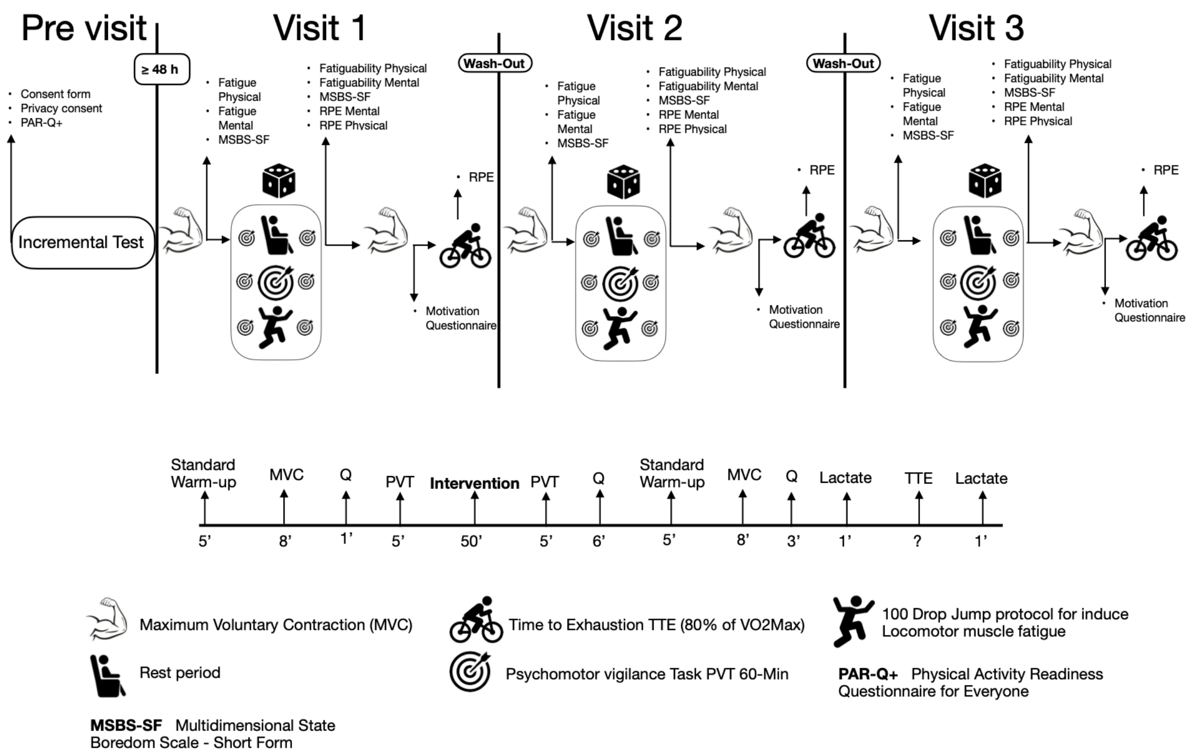


Fig. 16 Schematic of the experimental protocol

The diagram illustrates the sequence of laboratory visits and the procedures followed by the elite cyclists. It shows the division into phases (familiarization, mental fatigue intervention, locomotor fatigue intervention, control) and the tests performed (MVC, PVT, TTE, physiological and psychological measures). This scheme provides a clear overview of the experimental design.

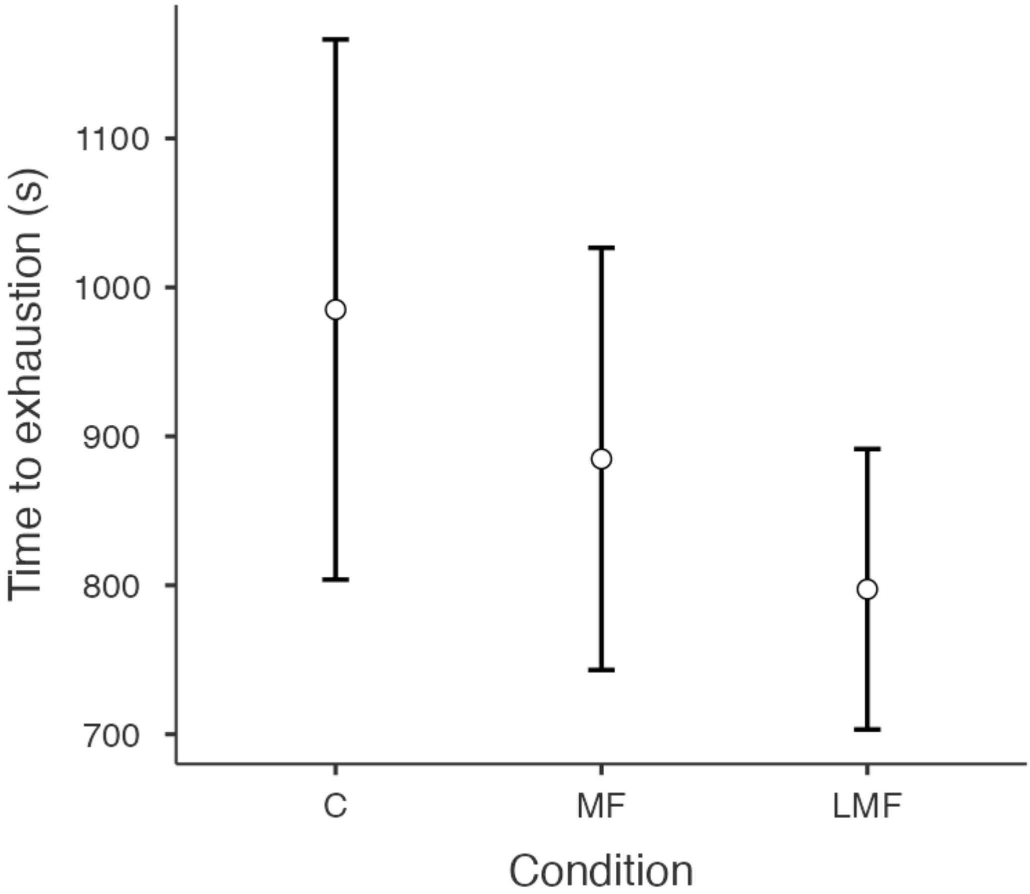


Fig.17 Time to Exhaustion (TTE) across interventions

The graph reports the mean time to exhaustion values in the three conditions: control (C), mental fatigue (MF), and locomotor muscle fatigue (LMF).

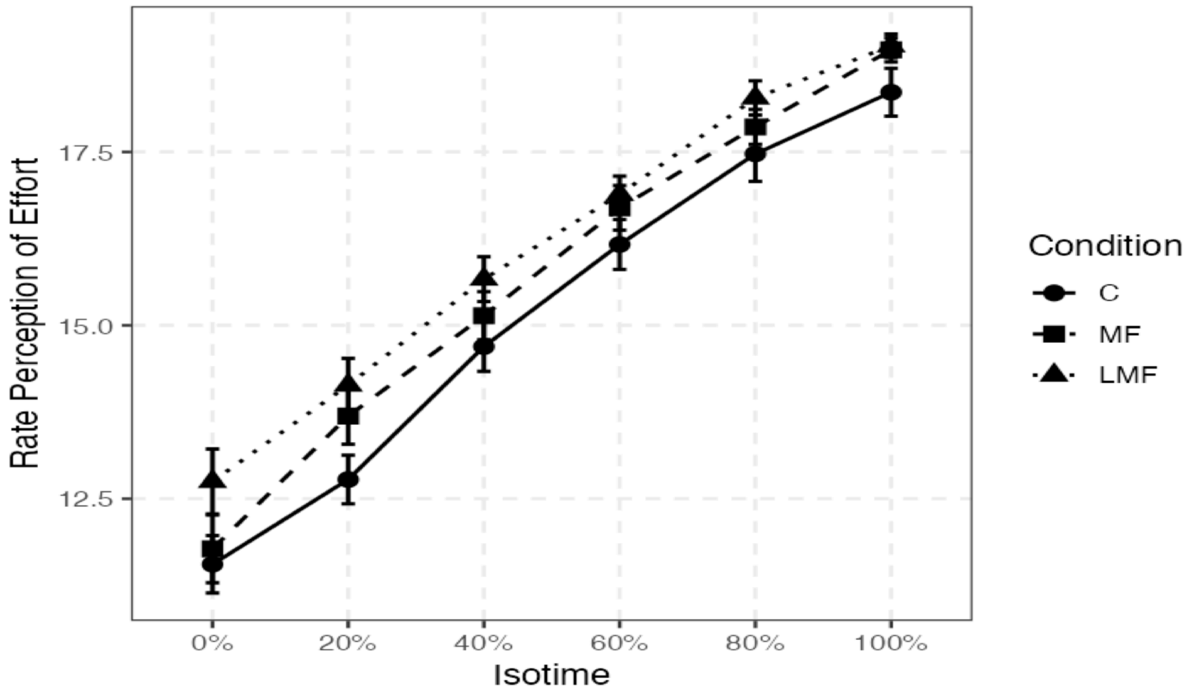


Fig. 18 Perceived exertion (RPE) at iso-time

The graph shows the evolution of RPE during the time-to-exhaustion tests.

Comparison	Time/Condition	Mean ± SD	p-value
C vs MF	C	15.2 ± 1.39	
	MF	15.7 ± 1.06	0.094
C vs LMF	C	15.2 ± 1.39	
	LMF	16.1 ± 1.01	**0.009****
MF vs LMF	MF	15.7 ± 1.06	
	LMF	16.1 ± 1.01	**0.031***

Tab. 4 reports the estimated marginal means (± SD) of rate of perceived exertion and post hoc comparisons between conditions. No significant difference emerged between **C** (15.2 ± 1.39) and **MF** (15.7 ± 1.06; p = 0.094). However, **C** showed significantly lower values compared to **LMF** (16.1 ± 1.01; p = 0.009). In addition, **MF** was lower than **LMF** (p = 0.031), although this difference did not remain significant after Tukey adjustment (p = 0.075).
* *p* < .05; ** *p* < .01; *** *p* < .001

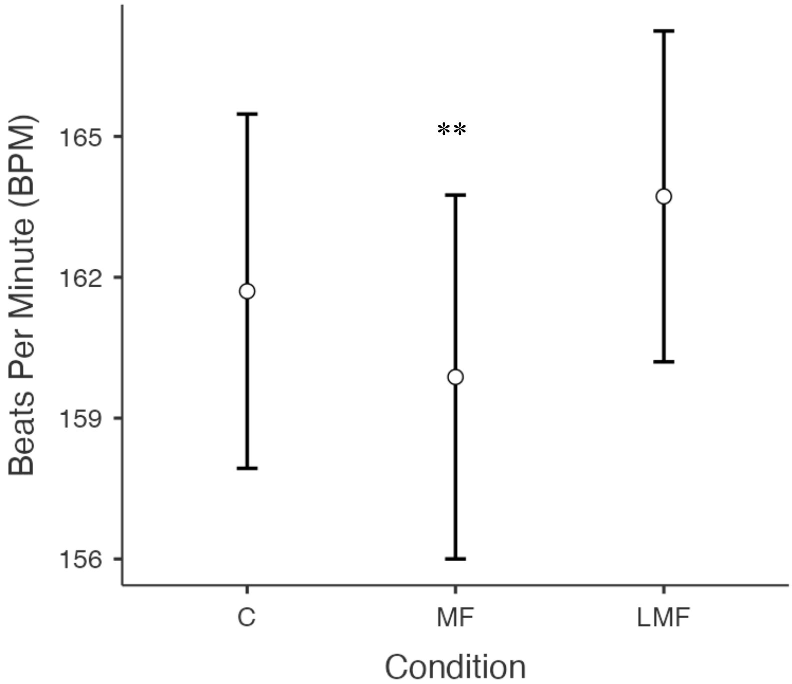


Fig.19 The estimated marginal means of heart rate (beats per minute) across the three experimental conditions, with error bars representing 95% confidence intervals.

Comparison	Time/Condition	Mean ± SD (beats/min)	p-value
C vs MF	C	162 ± 16.0	
	MF	160 ± 16.4	0.194
C vs LMF	C	162 ± 16.0	
	LMF	164 ± 14.9	0.088
MF vs LMF	MF	160 ± 16.4	
	LMF	164 ± 14.9	**0.009** **

Tab. 5 presents the estimated marginal means (± SD) and post hoc comparisons of heart rate (beats per minute) across conditions. No significant differences were observed between **C** (162 ± 16.0 BPM) and **MF** (160 ± 16.4 BPM; p = 0.194), nor between **C** and **LMF** (164 ± 14.9 BPM; p = 0.088). However, a significant difference emerged between **MF** and **LMF**(p = 0.009), with LMF showing higher heart rate values compared to MF. * $p < .05$; ** $p < .01$; *** $p < .001$

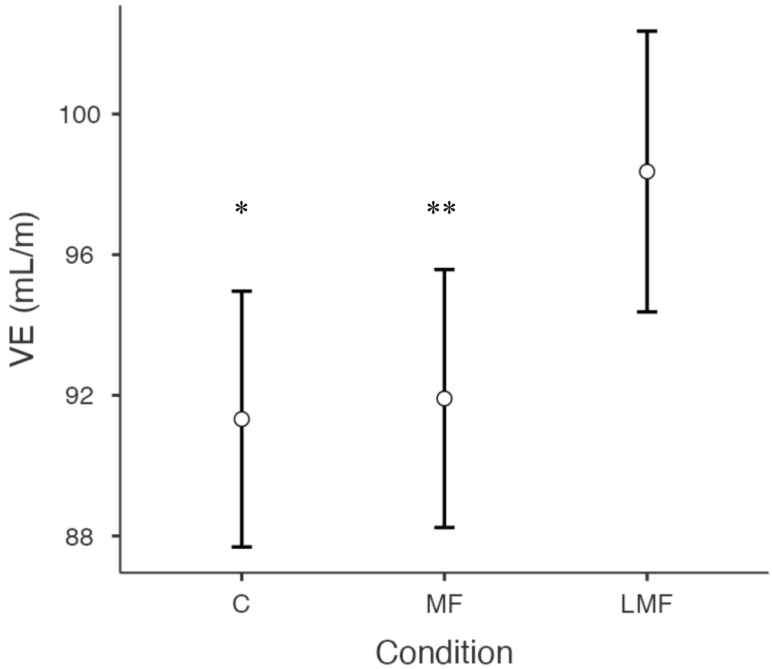


Fig. 20 shows the estimated marginal means of minute ventilation (VE, mL/min) across the three experimental conditions, with error bars indicating the 95% confidence intervals.

Comparison	Time/Condition	Mean ± SD (mL/min)	p-value
C vs MF	C	91.3 ± 15.5	
	MF	91.9 ± 15.6	0.755
C vs LMF	C	91.3 ± 15.5	
	LMF	98.4 ± 16.9	**0.019***
MF vs LMF	MF	91.9 ± 15.6	
	LMF	98.4 ± 16.9	**0.007****

Tab. 6 presents the estimated marginal means (± SD) and post hoc comparisons of ventilation (VE). No significant difference was found between **C** (91.3 ± 15.5 mL/min) and **MF** (91.9 ± 15.6 mL/min; p = 0.755). However, **LMF** (98.4 ± 16.9 mL/min) showed significantly higher values compared to both **C** (p = 0.019) and **MF** (p = 0.007). * $p < .05$; ** $p < .01$; *** $p < .001$

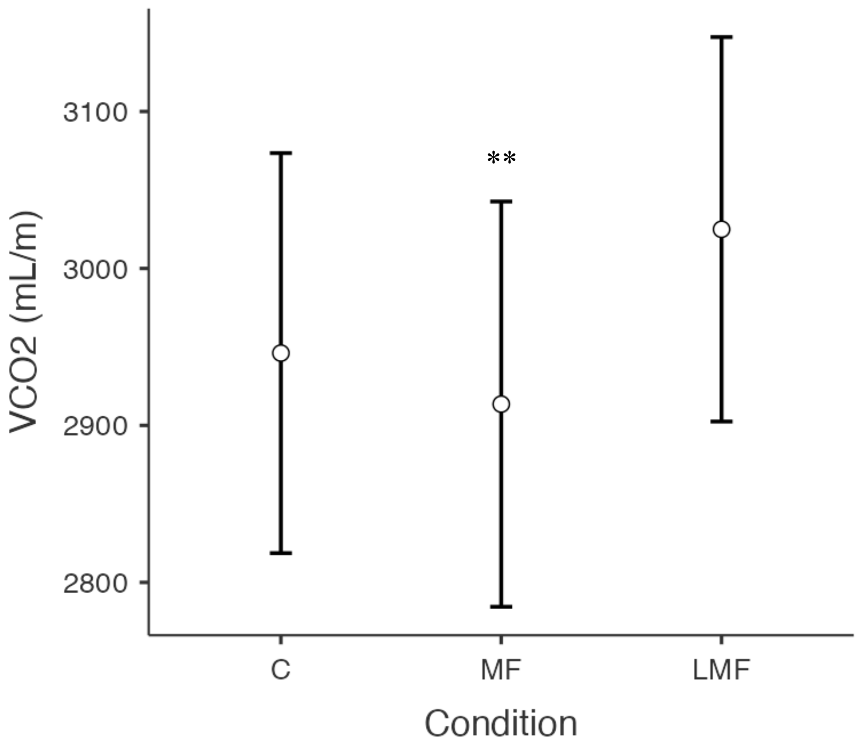


Fig. 21 illustrates the estimated marginal means of carbon dioxide output (VCO_2, mL/min) across the three conditions.

Comparison	Condition	Mean ± SD (mL/min)	p-value
C vs MF	C	2946 ± 540	
	MF	2914 ± 547	0.426
C vs LMF	C	2946 ± 540	
	LMF	3025 ± 519	0.108
MF vs LMF	MF	2914 ± 547	
	LMF	3025 ± 519	**0.002****

Tab. 7 shows the post hoc comparisons between conditions. No significant differences were found between **C** and **MF** (p = 0.426) or between **C** and **LMF** (p = 0.108). In contrast, a significant difference was observed between **MF** and **LMF** (p = 0.002), with MF showing lower values compared to LMF. * $p < .05$; ** $p < .01$; *** $p < .001$

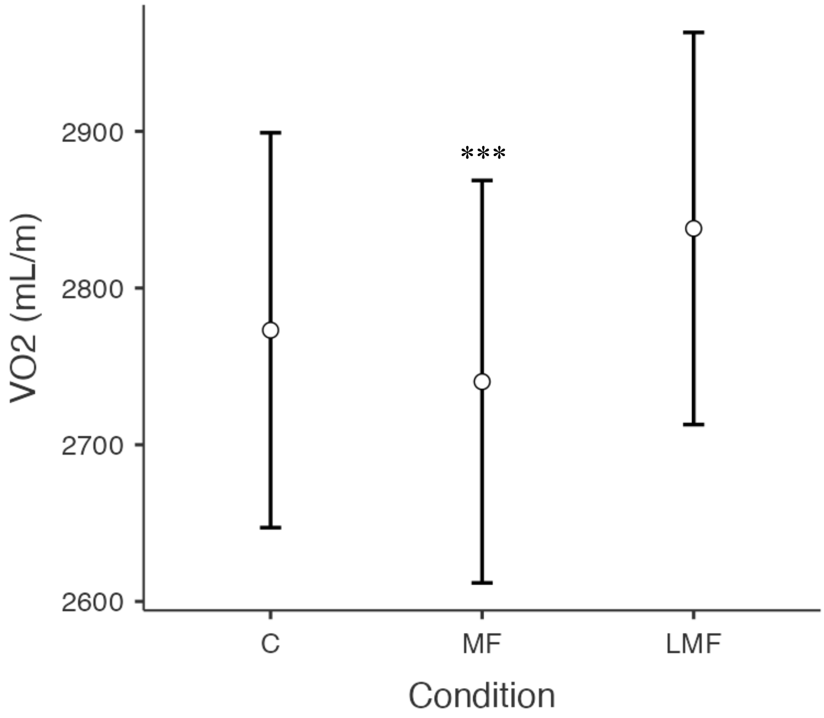


Fig. 22 illustrates the estimated marginal means of VO_2 across the three experimental conditions, with error bars representing 95% confidence intervals.

