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Balance control is impaired by mental fatigue due to the fulfilment of a continuous cognitive task or by the watching of a documentary

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Abstract

The aim of the present study was to evaluate the effects of mental fatigue (MF) induced by a 90-min continuous demanding cognitive task on balance control. Twenty healthy young participants were recruited. They had to perform three postural tasks (on a stable support with eyes open, with eyes closed and on a wobble board) while standing on a force platform before and after watching a documentary in a control condition or carrying out a prolonged continuous demanding cognitive task (AX-continuous performance test—AX-CPT) in a MF condition. Results showed that performing the AX-CPT generated MF since participants felt a higher subjective workload from the NASA Task Load Index after the AX-CPT than after view-ing the documentary. Both the AX-CPT and the viewing of the documentary impaired balance control, mainly by affecting postural regulatory mechanisms which evolved towards a less automatic and less complex regulation mode with an increased participation of cognitive resources. MF generated by the AX-CPT affected balance control by compromising the attentional processing, while the deleterious influence of watching a documentary on postural control could stem from an adverse effect of prolonged sitting on balance control during subsequent standing.

Keywords Balance control · Mental fatigue · Subjective workload · Postural regulation · Attentional resources

Introduction

Mental fatigue (MF) occurs during and after prolonged periods of cognitive activity and leads to a decrease in commitment to the task at hand with feelings of “tiredness” or “exhaustion” (Boksem et al. 2005; Boksem and Tops 2008; Marcora et al. 2009; Pageaux et al. 2013; Smith et al. 2019). MF is a complex phenomenon that affects further cortical structures such as anterior cingulate cortex and prefrontal cortical areas (Martin et al. 2018; Rozand et al. 2015; Smith et al. 2018). These brain structures are involved in many executive functions such as inhibitory control and attentional processes (Boksem and Tops 2008), planning process and action monitoring (Boksem et al. 2005) and effort-based decision-making (Schweimer and Hauber 2006). Hence, the changes in brain functioning due to MF can impair cognitive performances, while increasing response time and decreasing response accuracy in various cognitive tasks, e.g. Stroop task, psychomotor vigilance task, and AX-continuous performance test (Boksem et al. 2005; Boksem and Tops 2008; Marcora et al. 2009; Pageaux et al. 2013; Smith et al. 2019). MF can also have a negative impact on sports performances, especially in endurance activities where MF increases perception of effort, thus leading to a precocious exercise disengagement and a decline in performance which is neither due to cardiorespiratory, metabolic, nor neuromuscular mechanisms (Marcora et al. 2009; Martin et al. 2018; Pageaux et al. 2013; Smith et al. 2015). MF also affects motor skills performances (Le Mansec et al. 2017; Pageaux and Lepers 2018; Rozand et al. 2015; Smith et al. 2018). Indeed, Le Mansec et al. (2017) reported that MF could impair table tennis performance by decreasing ball speed and accuracy. Smith et al. (2018) concluded that MF impaired soccer-specific physical, technical, and perceptual/

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cognitive performances due to impairments in many executive functions such as decision-making, attentional allocation and action preparation. These impairments of executive functions induced by MF can also affect fine motor skills such as pointing tasks involving speed–accuracy trade-off by increasing the duration of the movement (Rozand et al. 2015).

Standing balance control is a fundamental motor skill, which requires a given level of attention to maintain an upright stance without falling, especially when the balance requirements increase while increasing the difficulty of the postural task (Lajoie et al. 1993; Palluel et al. 2010). Hence, MF is likely to impair balance control which can have many negative impacts, since proper balance control is crucial to perform work-related activities efficiently and safely and to optimize motor performance while preventing falls and fall-related injuries (Paillard 2017). However, to our knowledge, only one study has explored the effects of a prior demanding sustained attention task on balance control (Deschamps et al. 2013). Although these authors did not report any deleterious effects of MF on balance control, the cognitive task they implemented (two bouts of a 15 min psychomotor vigilance test separated by a 1–3 min rest period) appears inappropriate to induce MF. Indeed, Van Cutsem et al. (2017) emphasized that only continuous cognitive activity lasting more than 30 min can generate MF. Moreover, they did not assess MF while monitoring participant's physiological or subjective responses. Hence, the question about the influence of MF due to a sustained cognitive task on balance control remains open.