Comparison	Condition	Mean ± SD (mL/min)	p-value
C vs MF	C	2773 ± 533	
	MF	2740 ± 542	0.427
C vs LMF	C	2773 ± 533	
	LMF	2838 ± 531	0.107
MF vs LMF	MF	2740 ± 542	
	LMF	2838 ± 531	**< .001*** **

Tab. 8 reports VO_2 means (± SD) and post hoc comparisons. No significant differences were found between C and MF (p = 0.427) or C and LMF (p = 0.107). In contrast, LMF showed significantly higher VO_2 than MF (p < .001). * $p < .05$; ** $p < .01$; *** $p < .001$

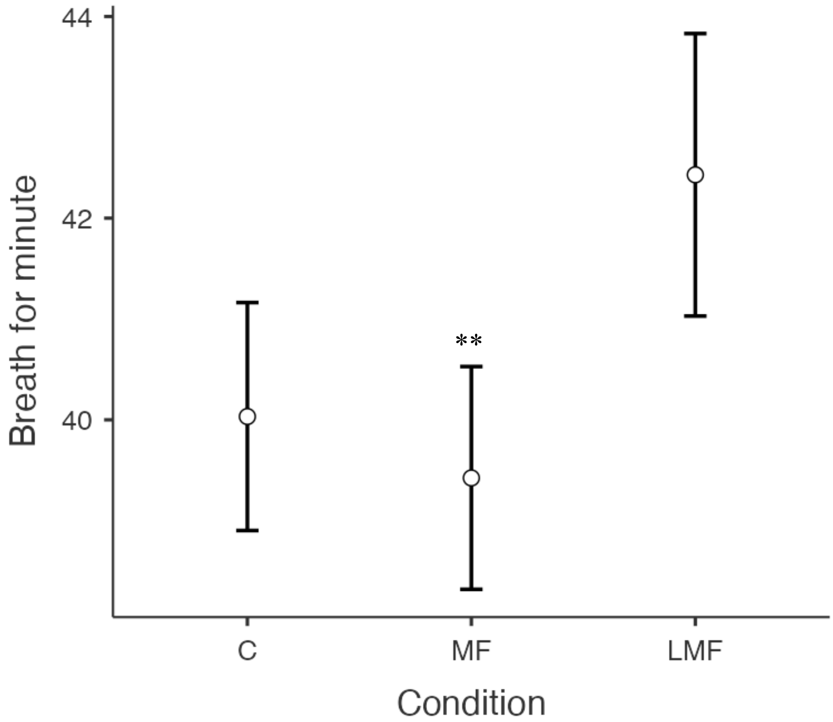


Fig. 23 illustrates the estimated marginal means of breathing frequency across the three experimental conditions.

Comparison	Time/Condition	Mean ± SD (breaths/min)	p-value
C vs MF	C	40.0 ± 4.80	
	MF	39.4 ± 4.67	0.502
C vs LMF	C	40.0 ± 4.80	
	LMF	42.4 ± 5.27	0.058
MF vs LMF	MF	39.4 ± 4.67	
	LMF	42.4 ± 5.27	**0.007****

Tab. 9 presents breathing frequency (± SD) across conditions. No difference emerged between **C** and **MF** (p = 0.502), while **C vs LMF** showed a trend (p = 0.058). **LMF** was significantly higher than **MF** (p = 0.007). * *p* < .05; ** *p* < .01; *** *p* < .001

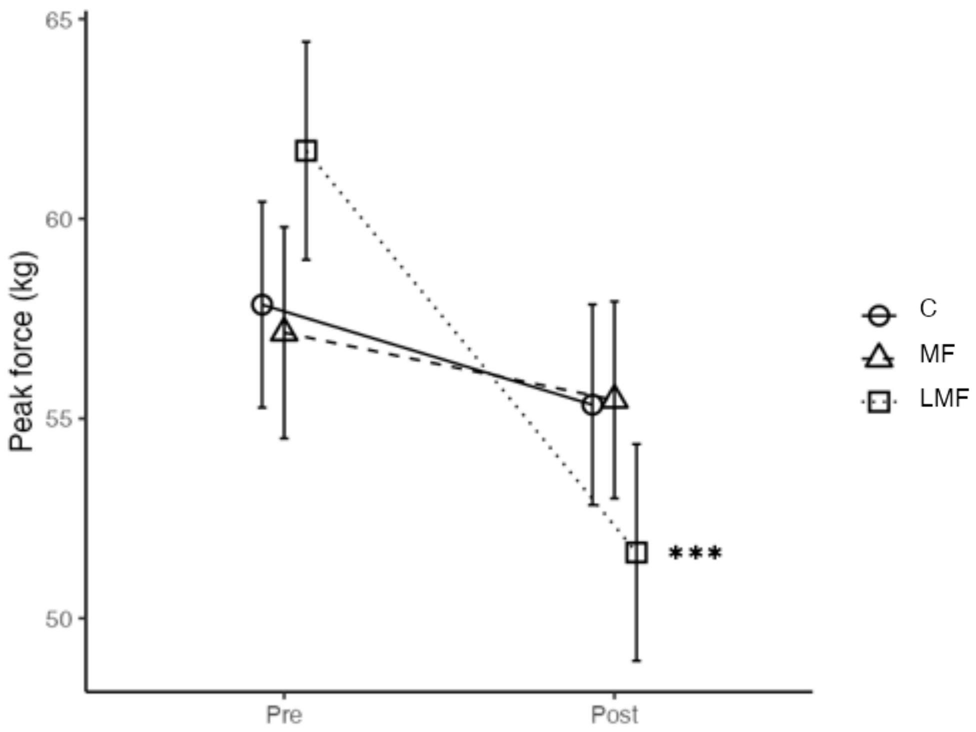


Fig. 24 displays estimated marginal means of peak force with error bars representing SE. C: control; PVT: Mental Fatigue; DJ: Locomotor muscle fatigue

Comparison	Time/Condition	Mean ± SD	p-value
C	PRE	57.8 ± 10.9 kg	
	POST	55.3 ± 10.7 kg	0.056
MF	PRE	57.1 ± 11.2 kg	
	POST	55.5 ± 10.4 kg	0.247
LMF	PRE	61.7 ± 11.6 kg	
	POST	51.6 ± 11.5 kg	**< .001*** **
C vs MF	C POST	55.3 ± 10.7 kg	
	MF POST	55.5 ± 10.4 kg	0.969
C vs LMF	C POST	55.3 ± 10.7 kg	
	LMF POST	51.6 ± 11.5 kg	0.140

Tab. 10 reports peak force values (± SD) and post hoc comparisons across time and conditions. In the **C** condition, peak force showed a trend toward reduction from pre (57.8 ± 10.9 kg) to post (55.3 ± 10.7 kg; p = 0.056), though this did not reach significance. In the **MF** condition, values remained stable from pre to post (p = 0.247). In contrast, the **LMF** condition exhibited a marked and significant decline from pre (61.7 ± 11.6 kg) to post (51.6 ± 11.5 kg; p < .001). At the post measurement, no significant differences were observed between conditions: **C vs MF** (p = 0.969), **C vs LMF**(p = 0.140), and **MF vs LMF** (p = 0.227). Overall, the results suggest a clear reduction in peak force in LMF, with a possible trend toward decline also emerging in C, while MF remained stable. * *p* < .05; ** *p* < .01; *** *p* < .001

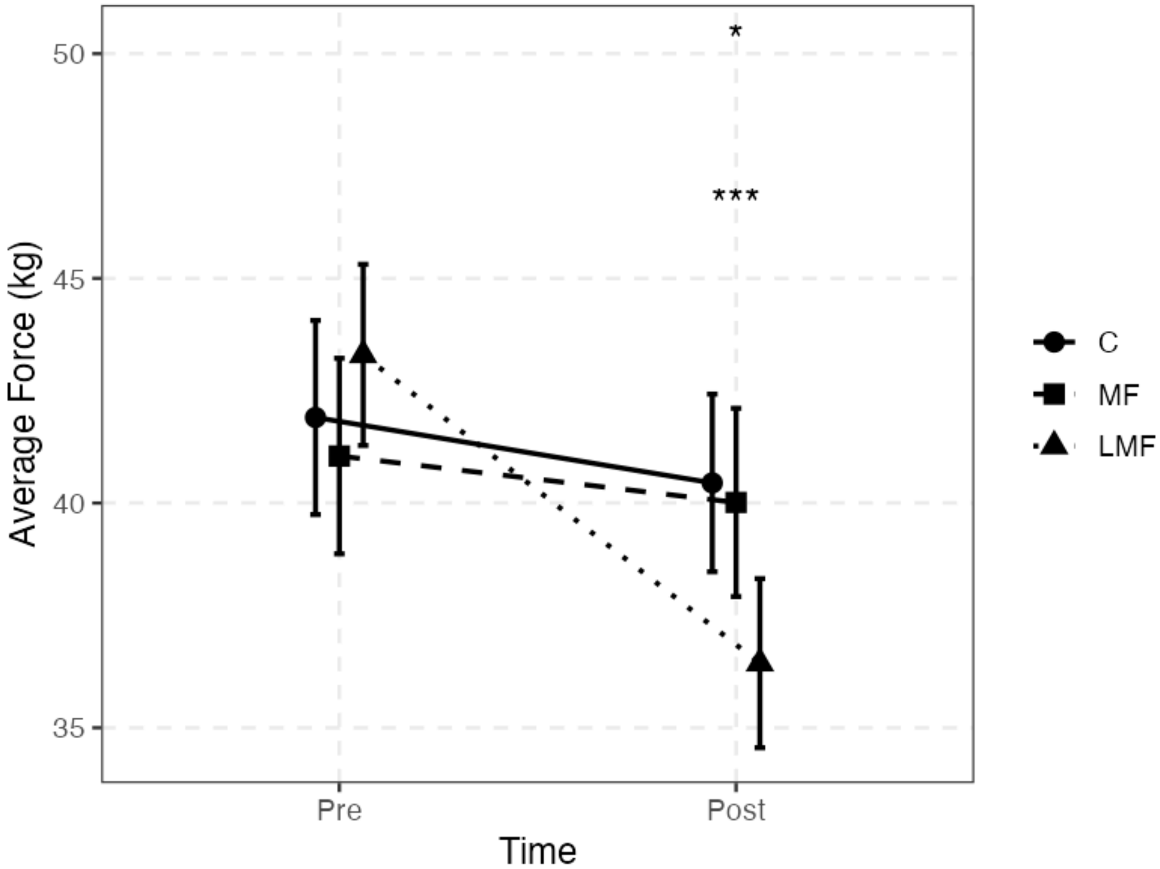


Fig.25 illustrates the estimated marginal means of average force across time and conditions with error bars representing SE.

Comparison	Time/Condition	Mean ± SD	p-value
C	PRE	41.9 ± 9.1 kg	
	POST	40.4 ± 8.6 kg	0.213
MF	PRE	41.0 ± 9.3 kg	
	POST	40.0 ± 8.9 kg	0.521
LMF	PRE	43.3 ± 9.0 kg	
	POST	36.4 ± 8.0 kg	**< .001*** **
C vs MF	C POST	40.4 ± 8.6 kg	
	MF POST	40.0 ± 8.9 kg	0.854
C vs LMF	C POST	40.4 ± 8.6 kg	
	LMF POST	36.4 ± 8.0 kg	**0.019***
MF vs LMF	MF POST	40.0 ± 8.9 kg	
	LMF POST	36.4 ± 8.0 kg	**0.045***

Tab. 11 presents average force values (± SD) and post hoc comparisons across conditions and time. No significant pre–post changes were detected in **C** or **MF** ($p = 0.213$ and $p = 0.521$, respectively). In contrast, **LMF** showed a significant reduction from pre (43.3 ± 9.0 kg) to post (36.4 ± 8.0 kg; $p < .001$). At the post measurement, **LMF** demonstrated significantly lower average force compared with both **C** ($p = 0.019$) and **MF** ($p = 0.045$), while C and MF did not differ from each other ($p = 0.854$). These findings indicate that the decline in force production was specific to LMF, with post-task deficits distinguishing it from the other conditions. * $p < .05$; ** $p < .01$; *** $p < .001$

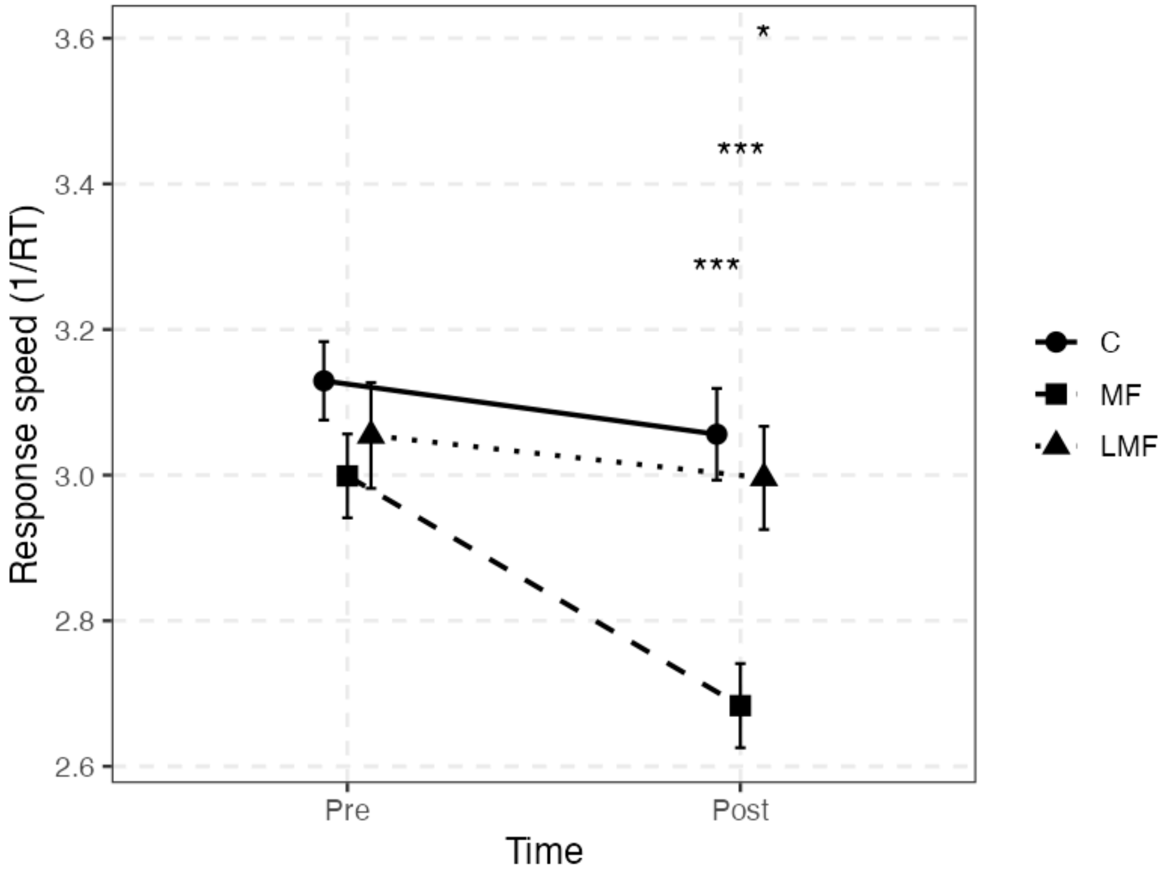


Fig. 26 shows the estimated marginal means of response speed (1/RT) across time and conditions with DS bars.

Comparison	Time/Condition	Mean ± SD (1/RT)	p-value
C	PRE	3.13 ± 0.23	
	POST	3.06 ± 0.26	**0.038***
MF	PRE	3.00 ± 0.24	
	POST	2.68 ± 0.24	**< .001*****
LMF	PRE	3.05 ± 0.31	
	POST	3.00 ± 0.30	0.132
C vs MF	C POST	3.06 ± 0.26	
	MF POST	2.68 ± 0.24	**< .001*****
C vs LMF	C POST	3.06 ± 0.26	
	LMF POST	3.00 ± 0.30	0.175
MF vs LMF	MF POST	2.68 ± 0.24	
	LMF POST	3.00 ± 0.30	**< .001*****

Tab. 12 presents response speed values (± SD) and post hoc comparisons. A small but significant pre–post reduction was observed in **C** (p = 0.038), while **MF** showed a marked decline (p < .001). No significant change was found in **LMF** (p = 0.132). At the post measurement, **MF** exhibited significantly lower response speed compared with both **C** (p < .001) and **LMF** (p < .001). No difference emerged between **C** and **LMF** (p = 0.175). These results highlight a selective deterioration in the MF condition, while C and LMF maintained stable response performance. * $p < .05$; ** $p < .01$; *** $p < .001$

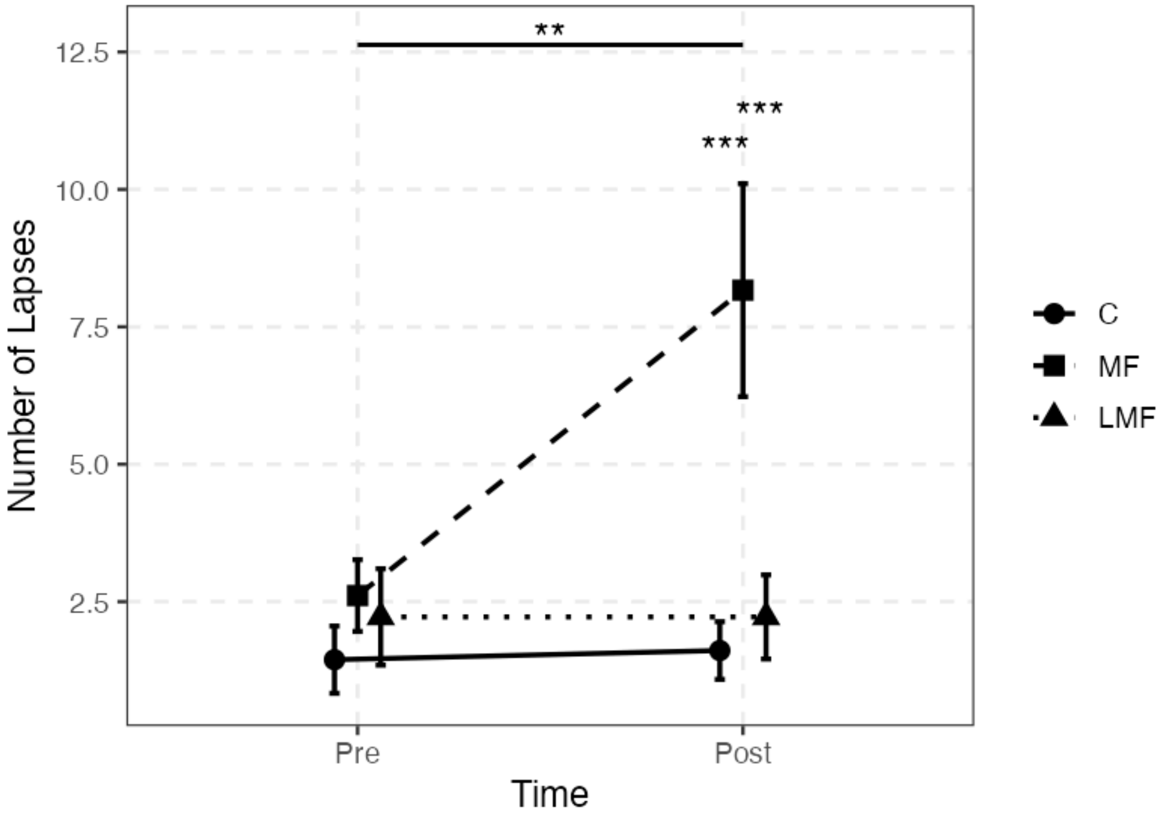


Fig. 27 illustrates the estimated marginal means of lapses across time and conditions with SE bars.

Comparison	Time/Condition	Mean ± SD	p-value
C	PRE	1.44 ± 2.60	
	POST	1.61 ± 2.23	0.736
MF	PRE	2.61 ± 2.77	
	POST	8.17 ± 7.34	**0.004****
LMF	PRE	2.22 ± 3.72	
	POST	2.22 ± 3.25	1.000
C vs MF	C POST	1.61 ± 2.23	
	MF POST	8.17 ± 7.34	**0.001****
C vs LMF	C POST	1.61 ± 2.23	
	LMF POST	2.22 ± 3.25	0.119
MF vs LMF	MF POST	8.17 ± 7.34	
	LMF POST	2.22 ± 3.25	**< .001*****

Tab. 13 reports number of lapses (± SD) and post hoc comparisons. In **C** and **LMF**, no significant pre–post changes were observed. In contrast, **MF** showed a strong increase in lapses from pre (2.61 ± 2.77) to post (8.17 ± 7.34; p = 0.004). At post, **MF** displayed significantly more lapses than both **C** (p = 0.001) and **LMF** (p < .001), while no difference was found between **C** and **LMF** (p = 0.119). These findings highlight a selective worsening of performance in the MF condition. * $p < .05$; ** $p < .01$; *** $p < .001$

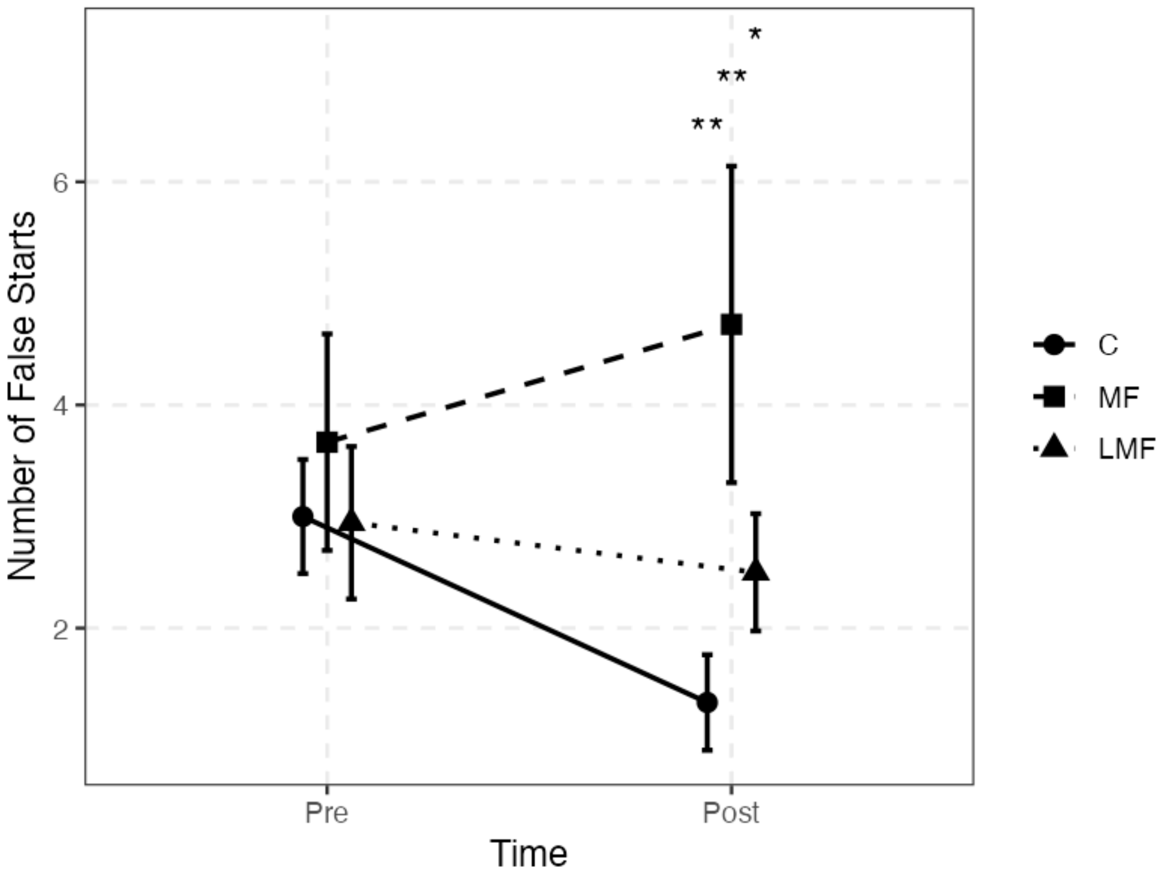


Fig. 28 shows the estimated marginal means of false starts across time and conditions with SE bars.

Comparison	Time/Condition	Mean ± SD	p-value
C	PRE	3.00 ± 2.17	
	POST	1.33 ± 1.82	**0.002****
MF	PRE	3.67 ± 4.12	
	POST	4.72 ± 6.01	0.231
LMF	PRE	2.94 ± 2.90	
	POST	2.50 ± 2.23	0.435
C vs MF	C POST	1.33 ± 1.82	
	MF POST	4.72 ± 6.01	**0.019***
C vs LMF	C POST	1.33 ± 1.82	
	LMF POST	2.50 ± 2.23	**0.002****
MF vs LMF	MF POST	4.72 ± 6.01	
	LMF POST	2.50 ± 2.23	0.083

Tab. 14 shows the number of false starts (± SD) and post hoc comparisons. In the **C condition**, false starts significantly decreased from pre to post (p = 0.002). No pre–post changes were found in **MF** or **LMF**. At post, **C** had significantly fewer false starts compared to both **MF** (p = 0.019) and **LMF** (p = 0.002), while the difference between **MF** and **LMF**showed only a trend (p = 0.083). * $p < .05$; ** $p < .01$; *** $p < .001$

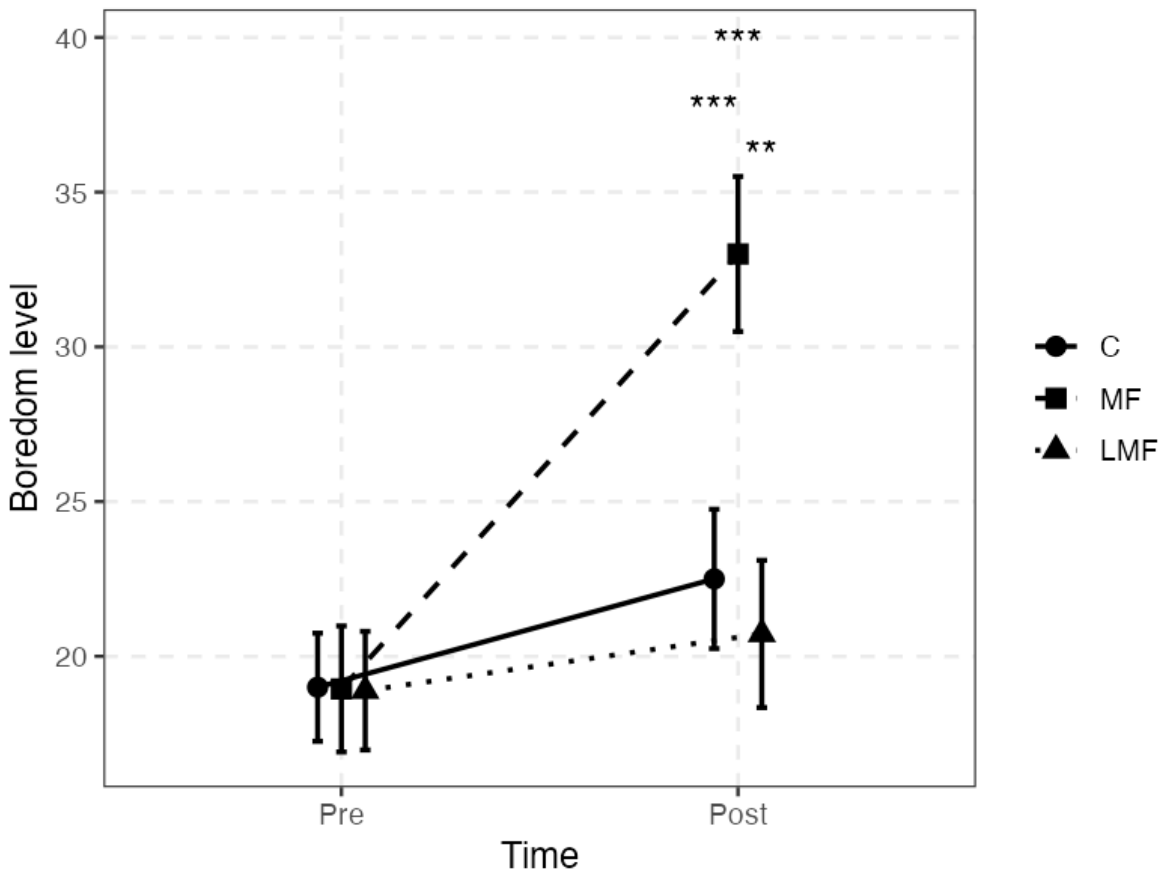


Fig. 29 displays mean boredom levels across conditions at pre- and post-test.

Comparison	Time/Condition	Mean ± SD	p-value
C	Pre	19.0 ± 7.4	
	Post	22.5 ± 9.5	**0.025***
MF	Pre	18.9 ± 8.7	
	Post	33.0 ± 10.6	**< .001*****
LMF	Pre	18.9 ± 8.1	
	Post	20.7 ± 10.1	0.114
C vs MF	C Post	22.5 ± 9.5	
	MF Post	33.0 ± 10.6	**< .001*****
MF vs LMF	MF Post	33.0 ± 10.6	
	LMF Post	20.7 ± 10.1	**< .001*****

Tab. 15 shows the mean of Boredome score ± SD. Post hoc analyses revealed a significant increase in boredom from pre- to post-test in the MF condition ($p < .001$), whereas no changes were observed in the C and LMF conditions ($p = 0.193$ and $p = 0.570$, respectively). At post-test, boredom levels were significantly higher in MF compared with both C ($p < .001$) and LMF ($p = .001$). * $p < .05$; ** $p < .01$; *** $p < .001$

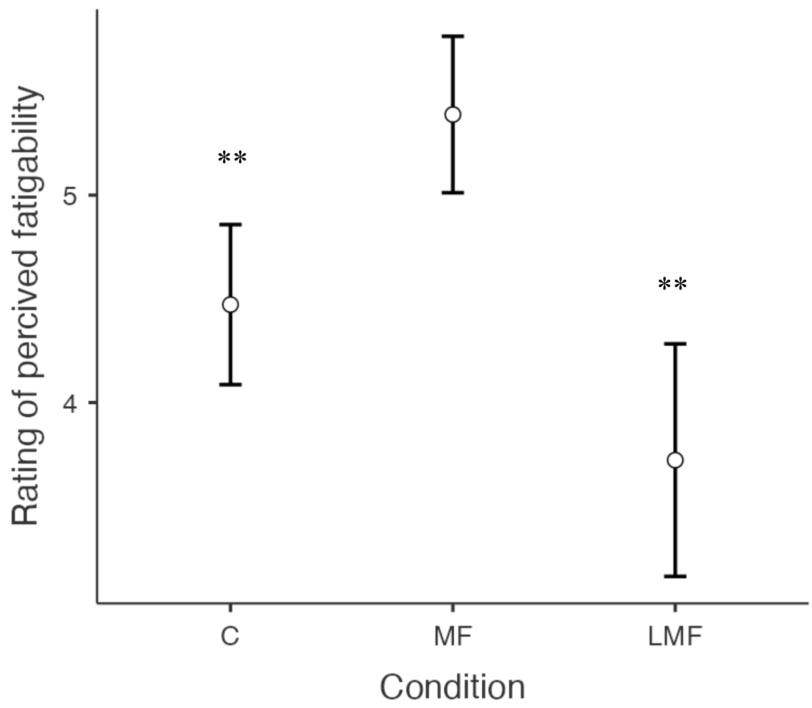


Fig. 30 presents mean ratings of mental perceived fatigability (± SD) across conditions.

Comparison	Condition	Mean ± SD	p-value
C vs MF	C	4.47 ± 0.8	
	MF	5.39 ± 0.8	**0.008****
C vs LMF	C	4.47 ± 0.8	
	LMF	3.72 ± 1.1	0.120
MF vs LMF	MF	5.39 ± 0.8	
	LMF	3.72 ± 1.1	**0.002****

Tab. 16 shows the mean ± SD of perceived fatigability score. Post hoc analyses indicated that mental perceived fatigability was significantly higher in MF compared with C (p = 0.008) and LMF (p = 0.002). No significant difference was observed between C and LMF (p = 0.120). * $p < .05$; ** $p < .01$; *** $p < .001$

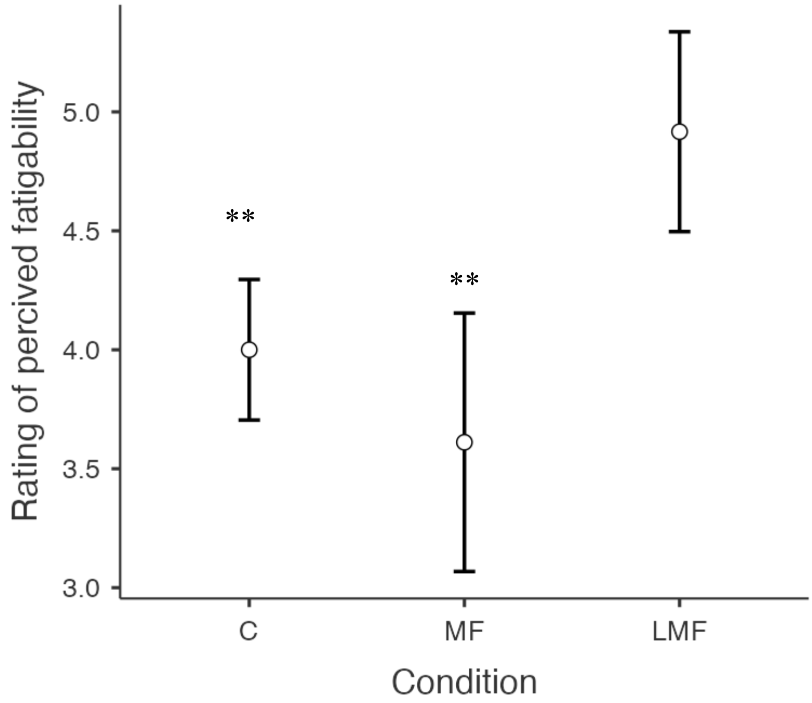


Fig. 31 illustrates mean ratings of physical perceived fatigability (± SD) across conditions.

Comparison	Condition	Mean ± SD	p-value
C vs MF	C	4.00 ± 0.6	
	MF	3.61 ± 1.1	0.343
C vs LMF	C	4.00 ± 0.6	
	LMF	4.92 ± 0.8	**0.003****
MF vs LMF	MF	3.61 ± 1.1	
	LMF	4.92 ± 0.8	**0.004****

Tab. 17 shows the mean ± SD of perceived fatigability score. Post hoc analyses revealed significantly higher perceived physical fatigability in LMF compared with both C (p = 0.003) and MF (p = 0.004). No significant difference was found between C and MF (p = 0.343).

CHAPTER 4

MENTAL FATIGUE IN ESPORTS: ACTIVE GAMING VS. SPECTATING

TULLIO SENATORE, MUZHI ZHANG, SAMUELE MARCORA

ABSTRACT

The rapid growth of esports participation is well established; however, increasing attention is being directed toward the expanding phenomenon of spectatorship. Esports performance requires prolonged perceptual–cognitive processing and emotional engagement, yet the psychophysiological demands of active gameplay compared with passive viewing remain poorly quantified. This study compared acute cognitive, psychological, and perceived workload responses to two hours of competitive first-person shooter gameplay versus passive spectating.

Sixteen amateur esports players completed two experimental sessions (Play and Watch) in a randomized crossover design. Before and after each session, participants performed a psychomotor vigilance task (PVT) and a game-specific shooting accuracy test and completed measures of perceived fatigue (ROF), mood (BRUMS), boredom (MSBS), and workload (NASA-TLX).

Response speed in the PVT declined over time in both conditions ($p = 0.004$), with no between-condition differences, while shooting accuracy showed a trend toward a time × condition interaction ($p = 0.073$), decreasing after Play and slightly improving after Watch. Subjective fatigue increased significantly after Play ($p < 0.001$) but not after Watch. Total mood disturbance increased only after Play ($p = 0.002$), accompanied by reduced vigor and increased fatigue subscale scores (both $p < 0.01$). Perceived workload was substantially higher during Play than Watch ($p < 0.001$), particularly for mental demand, physical demand, and effort. Boredom and inattention increased similarly in both conditions.

These findings indicate that competitive esports gameplay elicits greater mental workload, fatigue, and mood disturbance than passive viewing, despite modest cognitive performance changes. The results support the view that esports competition represents a meaningful psychophysiological stressor and provide empirical evidence for conceptualizing esports as a sport-like performance domain.

INTRODUCTION

The question of whether esports can be considered a sport has moved beyond online gaming forums and into the realm of traditional sport science, particularly following the growing institutional recognition of competitive gaming. A key milestone in this process has been the International Olympic Committee's creation of the Esports Olympic Games, scheduled for 2027 in Riyadh, which signifies greater legitimacy without fully resolving the scientific debate (International Olympic Committee, 2023). The present study contributes to this debate with an innovative protocol, aiming to provide empirical evidence in support of the claim while acknowledging that it does not settle the broader question regarding the sporting nature of esports performance.

From a performance standpoint, elite Esports demand many of the same perceptual–cognitive systems required in traditional sports. Meta-analytic evidence demonstrates that experience with action video games is associated with advantages in attentional control and spatial cognition (Bediou et al., 2018; Bediou et al., 2023). Professional players, in particular, devote greater visual attention to key game elements and employ distinctive gaze strategies, such as shorter fixations and wider horizontal scan paths (Jeong et al., 2024; Luo et al., 2025). From an ecological dynamics perspective, these performances reflect a continuous perception–action coupling in which performers extract task-relevant information and adjust their behavior in real time. Whereas in field sports such information includes the movement of opponents and ball trajectories, in esports it consists of dense on-screen signals (enemy vectors, object states, tactical overlays) that must be processed under severe time pressure.

Esports viewing has emerged as a rapidly expanding global phenomenon, paralleled by a growing body of literature. Spectators follow esports for reasons similar to those of fans of traditional sports (Rietz and Hallmann, 2023), and digital content consumption has been linked to real-life satisfaction and behavioral intentions (Kim and Kim, 2020). The parallels between esports and traditional sports now extend beyond the distinction between professional and amateur players, encompassing also the emotional and behavioral responses of audiences. In fact, live debates and chat interactions following esports events often suggest even greater engagement than in traditional sports. Spectator behavior strongly shapes the sense of presence and interaction during esports events (Cauteruccio and Kou, 2023). While factors such as commentary, player characteristics, event appeal, and scheduling are common to both contexts, elements such as chat-room dynamics, streamer characteristics, streaming quality, and virtual rewards appear unique to esports (Qian et al., 2020).