Consequently, the aim of the present study was to evaluate the effects of MF induced by a prolonged continuous demanding cognitive task on balance control. A subjective workload assessment was implemented to assess participants' MF state. Because postural tasks are more cognitively demanding in more challenging conditions (Lajoie et al. 1993; Palluel et al. 2010), balance control was investigated in tasks with various levels of difficulty, by means of computerized posturography before and immediately after either watching a documentary or carrying out a prolonged continuous demanding cognitive task. Both linear and non-linear postural sway metrics were calculated. By analysing the temporal structure of postural sway variations, non-linear metrics provide information about the complexity of postural sway and the associated regulatory mechanisms of balance control: a reduced postural sway complexity characterises a less automatized control with an elevated cognitive contribution, i.e. an impaired control with a reduced ability to adapt to the environmental changes (Ramdani et al. 2009; Reinert et al. 2017; Vaillancourt and Newell 2002). It was expected that MF, especially in the most challenging postural tasks, would induce a significant impairment in balance control with a reduced postural sway complexity.

Materials and methods

Subjects

Twenty healthy young right-handed subjects—thirteen men and seven women [age 21.8 (1.7) years old, height 173.6 (9.8) cm, weight 69.4 (11.5) kg, mean (SD), respectively] volunteered to participate in this study. Exclusion criteria were a documented balance control disorder or a medical condition that might affect balance control, a neurological or mental disorder and a musculoskeletal impairment in the past 2 years. Participants were asked to avoid strenuous activity 48 h before the data collection session and not to consume caffeine, alcohol, cigarettes or any psychoactive substances the day of the experiment. They all gave their written informed consent to participate in the experiment, which was approved by the University's Institutional Review Board in accordance with the Declaration of Helsinki.

Protocol and experimental conditions

Balance control was assessed before (PRE) and after (POST) watching a documentary (control condition) or carrying out a prolonged continuous demanding cognitive task (MF condition).

The MF condition corresponded to performing the AX-continuous performance task (AX-CPT) for 90 min on a computer (Cedrus, SuperLab®6, USA). In this test, sequences of letters (24-point uppercase Arial font) were visually presented at the centre of a black screen one at a time in a continuous fashion (Marcora et al. 2009; Pageaux et al. 2013). Target trials were defined by a paired cue–probe in which the cue was the letter A and the probe was the letter X. Non-target trials were represented by three trial types: B–X trials, in which an invalid cue (non-A) preceded the probe (X); A–Y trials, in which a valid cue (A) was followed by a non-probe (non-X); and B–Y trials, in which an invalid cue was followed by an invalid probe. All trials were presented in a pseudorandom order, with the following frequency of occurrence: 70% of target trials (A–X), 10% of non-target trials B–X, 10% of non-target trials A–Y and 10% of non-target trials B–Y. Target and non-target trials were presented with red letters. To increase task difficulty, two white letters were presented as distractors between cue and probe red letters. Each letter (red and white) was presented during 300 ms, followed by 1200 ms interval. A cross appeared at the centre of the screen during 300 ms to distinguish each trial. Participants were instructed to press the letter "p" of the keyboard on target trials and the letter "q" of the keyboard on non-target trials. Any incorrect or missing response induced a beep sound transmitted to the participants by headphones to increase speed and accuracy. The 90 min test was split into ten blocks of 9 min and the percentage of correct responses (CR) and time of correct response (TCR) were continually calculated in each block.

The control condition consisted of watching a 90 min documentary about animal migrations, "Earth" (directed by Fothergill and Linfield 2007).

A randomized cross-over study design was implemented, in which each participant performed both experimental conditions in a counterbalanced order separated by a period of 7 days. In both experimental conditions, participants were sitting in the same chair in front of the same computer screen, without receiving specific instruction about their sitting posture. The delay between the end of the documentary or the AX-CPT and the balance control assessment was approximately 10 s. Participants took about three steps from the chair to the force platform. To avoid a time-of-day effect which could have acted as a confounding factor (Deschamps et al. 2013), each participant was assessed at the same time in both control and MF conditions (half of participants were assessed at 10:00 and the other half at 15:00).