It is evident that being “glued to the screen” is a shared characteristic of both esports play and viewing. Prolonged screen exposure has been shown to deplete cognitive resources needed for emotion regulation (Oswald et al., 2023). Physiological evidence, although still incomplete, is promising. Competitive gaming has been shown to increase heart rate and alter heart rate variability profiles consistent with sympathetic activation (André et al., 2020; Welsh et al., 2023). Expanding this discussion within a psychobiological model, coordinated cardiovascular and neuroendocrine responses can occur even in the absence of physical effort (Salvador and Costa, 2009). This argument extends also to spectatorship: heart rate changes have been reported during emotionally engaging sports viewing (Khairy et al., 2017; Zimmerman et al., 2010), along with vagal withdrawal, increased vasomotor sympathetic activity, and blood pressure dynamics distinct from those observed during exercise despite comparable heart rate elevations.(Piira et al., 2011).

Related research also shows that physiological state can influence performance. For example, a brief bout of high-intensity cardiovascular exercise enhanced performance in League of Legends in a randomized trial (de Las Heras et al., 2020), while other intervention studies have reported benefits from structured warm-ups or exercise protocols (dos Santos et al., 2024). These findings reinforce the rationale for analyzing how active gameplay, as opposed to spectating, modulates psychophysiological demands.

This framework motivates a fundamental empirical question: does active esports play generate classic cognitive, behavioral, and physiological responses of active engagement that exceed those elicited by passive viewing? To address this gap, the present study employed a within-subjects design comparing two-hour sessions of competitive *Valorant* under play and viewing conditions. By assessing performance, mood, fatigue perceived, mental workload, and physiological indices, we provide preliminary evidence testing the hypothesis that gameplay imposes greater psychophysiological demands than spectating. These results represent the first step of a broader research program on cognition, mental fatigue, and physiology in esports, to be expanded with EEG measures during play and experimental interventions involving physical exercise and food integration. To our knowledge, only one previous study—although with different aims—has adopted a comparable design: in overweight/obese men, playing video games elicited an acute stress response compared with watching non-violent television, which was further associated with increased subsequent food intake (Siervo et al., 2018). Instead, the aim of this study was to determine whether actively participating in a medium-to-long session of a first-person shooter (FPS) video game increased perceived fatigue, altered mood states, and impaired reaction times, attentive performance, and generate greater cardiac responses than passively watching the same game for the same duration, as a consequence of the additional mental fatigue induced by active participation.

METHODS AND MATERIALS

Subjects

Sixteen amateur video gamers were recruited for the main study. All participants reported several years of esports experience and a regular weekly load of gameplay and spectating activities. Sample size for the main study was determined a priori using *G*Power analysis, based on effect sizes obtained in a previously conducted pilot experiment (Appendix C). The main study adopted a randomized, counterbalanced crossover design. Exclusion criteria included any neurological, muscular, skeletal, tendon, or ligament injury of the upper or lower extremities within the past six months, a history of cardiovascular disease, or the current use of medications known to influence cardiac or endocrine function (e.g., blood pressure, heart rate). All participants were fully informed about the study's purpose, procedures, and potential risks, and provided written informed consent. This study was conducted following the Declaration of Helsinki.

A pilot study was conducted beforehand with ten participants (4 female and 6 male). The pilot followed the same general structure as the official protocol but was implemented with reduced methodological rigor in order to accelerate data collection. Specifically, all participants completed the gameplay condition first and the spectating condition second, without randomization or counterbalancing. Moreover, all ten participants performed their sessions simultaneously in the same laboratory environment. Importantly, no physiological measures were collected during the pilot; only behavioral, mood and perceptual outcomes were assessed. The pilot results were used to guide protocol refinement and power analysis for the main study.

Procedures

Each participant in the main study attended the laboratory on two separate occasions. The randomized, counterbalanced design ensured that half of the subjects commenced with the gameplay condition (P), while the other half began with the spectating condition (W). At the first visit, eligibility was verified through initial screening, followed by a 30-min familiarization period. During this session, participants were instructed on the purpose and procedures of the study, provided written consent, and reported their esports background (years of FPS experience, weekly hours of gameplay, weekly hours of spectating). They were also explicitly instructed not to abandon sessions, use smartphones, or engage in distracting behaviors, as failure to comply would have resulted in exclusion from the study.

The pre-intervention phase included subjective fatigue ratings using the *Rating of Fatigue scale (ROF)*, assessment of boredom state via the *Multidimensional State Boredom Scale*, and mood evaluation with the *Brunel Mood Scale (BRUMS)*. All questionnaires were administered in validated Chinese translations, with the exception of the ROF, which was translated and back-translated by bilingual Chinese–English researchers.

Cognitive performance was assessed through a 5-min *Psychomotor Vigilance Task (PVT)* to measure attention and vigilance, and a game-specific skill test. In the skill test, participants completed a 30-s task aiming to achieve the maximum number of headshots. The test was repeated three times in each condition, and the mean score was recorded. Physiological data were collected using POLAR H10 chest straps for heart rate monitoring.

The intervention phase differed only in modality: in the gameplay condition, participants played a first-person shooter game for 120 minutes, whereas in the spectating condition they passively watched 120 minutes of pre-recorded gameplay. Intervention order was randomized using a web-based program (www.randomization.com).

At the conclusion of each session, participants repeated the same battery of assessments administered during the pre-intervention phase, including fatigue, boredom, mood, and cognitive performance, as well as the *NASA-TLX* workload scale (validated Chinese version).

Each experimental visit therefore comprised three phases (figure 32): (i) Pre-intervention (25 min) – baseline assessments of cognitive performance and questionnaires; (ii) Intervention (120 min) – active gameplay or spectating of FPS content; (iii) Post-intervention (25 min) – repeated assessments of cognitive and psychological measures.

A washout period of at least 48 h was implemented between conditions to minimize carry-over effects.

ROF

Subjective fatigue was assessed using the Rating of Fatigue scale (ROF), an 11-point numerical instrument ranging from 0 ("完全不疲劳", "not fatigued at all") to 10 ("完全力竭，精疲力尽", "total fatigue & exhaustion, nothing left"), accompanied by verbal and diagrammatic anchors to facilitate comprehension. Participants were asked to indicate their current level of perceived fatigue, and the scale has been shown to possess strong validity both during exercise, in recovery phases and in general in different moments of the day (Micklewright et al., 2017).

Multidimensional State Boredom Scale (MSBS)

State boredom was evaluated with the Multidimensional State Boredom Scale (MSBS). Originally developed and validated as a 29-item instrument capturing four dimensions of boredom—disengagement, low arousal, inattention, and distorted time perception (Fahlman et al., 2013)—the MSBS provides a robust measure of situational boredom. For the present study, the validated 19-item version proposed by Ng et al. (2015) was employed, which retains the multidimensional structure while improving efficiency and reducing participant burden. Participants rated each item on a 5-point Likert scale ranging from 1 ("非常不同意", "strongly disagree") to 5 ("非常同意", "strongly agree"), based on their feelings "right now". Subscale scores were calculated by summing responses for each dimension, with higher scores indicating greater levels of boredom. The 19-item MSBS has demonstrated good construct validity and reliability, and has been shown to capture cross-cultural differences in the subjective experience of boredom (Ng et al., 2015).

BRUMS

Mood states were measured using the Brunel Mood Scale (BRUMS), a 24-item questionnaire designed to capture six transient affective dimensions: tension, depression, anger, vigor, fatigue, and confusion. Each item is rated on a five-point Likert scale, anchored from "一点都没有" ("not at all") (0) to "非常多" ("extremely") (4), in response to the question "How do you feel right now?". Subscale scores were obtained by summing the four relevant items, yielding possible scores between 0 and 16 for each dimension. To provide an overall index of psychological state, the Total Mood Disturbance (TMD) score was calculated by summing the negative mood dimensions (tension, depression, anger, fatigue, and confusion) and subtracting the vigor score, with higher TMD values indicating a more negative mood profile. The BRUMS has demonstrated strong psychometric properties across sport and exercise contexts, including validation in Chinese populations (Terry et al., 2003; Terry and Lane, 2010; Zhang et al., 2014).

PVT

Sustained attention and vigilance were assessed using the Psychomotor Vigilance Task (PVT). The PVT is a simple visual reaction time test first used as a research tool by Dinges and Powell (1985). In this study implemented via a Windows-based platform (PC-PVT 2.0), which has been validated for accurate measurement of reaction times and lapses (Reifman et al., 2018). The test requires participants to respond as quickly as possible to the appearance of a visual stimulus presented at random interstimulus intervals (between 2 and 10 seconds). Each session lasted 5 minutes, and outcome variables included mean reaction time, number of lapses (responses >500 ms) and false start (responses <150 ms or responses before that stimulus appears). The PC-PVT 2.0 software has been extensively used in research on fatigue and sleep deprivation, demonstrating robust psychometric properties and reliability for laboratory and field applications (Reifman et al., 2018).

Game Performance Task

Game-specific motor performance was assessed using the skill challenge mode integrated in *Fearless Pioneers*. This test mode replicates the mechanics of *Valorant*, allowing players to select a weapon and attempt to perform as many headshots as possible within 30 seconds against automatically respawning targets. The task was performed before and after the intervention sessions (pre- and post-), providing an ecologically valid measure of in-game mechanical proficiency under time pressure.

Performance was quantified as the total number of headshots achieved within the 30-second window. Simultaneous HR monitoring via Polar H10 was conducted to synchronize physiological responses with motor performance outcomes.

The use of in-game aiming drills as a performance assessment has been supported by recent research, which highlights the reliability and validity of FPS training platforms (e.g., KovaaK's, Aim Lab) to quantify shooting accuracy, headshot precision, and shot frequency (Rogers et al., 2024; Spjut et al., 2022). In line with these findings, the Fearless Pioneers skill mode provides a standardized and repeatable framework to evaluate FPS-related motor skills in an experimental context.

NASA-TLX

Perceived workload was assessed with the NASA Task Load Index (NASA-TLX), a multidimensional tool designed to quantify subjective workload across six domains: mental demand, physical demand, temporal demand, performance, effort, and frustration. Each dimension was rated on a continuous 20-point scale, with the raw overall workload score derived from the mean of the six subscales. This approach has been widely adopted due to its practicality and sensitivity in both experimental and applied contexts (Hart and Staveland, 1988; Hart, 2006).

HR

Heart rate (HR) was continuously monitored using a Polar H10 chest-strap monitor (Polar Electro Oy, Kempele, Finland) in combination with the ECG Record for Polar H10 application (Apple App Store, iOS), which allowed the acquisition and storage of beat-to-beat recordings. The Polar H10 has been validated against electrocardiography for accurate detection of inter-beat intervals (IBI) and short-term HRV analysis (Gilgen-Ammann et al., 2019; Hernando et al., 2018). Recordings were obtained at predefined experimental phases: (i) during the psychomotor vigilance task (PVT) pre- and post-intervention, (ii) during the skill-based performance task pre- and post-intervention, and (iii) continuously throughout the 120-min intervention sessions (Play or Watch). Raw IBI series were visually inspected, and artifacts were corrected using standard criteria before analysis. Mean HR was extracted for each time window to characterize cardiovascular responses to cognitive and performance tasks as well as to prolonged gaming exposure. The inclusion of HR monitoring was based on growing evidence that e-sports activities induce significant autonomic responses, with reductions in parasympathetic modulation and increases in sympathetic activation during competitive play (Wu et al., 2025). Furthermore, motivational intensity theory highlights HR as an objective marker of mobilized mental effort (Gendolla, 2025). Finally, the acquisition and reporting of HR outcomes were aligned with the SPeCIFY guidelines (Schampheleer et al., 2025), ensuring methodological transparency and minimizing confounding factors in mental fatigue research.

Interventions

Participants completed two experimental sessions in a randomized crossover design: an active gameplay session (Play) and a passive spectating session (Watch). Each session lasted 120 minutes and was conducted on separate days, under identical laboratory conditions.

In the Play condition, participants engaged in competitive *Valorant* matches (*Riot Games, Los Angeles, USA*) using the official qualifying mode of the game client. Each match followed the competitive format, in which the first team to achieve 14 round victories was declared the winner. At the conclusion of a match, participants immediately began a new one, ensuring continuous gameplay for the entire 120-minute session. Standardized equipment was provided, including a desktop computer (144 Hz monitor, gaming mouse, and keyboard) and over-ear headphones. Ambient lighting, temperature, and noise were kept constant across participants.

In the Watch condition, participants viewed two pre-recorded professional *Valorant* matches (~60 minutes each; total duration: 120 minutes). Recordings were obtained from the Bilibili platform (Bilibili Inc., Shanghai, China) and included live commentary from one of the most popular Chinese streamers specializing in *Valorant*. Each match was played in the same competitive mode (qualifying format, first to 14 rounds won). Participants were seated individually at a fixed distance from a 24-inch monitor (144 Hz refresh rate) and used over-ear headphones to ensure immersion and minimize distractions. No interaction with the game or other participants was permitted during viewing.

Statistical Analysis

All analyses were performed using Jamovi software (Version 2.3.22.0; The Jamovi Project, Sydney, Australia). For each dependent variable, a repeated measures ANOVA was conducted with Condition (*Play vs. Watch*) and Time (*Pre vs. Post*) as within-subject factors.

For heart rate (HR), two distinct analyses were performed. First, HR recorded during the PVT and Skill Game was compared between *baseline* and *post-intervention*, with Condition as a within-subject factor. Second, HR measured continuously throughout the intervention sessions (*Play* vs. *Watch*) was analyzed using repeated measures ANOVA across predefined temporal segments to capture within-session dynamics.

All results are reported as mean ± standard deviation (SD). Effect sizes were reported as partial eta squared (η^2p), and statistical significance was set at $p < .05$ (two-tailed).

RESULTS

Cognitive Performance

Psychomotor Vigilance Task (PVT)

Response speed (RS), false starts (FS), and lapses were analyzed across condition, time, and the time × condition interaction. A significant main effect of time was observed for RS ($p = 0.004$; $\eta^2_p = 0.620$), whereas no main effect of condition ($p = 0.259$) or time × condition interaction ($p = 0.128$) was detected (Figure 33). Both interventions influenced RS without between-condition differences (Play: pre 3.99 ± 0.37 vs. post 3.60 ± 0.37; Watch: pre 3.92 ± 0.28 vs. post 3.75 ± 0.46). Neither FS nor lapses were significantly affected by time, condition, or the interaction.

Skill Game

Performance, indexed by the average number of headshots achieved during the three 30-s trials, showed a trend toward a time × condition interaction ($p = 0.073$) (Figure 34). At baseline, participants displayed comparable performance across conditions (Play: 12.4 ±

2.58; Watch: 12.3 ± 2.57). Following the intervention, headshots decreased in Play (11.4 ± 2.28), whereas a slight increase was observed in Watch (12.8 ± 2.99).

Psychological and Behavior Measures

Rating of Fatigue (ROF)

ROF scores showed significant effects of time ($p < .001$; $\eta^2_p = 0.754$) and of the time × condition interaction ($p = 0.002$; $\eta^2_p = 0.676$) (Figure 35), whereas no main effect of condition was detected. In both interventions, athletes reported higher fatigue after the session compared to baseline (Play: pre 2.9 ± 1.45 vs. post 7.1 ± 1.85; Watch: pre 5.0 ± 0.94 vs. post 6.4 ± 1.97). Post hoc analyses revealed a significant increase in Play from pre to post ($p < .001$), while the increase in Watch did not reach statistical significance ($p = 0.111$). Post-intervention fatigue did not differ between conditions ($p = 0.354$), whereas baseline scores differed significantly ($p = 0.005$).

Mood (BRUMS)

Total mood disturbance (TMD) showed significant effects of time ($p = 0.022$; $\eta^2_p = 0.460$) and of the time × condition interaction ($p = 0.011$; $\eta^2_p = 0.531$), but no main effect of condition ($p = 0.629$). Post hoc analysis indicated a significant increase in TMD from pre to post in Play ($p = 0.002$; pre −2.50 ± 5.03 vs. post 15.00 ± 11.64), while no change was observed in Watch ($p = 0.234$; pre 4.40 ± 9.49 vs. post 11.20 ± 12.65). Between-condition comparisons revealed no significant differences post-intervention ($p = 0.374$), although Play and Watch differed significantly at baseline ($p = 0.040$). (Figure 36)

Tension demonstrated a significant time × condition interaction ($p = 0.037$; $\eta^2_p = 0.400$), although no main effects were found. Scores decreased slightly in Play (pre 0.70 ± 1.16 vs. post 0.50 ± 1.08) but increased in Watch (pre 0.50 ± 1.27 vs. post 1.10 ± 1.91) (Figure 37A). Due to high inter-individual variability and the small sample size, confidence intervals occasionally extended below zero, although negative scores were not possible. Anger revealed only a trend in the time × condition interaction ($p = 0.075$) and no significant effects of time or condition (Figure 37B). Depression did not differ significantly by condition, although trends were observed for both time ($p = 0.071$) and time × condition interaction ($p = 0.052$), with values tending to increase in both interventions and reaching higher values post-intervention in Play (2.50 ± 2.88) compared to Watch (1.60 ± 2.91) (Figure 37C).

Vigor showed significant main effects of condition ($p = 0.026$; $\eta^2_p = 0.439$) and time ($p = 0.006$; $\eta^2_p = 0.584$), as well as a significant time × condition interaction ($p = 0.048$; $\eta^2_p = 0.367$). Scores decreased in both conditions, with a greater reduction in Play (pre 7.10 ± 2.73 vs. post 3.00 ± 2.62) compared to Watch (pre 3.50 ± 3.37 vs. post 2.30 ± 2.11). Post hoc analysis confirmed a significant pre–post decrease in Play ($p = 0.002$), but not in Watch ($p = 0.265$) (Figure 37D). Fatigue subscale scores showed significant main effects of time ($p = 0.008$; $\eta^2_p = 0.563$) and time × condition interaction ($p = 0.012$; $\eta^2_p = 0.521$), but no main effect of condition. Significant increases were found in Play ($p < .001$; pre 1.8 ± 1.60 vs. post 7.3 ± 3.43), whereas no differences emerged in Watch ($p = 0.221$; pre 4.5 ± 2.68 vs. post 6.5 ± 4.70). (Figure 37E).

Confusion showed a trend toward increased values post-intervention compared to pre ($p = 0.056$), with higher scores after both interventions (Play: pre 1.8 ± 1.48 vs. post 4.8 ± 4.27; Watch: pre 1.5 ± 2.01 vs. post 3.0 ± 2.98), but no significant main effects or interactions were detected. (Figure 37F).

Multidimensional State Boredom Scale (MSBS)

Inattention scores showed a significant main effect of time ($p = 0.014$; $\eta^2_p = 0.508$), with values increasing from pre- to post-intervention in both conditions (Play: pre 6.10 ± 2.28 vs. post 9.8 ± 3.94; Watch: pre 7.10 ± 2.38 vs. post 9.8 ± 4.44) (figure 38). No main effect of condition ($p = 0.502$) or time × condition interaction ($p = 0.515$) was found. Time perception, disengagement, low arousal, and overall boredom scores were not significantly affected by time, condition, or interaction. Average boredom scores showed small changes but high variability (Play: pre 34.5 ± 6.58 vs. post 42.0 ± 24.41; Watch: pre 35.4 ± 12.40 vs. post 41.9 ± 19.89) (Figure 39).

NASA Task Load Index (NASA-TLX)

Overall workload was significantly higher in Play (12.70 ± 2.85) compared to Watch (9.57 ± 3.35) ($p < .001$; $\eta^2_p = 0.797$) (Figure 40). Among the subscales, significant differences emerged for Mental Demand ($p < .001$; $\eta^2_p = 0.723$), Physical Demand ($p = 0.004$; $\eta^2_p = 0.630$), and Effort ($p = 0.027$; $\eta^2_p = 0.435$), with consistently higher scores in Play. Performance showed only a trend ($p = 0.091$), while Frustration ($p = 0.160$) and Temporal Demand ($p = 0.604$) did not differ between conditions (Figure 41).

Summary of Statistical Outcomes

Significant main effects and interactions from repeated measures ANOVAs are summarized in Tables 18–19. Estimated marginal means are presented as mean ± SD, with post hoc comparisons reported where relevant.

DISCUSSION

The present study investigated the psychophysiological effects of a two-hour competitive *Valorant* session, comparing active play against passive spectating. Our findings indicate that, while both activities were associated with cognitive and psychological changes, the Play condition elicited greater subjective fatigue, mood disturbance, and perceived workload compared with Watch. These results reinforce the growing evidence that esports competition represents a substantial psychophysiological stressor, even in the absence of physical exertion (Andre et al., 2020; Palanichamy et al., 2021; Ketelhut and Nigg, 2024; Leis et al., 2024).

Both the Play and Watch conditions were designed to reflect authentic esports experiences. Active gameplay requires motor precision, cognitive flexibility, and sustained attention, while spectating involves perceptual processing, emotional engagement, and cognitive appraisal of strategy. Previous research has shown that esports spectatorship itself elicits psychological and behavioral responses, including arousal, engagement, motivation, and social identification, making it a valid comparison to active play (Jenny et al., 2017; Qian et al., 2020; Hamari and Sjöblom, 2017; Pizzo et al., 2018). Thus, including a passive viewing condition enhances ecological validity and provides insight into how different forms of esports exposure—playing versus watching—affect cognitive, emotional, and physiological outcomes.

Cognitive outcomes revealed modest differences across conditions. Performance in the Psychomotor Vigilance Task (PVT) declined from pre- to post-assessment in both conditions, although without significant differences between Play and Watch. Response speed tended to be slower after gameplay, whereas spectating was largely neutral. A similar trend emerged in the shooting accuracy task: the number of headshots decreased slightly following Play, while a very modest, non-significant increase was observed after Watch. These very small and opposite results reflect the ongoing debate regarding the relationship between esports and cognition. These very small and partially opposite effects indicate that the cognitive impact of both active play and spectating is not unidirectional. While some aspects of performance tended to deteriorate after gameplay (e.g., vigilance and shooting accuracy), others remained largely unchanged or showed minimal variation across conditions. This pattern suggests that short-term exposure to esports-related activities may differentially affect specific cognitive processes rather than producing uniform improvements or impairments (Sala et al., 2018).

Importantly, this mixed profile is consistent with the broader and still debated literature on video games and cognition, which reports heterogeneous findings depending on task characteristics, exposure duration, and individual differences. Accordingly, the modest and domain-specific changes observed here are more plausibly interpreted as reflecting subtle, task-dependent modulation—potentially influenced by transient mental fatigue—rather than robust or generalized cognitive effects (Sala et al., 2018; Bediou et al., 2018; Bediou et al., 2023).

It is important to note that the present study assessed only the acute effects of a single 120-minute session, which are not directly comparable to the long-term influences of habitual video gaming. Nevertheless, our findings highlight the complexity of interpreting the relationship between esports and cognition. Despite clear perceptions of fatigue and workload after Play, accompanied by slower PVT responses, no drastic decline was observed in motor skill performance. Conversely, the slight, non-significant improvement in shooting accuracy after Watch is noteworthy. When considered as an externally focused task—where attention may be directed toward observing the streamer's tactical choices rather than one's own gameplay—these results align with recent evidence showing that external focus does not necessarily improve accuracy in FPS tasks (Lamers et al., 2023). This stands in contrast to earlier claims that performance and learning advantages induced by an external focus generalize across task types, skill levels, and age groups (Wulf et al., 2013).

The concept of perceived fatigue was another central focus of this study. Results from the Rating of Fatigue (ROF) and the Brunel Mood Scale (BRUMS) showed robust differences. Fatigue and total mood disturbance increased significantly after Play but not after Watch. These findings support the Model of Neuroendocrine and Mood Responses to a Competitive Situation (Salvador and Costa, 2009), which links competition with acute psychophysiological stress responses. No post-intervention differences emerged between conditions, which may be attributed to baseline disparities; the Watch condition was tested in the evening, whereas Play occurred in the late afternoon. As this analysis is based on pilot data, ongoing data collection is expected to address these design inconsistencies. Beyond global scores, Play was associated with changes in specific subscales. In particular, vigor declined and fatigue increased significantly, whereas anger and depression only showed a trend toward increase in the time × condition interaction. Confusion, by contrast, displayed a trend across time but not in the time × condition interaction. Notably, tension tended to decrease following Play, while it increased after Watch. Although post hoc analyses did not reveal significant effects, an overall time × condition interaction was observed. It is plausible that tension—often considered a marker of musculoskeletal pain and anxiety-related states such as nervousness, panic, and worry (Brandt et al., 2016)—was particularly elevated in the post-Watch condition due, at least in part, to the timing of the experimental sessions. Specifically, assessments in the Watch condition were conducted later in the evening compared with the Play condition, resulting in the post-test being closer to participants' habitual bedtime.

In line with this interpretation, previous evidence indicates that better sleep quality is positively associated with lower levels of tension (Andrade et al., 2016). Accordingly, evening assessments may have captured additional variability related to circadian or sleep–wake regulation processes rather than effects attributable solely to the experimental manipulation.

The NASA-TLX results provide the clearest evidence that active gameplay imposed a greater workload than spectating. Gameplay yielded higher overall workload scores, particularly for mental demand, physical demand, and effort. These findings are consistent with recent evidence showing that active gaming tasks are cognitively demanding, irrespective of player population (Belluccio et al., 2025; Yu and Chan, 2024). Both Play and Watch generated a perceptible workload, but at different magnitudes. In line with Grier (2015), workload assessment should be interpreted relative to the type of activity. Based on these benchmarks, Play in our study reached the "high workload" range (>75% of reference tasks), while Watch fell within the "moderate workload" range (>25%), despite being passive. It should be noted that Grier's dataset was based on games very different from current eSports titles, limiting direct comparability.

A major strength of the present study lies in its experimental design, which for the first time directly compared active esports participation with passive spectating under ecologically valid competitive conditions. By employing a standardized 120-minute *Valorant* qualifying session, the study ensured external validity and relevance to competitive gaming. Another strength was the multimodal assessment of outcomes, including objective measures of cognitive performance, subjective workload, and mood states, with heart rate monitoring planned for the full dataset. Furthermore, the within-subjects design reduced interindividual variability and enhanced sensitivity to detect condition-related changes.

Several limitations should be acknowledged. The sample was modest and limited to amateur participants, restricting generalizability to professional esports players. However, participants reported substantial prior gaming exposure, with a mean gaming experience of 6.7 years (SD = 3.06), an average of 27.3 hours of gameplay per week (SD = 12.83), and 9.0 hours of weekly game-related viewing (SD = 10.41). The study was confined to a single title (*Valorant*) and one version of the game, potentially limiting extrapolation to other genres or game modes. Additional limitations of the pilot design include the absence of randomization and differences in testing times between conditions. It is also important to note that the present discussion is based on pilot data; a follow-up study is currently underway with finalized data collection to strengthen the robustness of these findings. Finally, the present work addressed only the acute effects of a single competitive session; longitudinal studies are warranted to examine cumulative fatigue, adaptation, and the risk of burnout across repeated play.

CONCLUSION

In conclusion, a two-hour competitive *Valorant* session significantly increased fatigue, mood disturbance, and workload among players, while spectating produced milder effects. These data contribute to a growing body of evidence demonstrating that esports competition is cognitively and physiologically demanding, with potential implications for performance, recovery, and long-term health. The present findings further support the conceptualization of esports as a sport, as competitive gaming induces clear psychophysiological and behavioral changes. Moreover, the results observed in the spectating condition also revealed measurable emotional and social impact, underscoring the broader influence of esports on audiences.

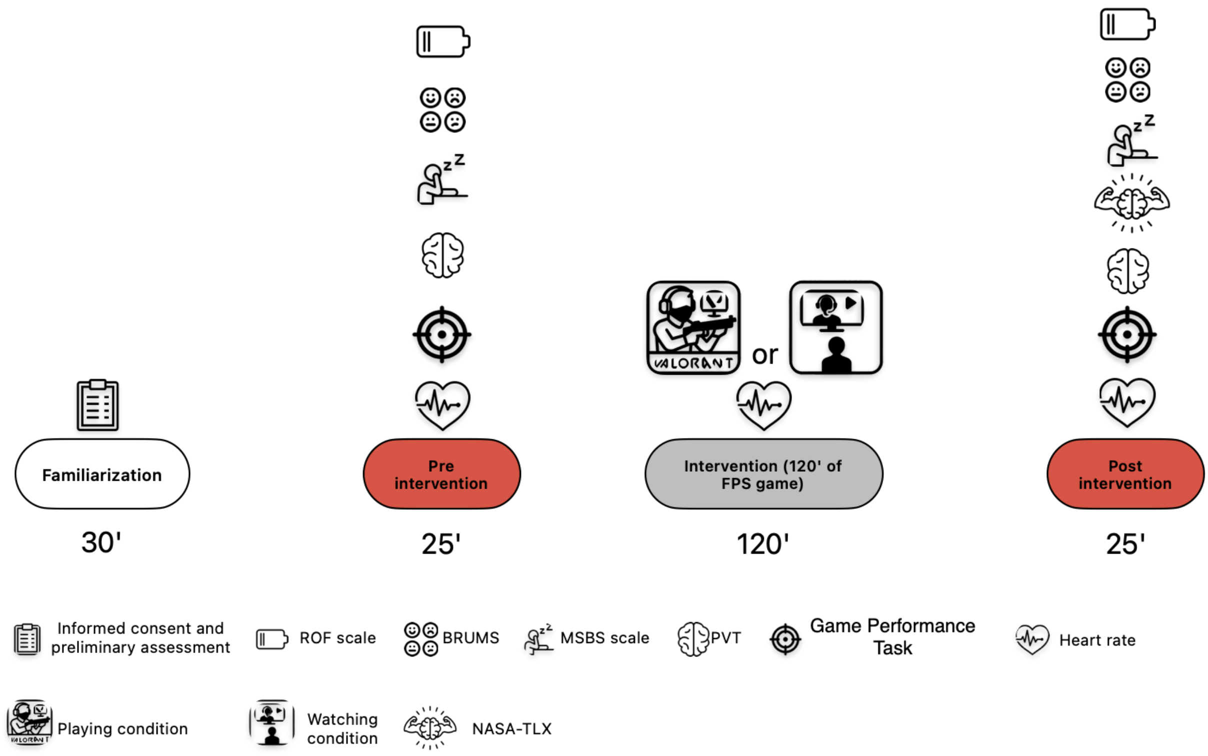


Figure 32. Experimental protocol.

The study consisted of four phases: familiarization, pre-intervention, intervention, and post-intervention. During familiarization (30 min), participants provided informed consent and completed preliminary assessments. The pre-intervention phase (25 min) included baseline measurements of fatigue (ROF), mood (BRUMS), multidimensional state of boredom (MSBS), vigilance (PVT), game performance, and heart rate. The intervention consisted of a 120-min session in one of two conditions, active play (*Valorant*) or passive spectating, during which heart rate was continuously monitored. In the post-intervention phase (25 min), all baseline assessments were repeated and the NASA-TLX scale was added to evaluate perceived workload. The bottom panel illustrates the icons representing each assessment and condition.

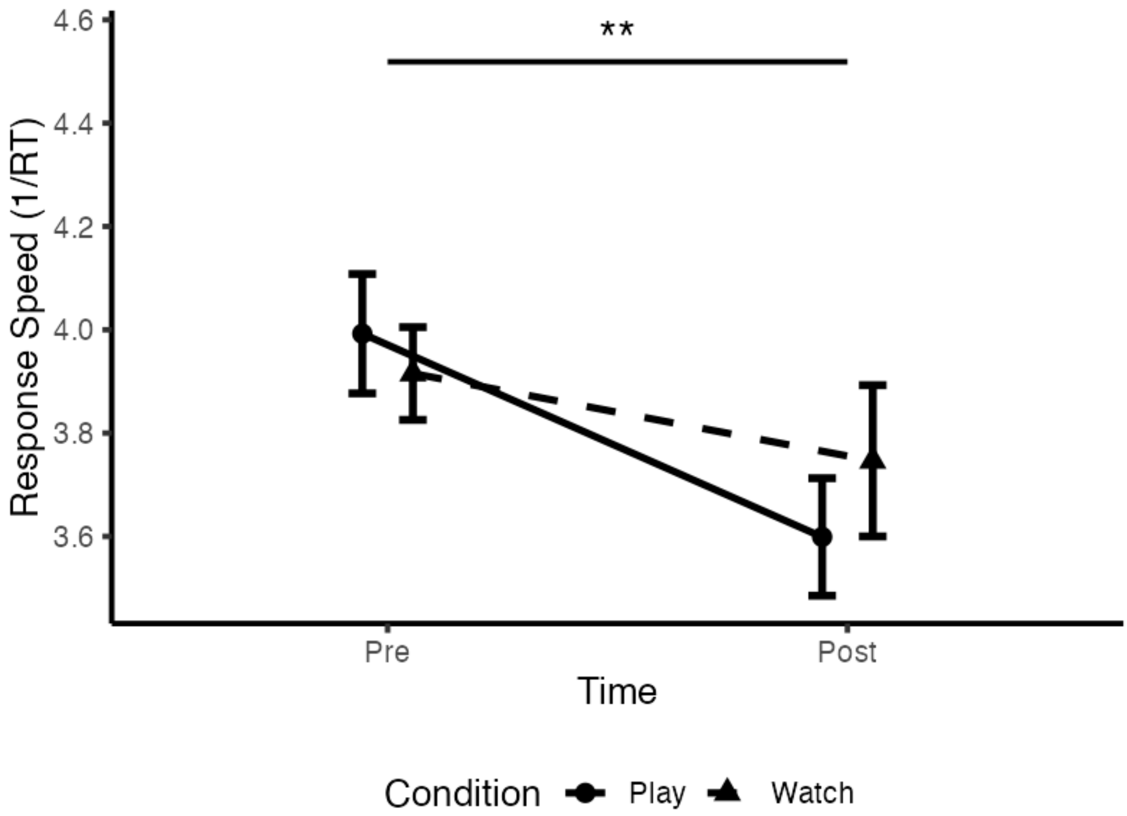


Figure 33. Response speed (1/RT) during the Psychomotor Vigilance Task (PVT) across time (pre vs. post) in the play and watch conditions. Both groups exhibited slower responses post-intervention, with a more marked decline following play. Values are estimated marginal means ± SE.

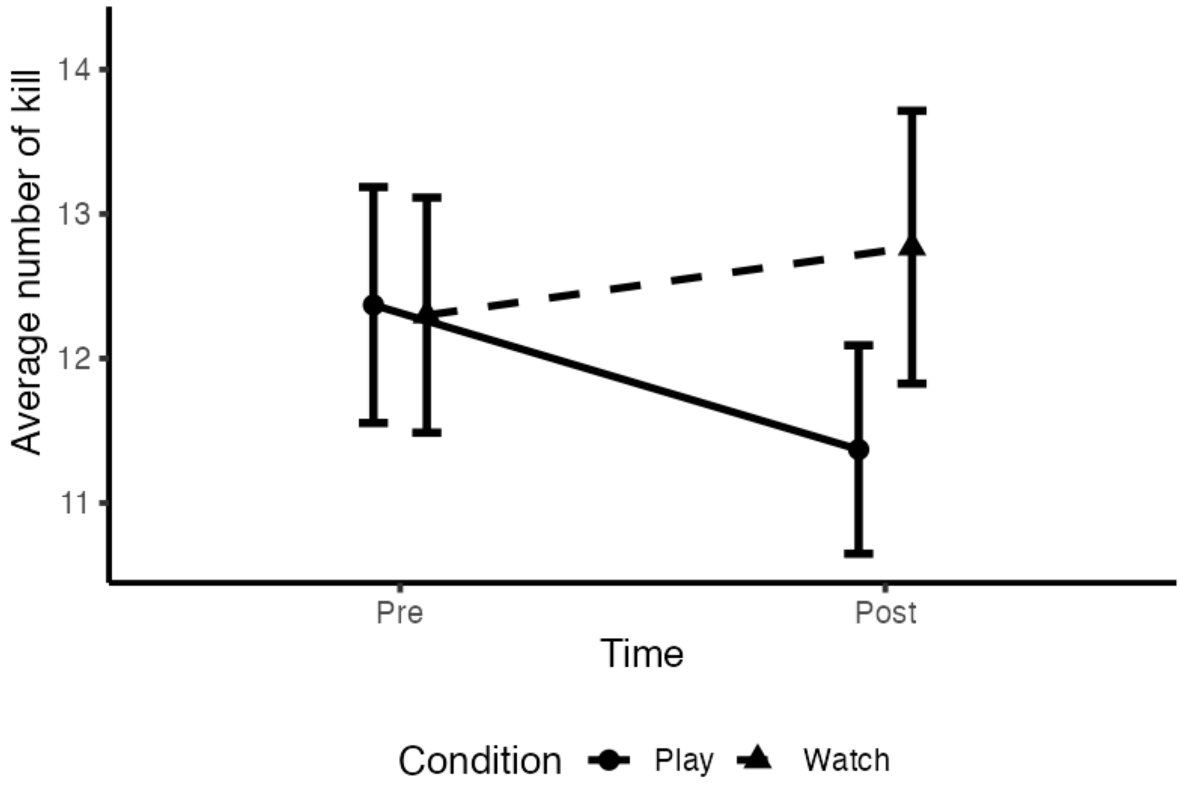


Figure 34. Average number of kills in the game performance task across time (pre vs. post) and condition (play vs. watch).

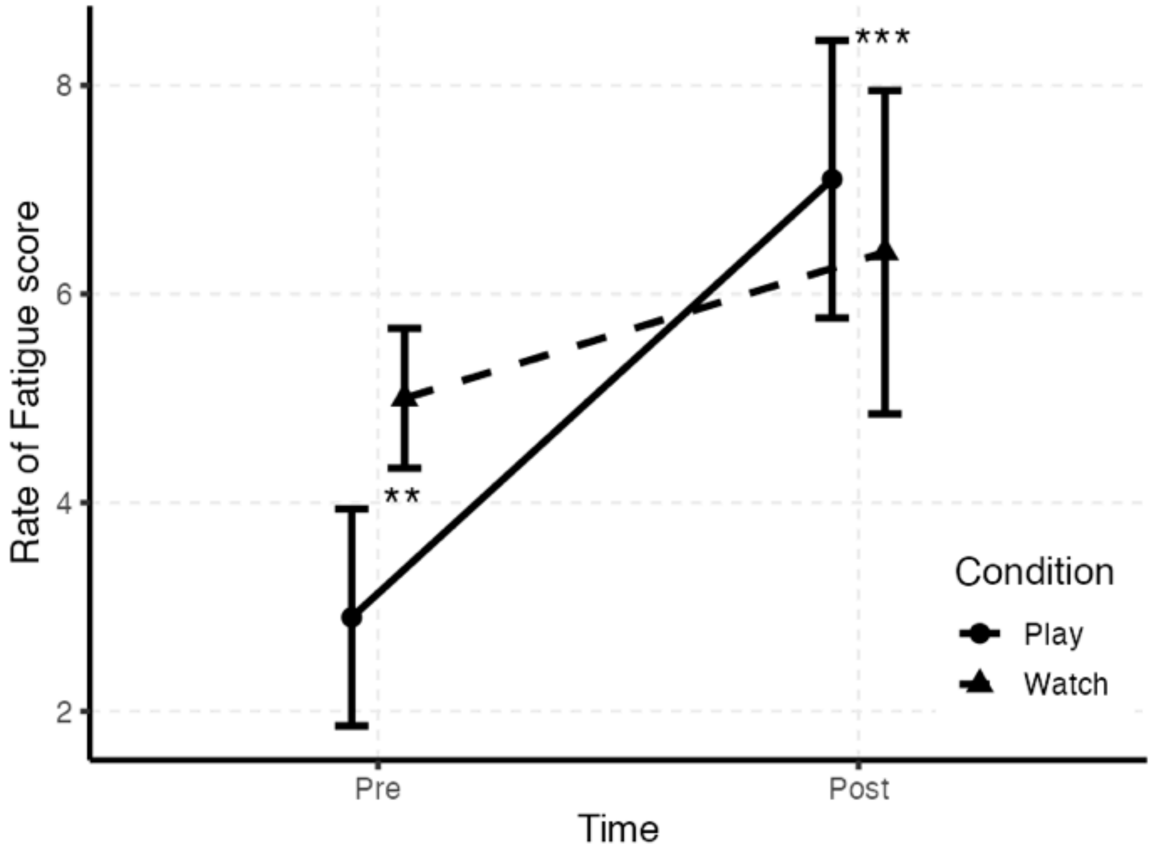


Figure 35. Rate of fatigue (ROF) across time (pre vs. post) in the play and watch conditions.

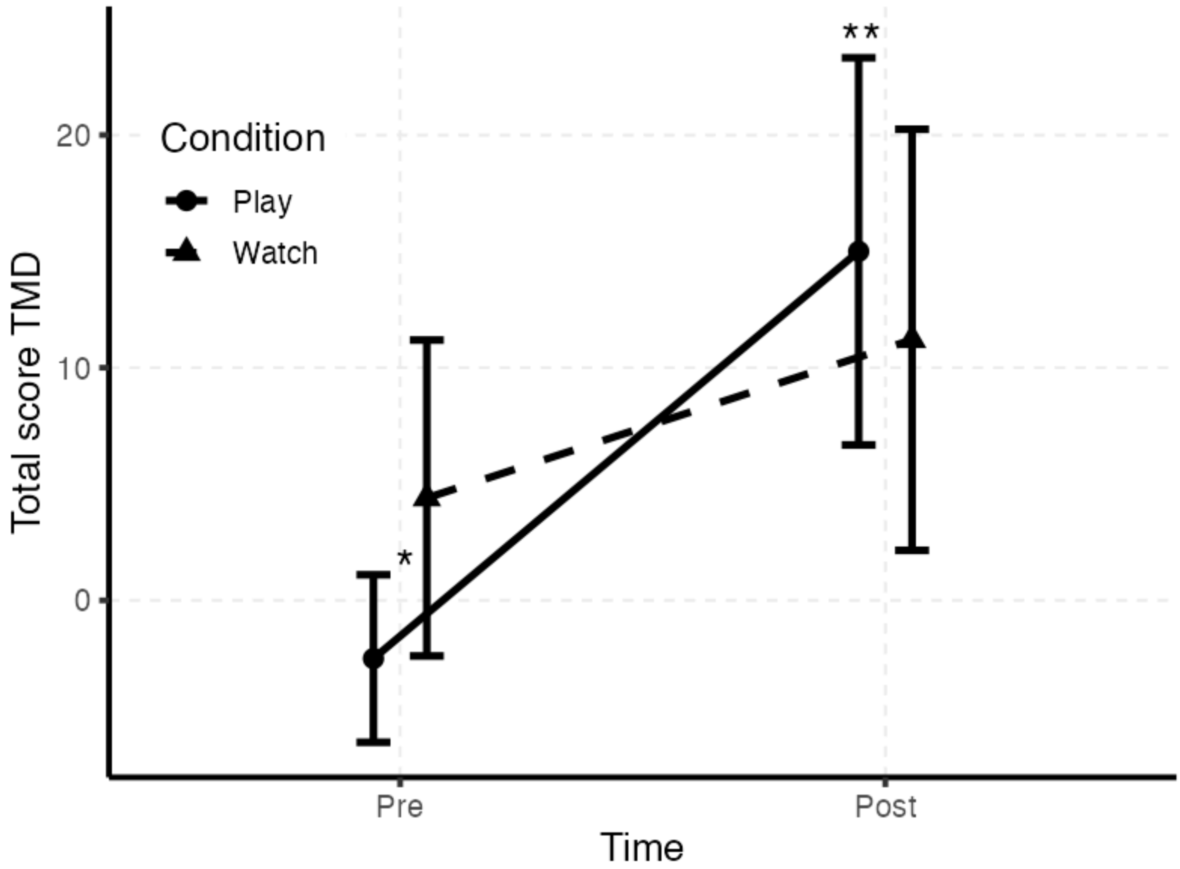


Figure 36. Total Mood Disturbance (TMD) across time (pre vs. post) in the play and watch conditions.

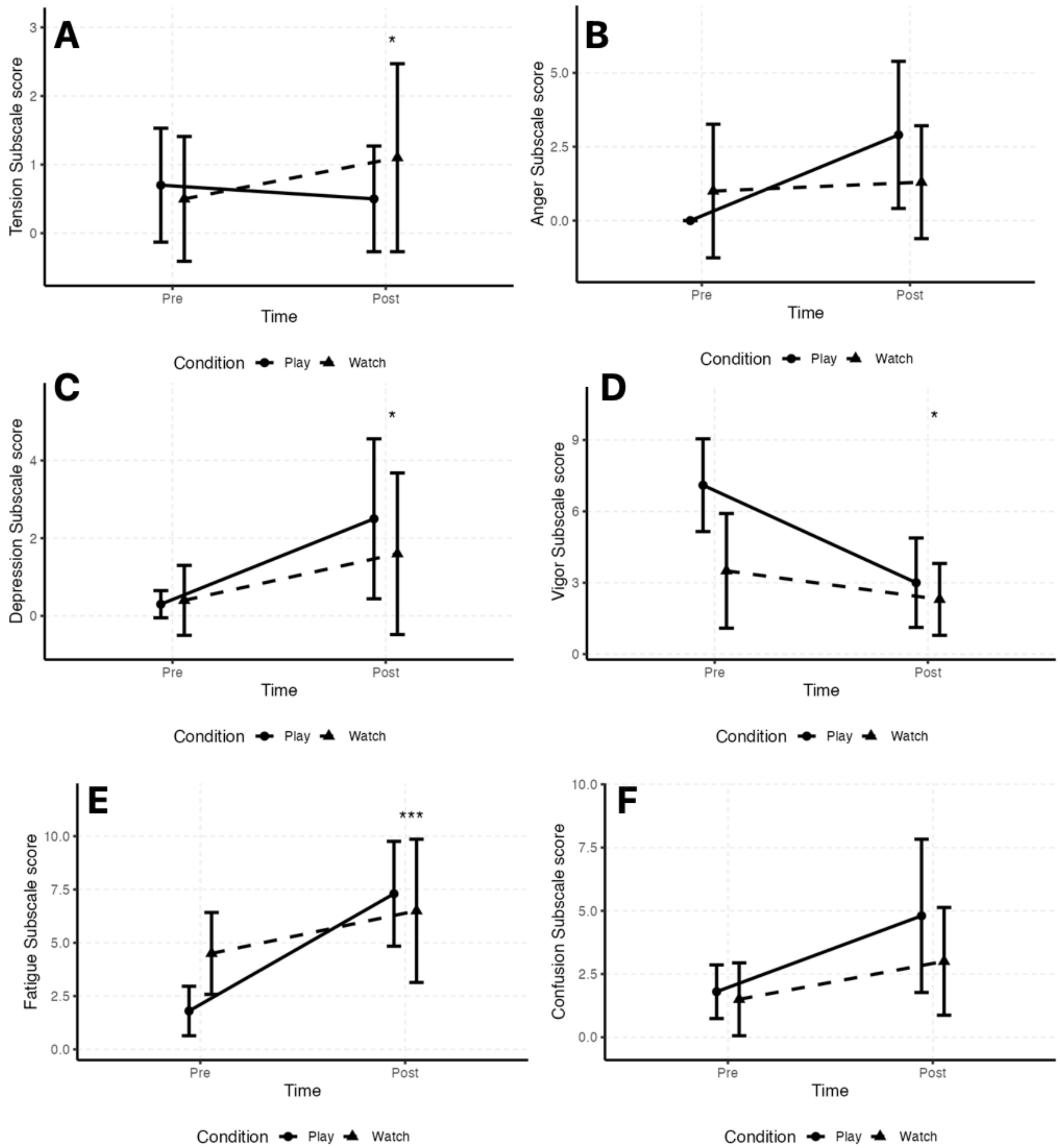


Figure 37. Subscales scores across time (pre vs. post) in the play and watch conditions. (A) Tension subscale values (B) Anger subscale values. (C) Depression subscale values (D) Vigor subscale values (E) Fatigue subscale values (F) Confusion subscale values. All Values are estimated marginal means ± SE.

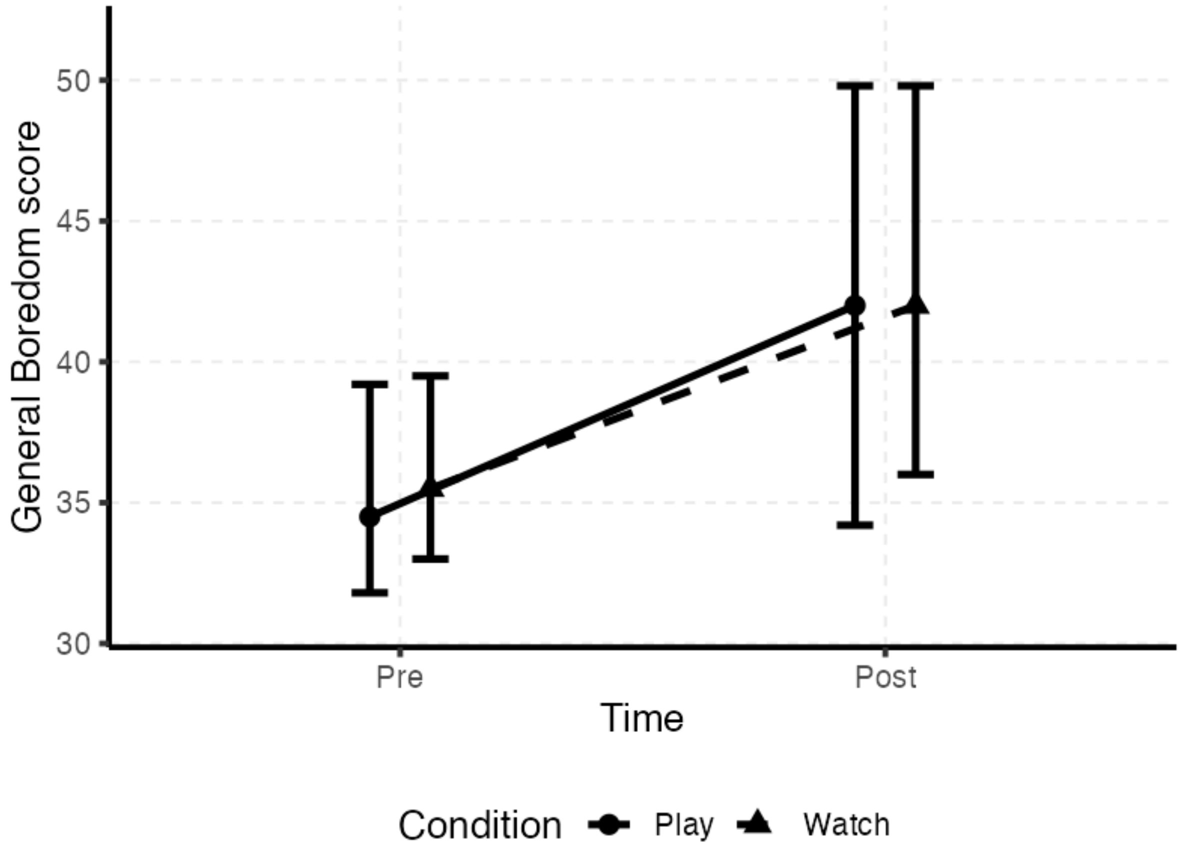


Figure 38. General boredom scores across time (pre vs. post) in the play and watch conditions.

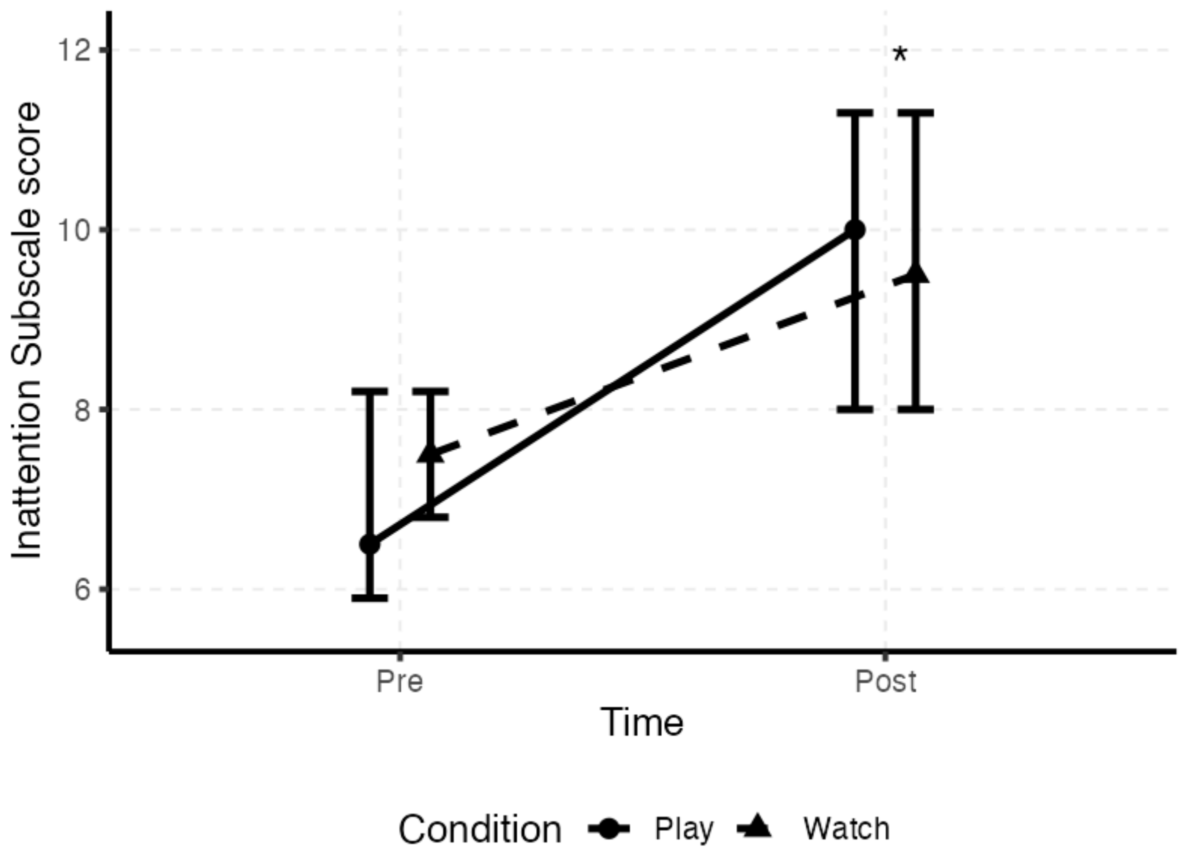


Figure 39. Inattention subscale scores across time (pre vs. post) in the play and watch conditions.

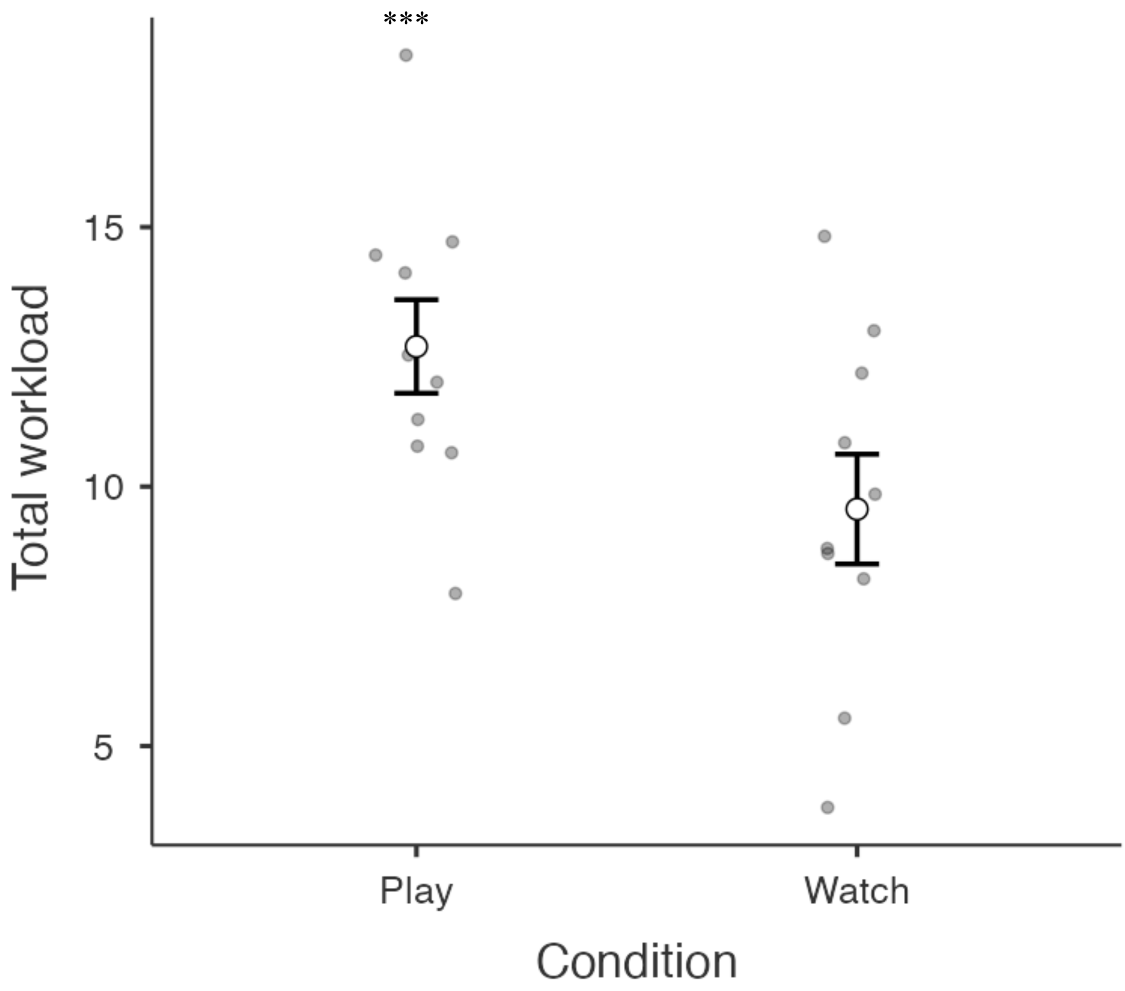


Figure 40. Total workload across conditions.

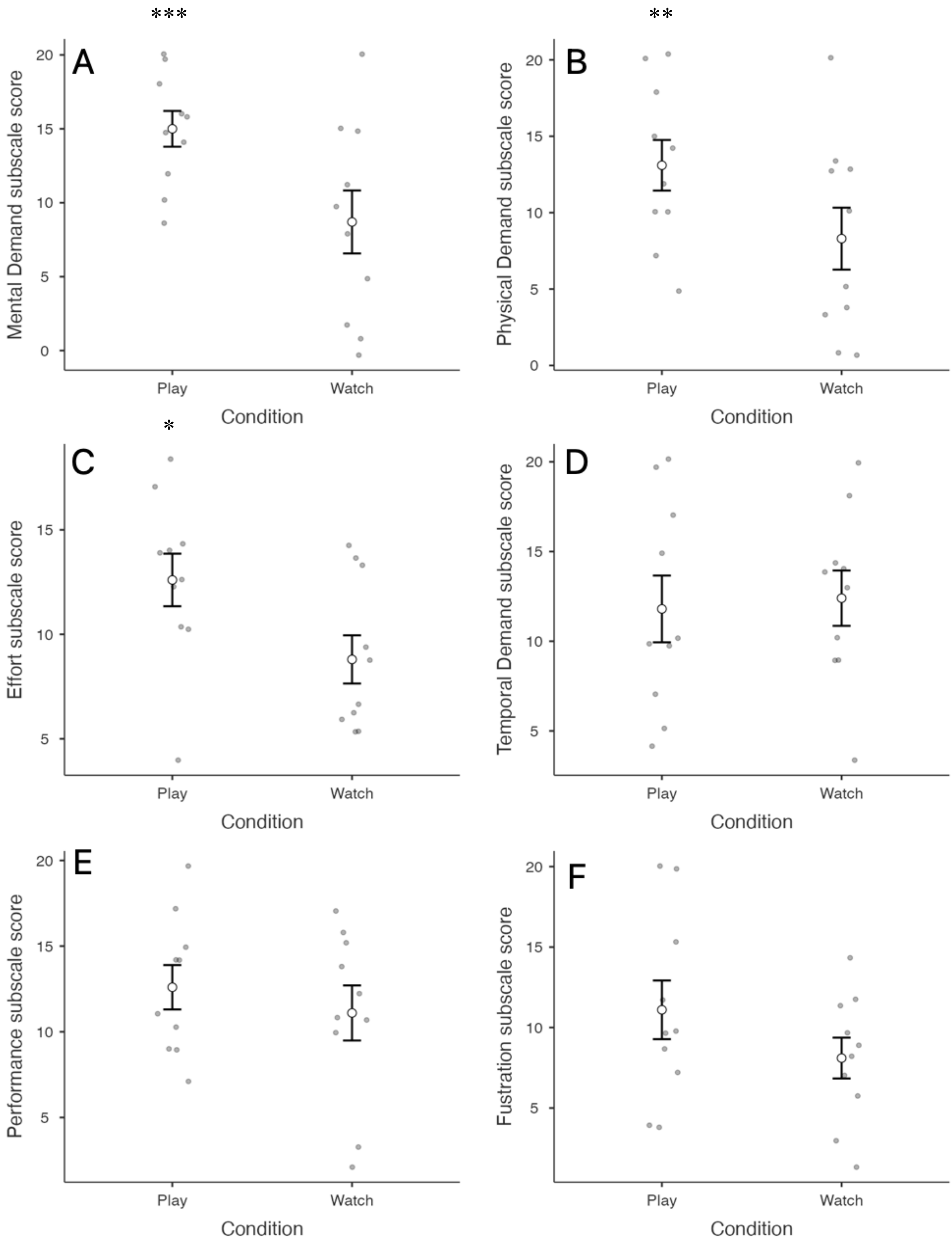


Figure 41. Demand subscale scores across conditions.

(A) Mental demand subscale scores (B) Physical demand subscale scores (C) Effort subscale scores (D) Temporal demand subscale scores (E) Performance subscale (F) Frustration subscale scores.

All values are estimated marginal means ± SE.

Variable	Comparison	Mean ± SD (Play)	Mean ± SD (Watch)	p	η^2□
Response Speed 1/RT	Time	3.99 ± 0.37	3.60 ± 0.37	**0.004**	**0.620**
Skill Test (Kills)	Time x condition			0.073	
	Pre vs Post Play	12.4 ± 2.58 11.4 ± 2.28			
	Pre vs Post Watch		12.3 ± 2.57 12.8 ± 2.99		
ROF	Pre vs Post Play	2.9 ± 1.45 7.1 ± 1.85		< .001	
	Pre vs Post Watch		5.0 ± 0.94 6.40 ± 1.97	0.111	
	Post Play vs Post Watch	7.1 ± 1.85	6.40 ± 1.97	0.354	
	Pre Play vs Pre Watch	2.9 ± 1.45	5.0 ± 0.94	0.005	
BRUMS – TMD	Pre vs Post Play	−2.5 ± 5.03 15.0 ± 11.64		**0.002**	
	Pre vs Post Watch		4.40 ± 9.49 11.20 ± 12.65	0.234	
	Post Play vs Post Watch	15.0 ± 11.64	11.2o ± 12.65	0.374	
	Pre Play vs Pre Watch	−2.5 ± 5.03	4.40 ± 9.49	**0.040**	

BRUMS – Tension	Pre vs Post Play	0.7 ± 1.16 0.5 ± 1.08		0.662
	Pre vs Post Watch		0.5 ± 1.27 1.1 ± 1.91	0.382
	Post Play vs Post Watch	0.5 ± 1.08	1.1 ± 1.91	0.217
	Pre Play vs Pre Watch	0.7 ± 1.16	0.5 ± 1.27	0.662
BRUMS – Vigor	Pre vs Post Play	7.1 ± 2.73 3.0 ± 2.62		**0.002**
	Pre vs Post Watch		3.5 ± 3.37 2.3 ± 2.11	0.256
	Post Play vs Post Watch	3.0 ± 2.62	2.3 ± 2.11	0.553
	Pre Play vs Pre Watch	7.1 ± 2.73	3.5 ± 3.37	**0.003**
BRUMS – Fatigue	Pre vs Post Play	1.8 ± 1.60 7.3. ± 3.43		**< .001**
	Pre vs Post Watch		4.5 ± 2.68 6.5 ± 4.70	0.221
	Post Play vs Post Watch	7.3. ± 3.43	6.5 ± 4.70	0.599
	Pre Play vs Pre Watch	71.8 ± 1.6	4.5 ± 2.68	**0.013**

BRUMS – Anger	time x condition			0.075
	Pre vs Post Play	0.0 ± 0.0 2.9 ± 3.5		
	Pre vs Post Watch		1.0 ± 3.2 1.3 ± 2.7	
BRUMS – Depression	time x condition			0.052
	Pre vs Post Play	0.3 ± 0.5 2.50 ± 2.88		
	Pre vs Post Watch		0.4 ± 2.3 1.60 ± 2.91	
Boredom – Inattention	Pre vs Post Play	6.10 ± 2.28 9.8 ± 3.94		0.014
	Pre vs Post Watch		7.1 ± 2.38 9.8 ± 4.44	0.077
	Post Play vs Post Watch	9.8 ± 3.94	9.8 ± 4.44	1.000
	Pre Play vs Pre Watch	6.10 ± 2.28	7.1 ± 2.38	0.237

Tab. 18 Post hoc comparisons are reported for variables in which a significant Time × Condition interaction was detected (PVT Response speed, Skill ability, ROF, Boredom–Inattention, and BRUMS subscales: Vigor, Fatigue, TMD). For variables showing only a statistical trend, marginal means and standard deviations are presented. * $p < .05$; ** $p < .01$; *** $p < .001$

Subscale	Play (Mean ± SD)	Watch (Mean ± SD)	p	η^2□
Overall Workload	12.7 ± 2.85	9.57 ± 3.35	**< .001**	0.797
Mental Demand	15.0 ± 3.8	8.7 ± 6.7	**< .001**	0.723
Physical Demand	13.1 ± 5.2	8.3 ± 6.4	**0.004**	0.630
Temporal Demand	11.8 ± 5.9	12.4 ± 4.9	0.604	
Performance	12.6 ± 4.1	11.1 ± 5.1	0.091	
Effort	12.6 ± 4.0	8.8 ± 3.6	**0.027**	0.435
Frustration	11.1 ± 5.7	8.1 ± 4.0	0.160	

Tab. 19 NASA-TLX subscale scores (Mean ± SD) are presented for the Play and Watch conditions, together with the results of between-condition comparisons (*p* values). Where significant, the associated partial eta squared (η^2□) effect size is also reported. * $p < .05$; ** $p < .01$; *** $p < .001$

CHAPTER 5

A RANDOMIZED CONTROLLED TRIAL OF VEILLONELLA ATYPICA FB0054 SUPPLEMENTATION ON ENDURANCE PERFORMANCE AND QUALITY OF LIFE IN ADULT AMATEUR CYCLISTS

TULLIO SENATORE, DANIELA PINTO, SAMUELE MARCORA

INTRODUCTION

The human gut microbiome is linked to many states of human health and disease. Elite athletes have a higher number of certain types of bacteria in their gut microbiomes (the commensal microbes populating the intestinal tract) that support or perhaps drive optimal levels of performance and recovery during and after challenging athletic events (O'Brien et al., 2022).

A proof-of-concept study recently published in Nature Medicine (Scheiman et al., 2019) showed that a specific bacterial strain of Veillonella bacteria, *V. atypica* was amplified in the gut microbiomes of 2015 Boston Marathon runners after the race. The same strain, when given to mice, enhanced the animals' running performance on a treadmill by 13 percent.

Preliminary results from pre-clinical functional testing in animal models indicate these probiotic candidates clear blood lactate and reduce inflammation in mice after exercise. Despite the reference genomes on National Center for Biotechnology Information (NCBI) , Scheiman et al. in the study mentioned above validated the production of propionate via mass spectrometry on isolates of these species. It appears, indeed, that *V. atypica* can convert lactate in the gastrointestinal tract to short-chain fatty acids (SCFAs), especially propionate. V. Atypical is not the only genera that can metabolize the SCFA but is the only one to do so through the methylmalonyl-CoA15 pathway. The use of this metabolic pathway distinguishes this genera from others that are theoretically able to use lactate through lactate dehydrogenase but do not possess the complete pathway to convert lactate to propionate (Cristoni et al., 2019). The effect of SCFAs on performance has not yet been demonstrated, and it should be understood whether there is an optimal level of SCFA concentration that can improve performance and/or recovery during exercise (Bongiovanni et al., 2021).

However, there is evidence to support the role of SCFAs as metabolic molecules regulating host cellular physiology by observing act at different organ and tissue sites (Morrison et al., 2016).

The potential beneficial effects of SCFAs are not limited to athletes but to a general population whether they are trained, active or sedentary. Recently, a study on the effect of a multi-strain Lactobacillus probiotic showed modulation of sleep quality, recovery from exercise and the composition of the gut microbiome in both the general population and elite athletes. (Bongiovanni et al., 2025)

Objective

The primary aim of this study is to assess the effect of 4-week supplementation with "Veillonella atypica FB0054" strain on endurance performance in adult amateur cyclists. The effect of such supplementation on lactate production and clearance will also be investigated as a potential mechanism for the hypothesised positive effect on endurance performance.
In addition, the effects of "Veillonella atypica FB0054" supplementation on quality of life, microbial dysbiosis index, microbiome composition and cognitive function will be explored as these effects are also relevant to the health of the general population.

METHODOLOGY

Study design

This study will be a randomized, double-blind, pretest–posttest controlled trial with matched allocation. Forty participants will be recruited and randomized in a one-to-one ratio into two treatment arms; sample number was calculated with GPower from the results observed in the study by Scheiman et al., 2019. Matching will be performed by age, sex, and performance level in order to minimize the effect of confounding variables. Performance level will be categorized following the criteria of McKay et al. (2022) , distinguishing between recreational or local competitors (Tier 1–2) and national or international competitors (Tier 3–5). Age will be stratified into two groups, 18–45 years and 46–60 years, since evidence shows an accelerated decline in cardiorespiratory fitness after 45 years (Jackson et al., 2009).. Randomization will be conducted using sealed envelopes prepared by an independent statistician, and allocation concealment will remain in place until after statistical analyses are completed.
Participants in the intervention arm will take one capsule of *Veillonella atypica* FB0054 containing 10 billion CFU each morning after breakfast, while those in the placebo arm will consume an identical capsule containing potato starch. All capsules will be identical in appearance, weight, and color. The treatment period will last four weeks (28 days). The protocol has been approved by the Independent Ethics Committee for Non-Pharmacological Clinical Trials (*Rif*: 2025/13).

Participants

Inclusion criteria

Eligible participants will be men and women aged 18–60 years who are accustomed to regular cycling. They must perform at least 150–300 minutes of moderate-intensity exercise or 75–150 minutes of vigorous-intensity exercise weekly, according to World Health Organization guidelines. Participants must agree to comply with the study instructions, attend laboratory visits as scheduled, and refrain from using any pharmacological treatments that could interfere with the study outcomes. They must not currently be enrolled in, or have participated in, another clinical trial within the past month. Written informed consent and a privacy agreement will be obtained before inclusion.

Exclusion criteria

Exclusion criteria include pre-existing gastrointestinal disorders such as irritable bowel syndrome, recent use of antibiotics (within the last month), or current use of probiotic supplements. Women who are pregnant, breastfeeding, or planning pregnancy during the study period will not be eligible. Participants with recent severe infections, major medical disorders, significant alcohol or drug abuse, or psychiatric conditions that may compromise adherence will also be excluded. Those who do not meet WHO activity guidelines or present medical conditions judged by the investigators as incompatible with safe participation will also be excluded.

Procedures

Participants will attend four laboratory visits, two before and two after the intervention. At baseline, the screening and familiarization visit (V0A) will include eligibility confirmation, anthropometric measurements, a ramp test for VO_2peak detection, and familiarization with Borg's 6–20 Rating of Perceived Exertion scale (Borg, 1998). Within 48 hours, a second pretest visit (V0B) will be conducted, including endurance performance tests, Wingate assessment, cognitive and psychological testing, and collection of biological samples. After four weeks of supplementation, the posttest phase will begin. The first post-intervention visit (V1A) will include reassessment of cardiorespiratory fitness and VO_2peak, while the final visit (V1B), scheduled within 48 hours, will repeat the endurance, cognitive, psychological, and biological assessments. This spacing ensures adequate recovery between maximal exercise tests.

During the study, participants will complete a training and dietary diary, including type and duration of activity, session RPE, and dietary records based on the Italian Food Frequency Questionnaire (Buscemi et al., 2025). Use of antibiotics or other interfering medications during the intervention will result in withdrawal from the study.

Assessments

Performance and physiology

Cardiorespiratory fitness will be assessed through a ramp test protocol starting at 70 W with continuous increments until volitional exhaustion or cadence failure. Anaerobic power and lactate responses will be evaluated with a 30-second Wingate test at 7.5% body mass resistance (Dotan & Bar-Or, 1983), while lactate kinetics will be monitored with repeated blood sampling (Weinstein et al., 1998). Endurance performance will be measured through a 10-minute constant workload test at 70% of peak power output, with lactate values at the fifth and tenth minute used to calculate predicted time to exhaustion following the equation described by Sassi et al. (2006). A five-kilometer time trial will also be performed as a validated measure of endurance capacity and proxy for maximal lactate steady state velocity (Campbell et al., 2007; Swensen et al., 1999; Harnish et al., 2001).

Cognitive and psychological measures

Executive function will be assessed with the Stroop task, while sustained attention and vigilance will be measured with the Rapid Visual Processing task (Chung et al., 2014). Psychological state will be evaluated with the Italian Mood Scale, administered during VO_2peak visits and weekly thereafter, as well as the Profile of Mood States and the General Sleep Disturbance Scale (Galeoto et al., 2023). Fatigue will be quantified with the

Rating-of-Fatigue scale (Micklewright et al., 2017) at baseline, during, and after endurance and Wingate tests, while perceived exertion will be measured with Borg's 6–20 (Borg, 1998) scale during the predicted time-to-exhaustion test.

Microbiome and metabolic profiling

Stool samples collected at baseline and post-intervention will be analyzed using 16S rRNA sequencing and shotgun metagenomics. Short-chain fatty acids will be quantified from stool extracts. Urine samples will be analyzed using LC-SASI-MS/MS to identify metabolic signatures of dysbiosis, including skatole, indican, and major bacterial metabolites (Cristoni et al., 2017; Hebert et al., 2018; Ng et al., 1971; Jager et al., 2019).

OUTCOMES

The primary outcomes will include endurance performance measured through VO_2peak, time trial results, and predicted time to exhaustion, as well as lactate accumulation and clearance and quality of life assessed by a validated 46-item questionnaire. Secondary outcomes will encompass sleep quality, fatigue perception, executive function, sustained attention, perceived exertion, and mood, alongside microbiota composition, short-chain fatty acid levels, and urinary dysbiosis profiles.

Safety and tolerability

Safety and tolerability will be assessed through continuous monitoring of adverse events and periodic physical examinations. Participants who require rescue or confounding medications during the trial will be withdrawn.

CHAPTER 6

GENERAL DISCUSSION

MAIN FINDINGS

This general discussion critically integrates the results of the studies conducted in this thesis, examining fatigue across its physical, mental, and cognitive dimensions and considering key modulators: motivation and boredom, circadian rhythms, and, finally, the potential influence of the gut microbiota on performance and perceived exertion. Overall, converging evidence indicates that fatigue is a multidimensional phenomenon in which peripheral and central neurophysiological mechanisms interact with perceptual, affective, and motivational factors (Wolff et al., 2021; Hopstaken et al., 2015; Giboin & Wolff, 2019; Marcora et al., 2009; Pattyn et al., 2018). Taken together, the three studies suggest that the boundaries between physical, mental, and cognitive fatigue are largely functional rather than contextual. The e-sport findings, in particular, highlight that even in the absence of muscular work, attentional engagement and motivational control remain decisive determinants of performance and perceived exertion.

The Boredom role

Starting from a theme that was not part of the initial hypotheses but increasingly captured my interest, we consider boredom: an aversive affective state defined by difficulty engaging attention despite having the motivation to do so. That is, the individual intends to focus but fails to do so and experiences a sense of frustrating mental effort (Eastwood et al., 2012). This condition differs from mere lack of interest because it still entails an (ineffective) investment of attentional resources (Mugon et al., 2018; Danckert & Eastwood, 2020). Mental fatigue, by contrast, is a psychobiological state of cognitive tiredness induced by prolonged cognitive demands that can impair both mental and physical performance—particularly in endurance sports—primarily through increased perceived effort (Marcora et al., 2009; Van Cutsem et al., 2017). Recent work shows that boredom and mental fatigue are intertwined: easy yet monotonous tasks can induce mental fatigue equal to or greater than that produced by cognitively demanding tasks (Wolff et al., 2021; Pickering et al., 2024). Sustaining attention on low-salience stimuli requires continuous self-control; this investment translates into fatigue and disengagement. Hence the proposal to distinguish "passive" mental fatigue (under-stimulation/boredom) from "active" mental fatigue (cognitive overload), which may yield similar outcomes (reduced vigilance, subjective exhaustion) while differing in etiology (Pattyn et al., 2018).

This distinction is crucial in sport: lengthy, repetitive training or low-stimulus race phases impose a double load—that of the task itself and that of boredom—which reduces tolerance to mental effort and favors performance decline. Experimental evidence shows that both “active” and “passive” tasks induce mental fatigue but with different subjective profiles (greater demandingness vs. greater boredom and lower motivation) and differential performance effects (Pickering et al., 2024). Moreover, when mental fatigue is induced while minimizing boredom and sleepiness (maintaining high engagement), it may not significantly impair subsequent physical or cognitive performance, suggesting that part of the deleterious effects traditionally attributed to mental fatigue actually derive from boredom (O’Keeffe et al., 2021).

Our findings align with the letteratures. We observed that both the Control (C) condition (watching documentary) and the mental fatigue (MF) condition (1-hour PVT) condition were boring, and both produced reductions in subsequent cognitive performance. The more pronounced cognitive decrements in MF may reflect a cumulative effect, whereby boredom and fatigue add up within the PVT: beyond requiring vigilance and fast reactions, the PVT is monotonous, thus potentially compounding both fatigue drivers. Consistently, in both studies the LMF (locomotor muscle fatigue) condition yielded better post-exercise cognitive performance than control, with no significant increase in boredom, underscoring the specificity of the underlying mechanisms. Supporting this view, in the third study—where boredom was compared too—neither watching nor playing produced observable differences in boredom relative to each other or to pre-intervention levels: we can conclude at this point that rather than what is watched (two hours of FPS) or how repetitive the game is (two hours of FPS), the degree of engagement appears to matter most.

The centrality of boredom also emerges from group comparisons: in our data, both elite athletes and healthy individuals rated PVT and Documentary as significantly boring; however, whereas MF was more boring than C in healthy adults, this difference did not emerge in elites. Thus, for elite athletes the “documentary” control is not truly neutral and may mask between-condition differences. Going forward, elite cohorts likely require alternative control conditions rather than emotional neutral documentaries. The literature confirms that boredom can impair cognitive performance (↓ vigilance, ↑ RT, ↑ errors) and physical performance (↓ endurance, ↑ RPE), with both acute and chronic consequences (reduced enjoyment, poorer adherence) (Wolff et al., 2021; Pickering et al., 2024; Weich et al., 2022). In ultra-endurance contexts, boredom can predict in-race crises more strongly than pain or physical fatigue, with much higher odds among bored athletes (Weich et al., 2022); maintaining engagement thus becomes a performance objective. As previously discussed, identifying an effective solution to what we have termed the "documentary dilemma" is far from straightforward. Although alternative solutions certainly exist, they would require further empirical examination to determine which could be considered truly neutral and sufficiently replicable across studies. In our investigations, viewing a documentary elicited increases in perceived boredom comparable to those observed following the mentally fatiguing intervention. As discussed above, this likely contributed to the reduced differences between the control and the mental fatigue conditions. Similarly, passive viewing of the eSport session produced levels of boredom and mental fatigue comparable to those observed after active gameplay. These findings suggest that passive viewing may not represent a genuinely neutral condition.

Allowing participants to freely use social media or their smartphones during the control period could represent a potential alternative; however, this experience is highly dependent on the specific content accessed and therefore lacks standardization and replicability. Providing a broader selection of multimedia content may offer another possibility, yet the issue remains that athletes may still experience boredom even when the material is of personal interest, as their primary motivation is to assess their own performance during the testing session (Shah et al., 2002). The use of virtual activities involving head-mounted displays may reduce boredom due to the heightened engagement associated with three-dimensional immersive environments. Moreover, virtual reality (VR) simulations have been shown to promote psychological relaxation and sustained cognitive attention (Kim, 2025). However, this approach raises the opposite concern: such flexible, immersive, and user-friendly interaction technologies have been shown to enhance cognitive and memory functions (Yang et al., 2022; Neumann et al., 2018; Thornton et al., 2005), potentially compromising the neutrality required of a control condition. In conclusion, the choice of an appropriate control condition depends on several factors, including the nature of the target problem, the scientific questions addressed by the trial, the time and resources available, the range of alternative treatments within the health care system, and the extent to which the experimental intervention has already been investigated (Hart et al., 2007).

Physical vs Mental Fatigue

In our healthy adult sample—and in line with prior work—mental fatigue impairs endurance without substantially altering peripheral parameters, chiefly by increasing perceived effort (Marcora et al., 2009; Van Cutsem et al., 2017). Locomotor muscle fatigue induced by 100 drop jump protocol (Skurvydas 2000) reduces maximal force and increases cardiorespiratory demand during subsequent exercise (e.g., >15% ↓MVC; ↑HR/ $\dot{V}O_2$/$\dot{V}CO_2$/VE/Bf). A relevant methodological point: Marcora et al. (2008)—as well as our elite cyclist study—using the 100 drop-jump protocol, did not observe increases in metabolic stress, indicating that performance decrements after LMF were not metabolically driven. As discussed later, performance level matters: elite participants (PL 4–5) and those in Marcora's sample (PL 3) displayed higher levels than our healthy adults; this likely explains the absence of metabolic stress in higher-PL cohorts and its presence in our PL 1–2 participants. Although their origins differ, both fatigue types advance the performance limit primarily by raising perceived effort and reducing tolerance to continuation (Marcora et al., 2009). In direct comparison, muscular fatigue tends to reduce time-to-exhaustion more markedly (~20% vs. ~10% for mental fatigue) and is accompanied by clear physiological signs (↑HR, ↑$\dot{V}O_2$, ↑VE), whereas mental fatigue acts predominantly on perceptual-cognitive domains (slower RTs, more vigilance lapses) (Van Cutsem et al., 2017; Hopstaken et al., 2015).

Interestingly, this pattern was also evident in the e-sport study: participants reported increased perceived physical demand and effort despite the complete absence of muscular activity. This finding indicates that physical sensations of effort can emerge purely from central processes associated with attentional regulation and cognitive control. Such evidence reinforces the psychobiological model of fatigue, suggesting that the boundaries between mental and physical effort are functional rather than structural.

It is plausible that, in our healthy adults, metabolic stress added to physical fatigue, producing the larger decrement. Therefore, to isolate the effects of muscular fatigue without metabolic stress, future studies should either recruit better-trained (non-elite) participants or adjust the fatiguing intervention.

Fatigue resilience in elite cyclist

A key finding of the present work is the fatigue resilience observed in elite cyclists: neither mental fatigue nor physical fatigue compromised endurance performance, even in the presence of indicators such as reduced attentional vigilance or increased perceived physical fatigue. RPE values were higher in LMF, yet did not differ significantly from control or MF. Thus, elite athletes tolerate fatigue more effectively—whether subjectively perceived or accompanied by slight cognitive decrements—while maintaining performance. This pattern aligns with prior evidence and supports the interpretation that professional athletes develop superior inhibitory control, motivational resources, and attentional strategies (Martin et al., 2016). That said, we note that the high boredom load induced by the viewing control may have affected outcomes, potentially yielding performances that are lower than what might be observed in the complete absence of extraneous performance-limiting factors. Nevertheless, while our mental-fatigue findings accord with Martin et al. (2016), we also provide the first evidence that elite cyclists exhibit resilience to laboratory-induced locomotor fatigue. A crucial methodological point, highlighted by comparison with Martin (2016), is the lack of a same-day double-bout design: testing multiple conditions within a single session would reduce between-day variability and enhance sensitivity to subtle differences in elite cohorts.

Moreover—as previously discussed—performance level matters. Considering our two studies and the work of Marcora et al. (2009), the same protocol produced metabolic effects only in participants with PL 1–2 (based on $\dot{V}O_2max$) (De Pauw et al., 2013), whereas no metabolic stress was observed in higher-PL samples. This further underscores the need to stratify by performance level, rather than relying solely on an elite vs. non-elite dichotomy.

KEY METHODOLOGICAL CONSIDERATIONS

Comparisons with the existing literature and cross-study insights from our research yield several operational recommendations: (i) Avoid "neutral" control conditions that are intrinsically boring—particularly with elite athletes—as they may flatten differences between experimental conditions; (ii) Consider same-day double-bout designs to reduce inter-day variability and increase sensitivity to subtle performance changes in elite participants; (iii) Stratify participants by performance level (PL/$\dot{V}O_2max$) rather than relying solely on an elite vs. non-elite dichotomy, since protocols isolating certain mechanisms in higher PL groups may not do so in lower ones; (iv) Recognize Esport research as equivalent in value to traditional sport science, given both its vast user base and the measurable physiological and psychological effects Esport activities exert on athletes; (v) When investigating circadian rhythms and perceptual responses, it is essential to account for each participant's chronotype and to differentiate between specific hours rather than broad "times of day"; (vi) Exploring microbiome effects on health and performance represents a promising new frontier in nutritional supplementation, with implications for both athletic and general populations.

Main Limitation

The studies presented in this thesis inevitably have some limitations. One of the main problems concerns the unbalanced composition of the samples, particularly when comparing mental and physical fatigue conditions. In both studies, the proportion of male participants was significantly higher than that of female participants, making it difficult to identify potential sex differences. In the elite cyclist group, the sample size was limited, and two additional participants, although recruited, had not yet completed all test sessions at the time of writing this thesis. Their absence led to consequent analysis and discussion with a lack of counterbalance in the order of testing, representing a methodological limitation. Similarly, in the group of healthy adults, despite homogeneity in peak power and VO_2max, the absence of a comparable sports background led to high interindividual variability, reducing the consistency of the results.

The role of boredom, already discussed in detail, also emerged as a limitation and source of further study, highlighting its potential influence on attention and performance. In addition,

all three studies were conducted in controlled laboratory environments that, despite efforts to reproduce field-like conditions, inevitably limit ecological validity.
Finally, it must be acknowledged that none of the three studies is yet ready for publication. Some final analyses and methodological adjustments are still in progress and will be completed in the near term. However, this does not exempt this thesis from this limitation, as the work is based on data and materials that are still being finalized.

FUTURE RESEARCH DIRECTION

The research program presented in this thesis will be continued and expanded to encompass additional facets of fatigue. The next immediate step will be the *Veillonella* study. What are currently only hypotheses—regarding the potential effects of probiotic supplementation, and *Veillonella atypica* in particular, on fatigue, cognitive functions, and quality of life—will begin to receive empirical indications. Subsequent studies would then need to explore the extent of this phenomenon: What is the minimum duration and dosage of supplementation needed to achieve observable effects? How long would the observed benefits persist? In essence, this line of research is likely to grow along with the rapidly evolving research on the microbiome and its involvement in sport. Our contribution is to extend the discussion from performance per se to fatigue and its perception.
A second theme highlighted in the Introduction—yet not represented among the completed studies reported here—concerns the relationship between perceived fatigue and circadian rhythms. A study I designed, which is still in its initial steps and therefore not reported in detail above, aims and will better propose in the near future to examine how time of day modulates the effects of exercise on cognition, perceived fatigue, and mood. In addition, this ambitious study evaluates whether pre- to post-exercise differences in gene expression are detectable and whether such molecular responses are themselves modulated by the timing of exercise.
In brief, the study was a randomized crossover study that examined how exercise timing (morning, noon, evening) modulates both molecular and cognitive–perceptual responses. The primary aim is to determine whether time of day elicits distinct patterns of gene expression in peripheral blood mononuclear cells (PBMCs) and whether these patterns align with changes in cognitive performance and subjective experience (mood, fatigue, motivation and exercise

enjoyment). By strictly controlling for diet, sleep, hydration, and chronotype, the study aims to isolate the specific effects of exercise timing. Preliminary pilot data suggest that while overall cognitive performance remains stable across the day, morning exercise may enhance vigilance and mood, indicating a wake-promoting effect, whereas later sessions may show mild fatigue-related slowing. Mood data mirror this pattern: participants generally report poorer pre-exercise mood in the morning, which improves markedly after exercise, while evening sessions begin from a more positive baseline with limited post-exercise change. Physiological measures, such as blood lactate, vary with exercise intensity but not with time of day, and rate of perceived exertion (RPE) increases predictably across the exercise regardless of session timing. Although these pilot results are encouraging, the study still requires completion of the gene-expression analyses, which will likely yield the most informative insights, and an expanded sample to confirm generalizability. The results discussed here derive from exploratory analyses in six participants; accordingly, they are presented as future research on circadian and microbiological dimensions rather than as definitive findings for this thesis.

None of the studies included in this dissertation have yet been published in peer-reviewed journals; however, the work on locomotor muscle fatigue and mental fatigue has been presented and discussed at different conferences. A key near-term objective is therefore to consolidate and disseminate these findings through publication in peer-reviewed journals.

In conclusion, in the immediate future it is unlikely that a single, unified explanation of fatigue will emerge. The mechanisms that generate fatigue are diverse, spanning physiological, cognitive, motivational, circadian, and maybe microbiome-related dimensions, and their all interrelations remain far from fully understood. Likewise, it is improbable that universally shared concepts and models of fatigue perception will be established in the short term. The purpose of this research agenda is therefore not to propose a comprehensive theory, but to progressively advance empirical knowledge in this vast and complex field. Each study contributes a piece to the broader puzzle, addressing current unanswered questions while laying the groundwork for those that will inevitably arise. Step by step—through investigations of mental, muscular, and mental fatigue, the roles of boredom and circadian rhythms, and novel insights into the gut–brain axis—this thesis emphasizes the importance of embracing the multifaceted nature of fatigue.

Ultimately, the goal is to continue clarifying as many components as possible, integrating them into a more comprehensive framework that, although unlikely to be universally definitive, may significantly advance both theory and practice in the understanding and management of fatigue

Influence of circadian rhythm

From the study briefly discussed above, we observe that time of day modulates both perceptions and performance: in line with the literature, cognitive functions such as attention show their best outcomes around midday and in the evening (Valdez et al., 2012; Valdez, 2019). In our pilot data, reaction times were generally preserved throughout the day, with a trend toward faster responses following morning exercise and slower responses after afternoon and evening sessions. However, attention levels appeared lower in the morning, although a “wake-promoting” effect was evident immediately after exercise.

Overall, the relationship between fatigue and performance is highly complex and characterized by substantial variability, with the literature itself often reporting inconsistent findings. Although exercise did not exert an “energizing” effect—as post-exercise fatigue increased regardless of time of day—the comparison of pre-exercise fatigue levels aligns with previous research, showing lower pre-session fatigue in the evening (Reilly et al., 2007; Knaier et al., 2022). Conversely, some studies indicate that morning exercise is more effective than afternoon sessions in enhancing short-term maximal performance and reducing fatigue (Hasni et al., 2025).

Our findings further highlight the importance of considering the specific hour, rather than broadly categorizing by time of day. Indeed, in our circadian rhythm study, the lowest perceived fatigue occurred in the evening (6 p.m.–8 p.m.), whereas in the “E-sport” study, participants reported higher fatigue when visiting the laboratory at 9 p.m. (watch condition) compared with 7 p.m. (play condition). A similar pattern emerged for mood: in the circadian rhythm experiment, pre-exercise mood was poorer in the morning and more positive in the evening, with the most pronounced post-exercise improvements observed at morning and midday sessions. In contrast, the E-sport study revealed better mood levels at 7 p.m. than at 9 p.m.

Taken together, these findings support the need—both in research and applied settings—to systematically account for time of day, sleep patterns, chronotype, and individual routines in experimental design, training scheduling, and pre-competition strategies (Valdez , 2019).

Microbiome and psychobiotic horizons

The modulation of the gut microbiota represents a promising intervention strategy to mitigate fatigue and optimize performance. Pioneering findings indicate that bacterial strains such as *Veillonella atypica* metabolize exercise-derived lactate into short-chain fatty acids (SCFAs)—primarily propionate—which may exert ergogenic effects (Scheiman et al., 2019). The upcoming Veillonella trial, already approved and ready to begin, aims to determine whether four weeks of supplementation can improve endurance, lactate clearance, cognitive performance, and perceived exertion in amateur cyclists.

In parallel, studies I have collaborated on investigating multi-strain probiotic formulations suggest potential benefits in professional athletes, particularly in improving sleep quality and recovery (Bongiovanni et al., 2025). However, the causal mechanisms and generalizability of these effects remain open questions, given the strong strain specificity, interindividual variability, and uncertain transferability to the general population. Addressing these issues will require well-controlled studies, multimodal assessment approaches, and independent replication.

The frontier of this research lies in the integration of nutritional and probiotic strategies with physical and mental training, while maintaining rigorous methodological standards and multidomain outcome measures.

Conclusions and perspectives

The results of this thesis show that performance under exertion depends on a balance between physiological capacity and psychological state. When the balance is altered-for perceived exhaustion, mental fatigue, boredom, motivational decline, or circadian mismatch - the threshold of perceived exertion is brought forward and a decline in performance is observed. At the same time, moderators and strategies emerge: in elites, motivational and attentional resources sustain performance even in the presence of perceived fatigue (Martin et al., 2016);

controlling boredom and maintaining engagement protect attention and decision-making (Wolff et al, 2021; Hopstaken et al., 2015); chronobiology offers practical levers for planning and interpreting loads and tests (Valdes, 2019;); microbiota opens up integrative intervention scenarios (Scheiman et al., 2019; Bongiovanni et al., 2025).

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APPENDIX A

Multidimensional state of Boredom scale - short form (MSBS-SF)

1. Time is passing by slower than usual
2. I am easily distracted
3. I seem to be forced to do things that have no value to me.
4. I feel bored
5. I'm wasting time that would be better spent on something else
6. My mind is wandering
7. I want something to happen but I'm not sure what
8. I feel like I'm sitting around waiting for something to happen

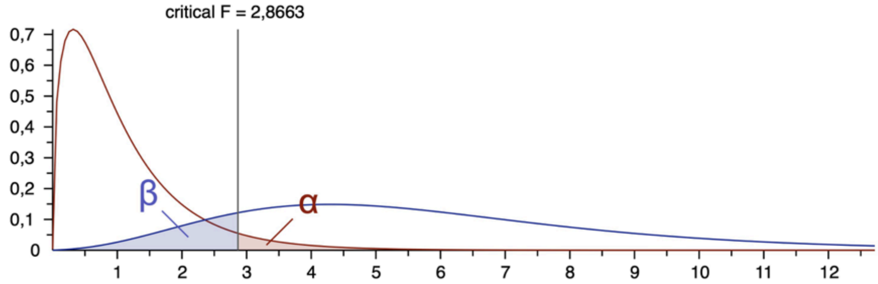
1 Strongly disagree; 2 = Disagree; 3 = Somewhat disagree; 4 = Neither agree nor disagree; 5 = Somewhat agree; 6 = Agree; 7 = Strongly agree.

APPENDIX B

Fatigue and Fatigability Scales

Fatigue Scale Items (Before Walking Test)	Score	Fatigability Scale Items (After Walking Test)
Extremely tired	7	Extremely more tired
Somewhat tired	6	Somewhat more tired
A little tired	5	A little more tired
Neither tired nor energetic	4	Neither more tired nor energetic
A little energetic	3	A little more energetic
Somewhat energetic	2	Somewhat more energetic
Extremely energetic	1	Extremely more energetic

APPENDIX C



F tests - ANOVA: Repeated measures, within factors

Analysis:	A priori: Compute required sample size		
Input:	Effect size f	=	0,35
	α err prob	=	0,05
	Power (1-β err prob)	=	0,8
	Number of groups	=	2
	Number of measurements	=	4
	Corr among rep measures	=	0,5
	Nonsphericity correction ε	=	1
Output:	Noncentrality parameter λ	=	13,7200000
	Critical F	=	2,8662656
	Numerator df	=	3,0000000
	Denominator df	=	36,0000000
	Total sample size	=	14
	Actual power	=	0,8499474