Balance assessment

Three postural tasks were carried out while asking participants to stand on a force platform (Stabilotest® Techno Concept, Mane, France) and to sway as little as possible during 60 s: on a stable support with their eyes open (STAT_EO), on a stable support with their eyes closed (STAT_EC) and on a wobble board (Balance-board, Sissel® GmbH, Bad Dürkheim, Germany) which was placed on the force platform (UNSTAB). In each postural task, participants stood with parallel feet with a 2 cm spacing between the heels, while legs were extended, and arms held alongside the body. Two familiarization trials were performed for each postural task before data acquisition to avoid any learning effect (Cug and Wikstrom 2014). Displacements of the centre of foot pressure (COP) were recorded with a sampling frequency of 40 Hz (Cherng et al. 2003). COP surface (COPS corresponding to 90% confidence ellipse), mean COP velocity on the mediolateral and anterior–posterior axes ($\dot{\mathrm{COPX}}$ and $\dot{\mathrm{COPY}}$) were calculated as traditional linear measures of the COP characterizing the amount of postural sway. The analysis of the dynamical features of postural sway variations was calculated from the sample entropy (SampEn). This variable was calculated from the resultant COP velocity using the method proposed by Ramdani et al. (2009). SampEn is determined as the negative of the natural logarithm of conditional probability, which is a sequence of data points, having repeated itself within a tolerance r for a window length m, which will also repeat itself for $m+1$ points, without allowing self-matches. SampEn was quantified using $m=3$ and $r=0.2$ (Reinert et al. 2017). SampEn provides information about the regulation processes involved in balance control. Low SampEn values reflect an intentional control of balance control with a strong cognitive contribution and reduced adaptive abilities. Conversely, high SampEn values are indicative of a more automatic and adaptive control of balance (Ramdani et al. 2009; Reinert et al. 2017; Vaillancourt and Newell 2002).

Workload assessment

The National Aeronautics and Space Administration—Task Load Index (NASA-TLX—Hart and Staveland 1988), translated and adapted by Maincent et al. (2005), was used to assess subjective mental workload after each experimental condition through a paper-and-pencil version. It allows for an understanding of the workload sources. This test relies on the following subsequent dimensions: mental demand (MD), physical demand (PD), temporal demand (TD), frustration level (FL), effort (EFFORT), performance (PERF) and on a global score (GS). Participants assessed each dimension using non-graduated bipolar scales, ranging from "low" to "high", for which scores were then calculated from 0 to 100. Next, in each paired combination of the six dimensions (totalling fifteen pairs), the participants chose the dimension which was the most related to the definition of workload, giving a weighting factor for all dimensions. Then, each dimension score was multiplied by the appropriate weight. A global score was then obtained by averaging all the weighted dimensions' scores.

Statistical analysis

Each postural task was analysed independently. Since most of the postural sway and cognitive variables did not show normal distribution (tested with the Shapiro–Wilk test), non-parametric tests were performed. Wilcoxon signed rank tests were applied to test for potential differences between the control and MF conditions at baseline (PRE). The relative increases (RI) were calculated from each postural sway variable as follows: $\mathrm{RI}=(\mathrm{POST}-\mathrm{PRE})/\mathrm{PRE}$. This makes it easy to obtain interpretable descriptors to characterize the evolution of the variables between PRE and POST measures while limiting the influence of the heterogeneity between participants at baseline (PRE). Univariate Wilcoxon–Mann–Whitney tests were applied to compare each RI ($\dot{\mathrm{COPS}}$_RI, $\dot{\mathrm{COPX}}$_RI, $\dot{\mathrm{COPY}}$_RI and SampEn_RI) to a zero reference value (Paillard et al. 2016). Wilcoxon signed rank tests were performed to compare RIs and NASA-TLX scores between the control and MF conditions. Effect sizes (r) were calculated by dividing the Z value by the square root of the number of observation (Pallant 2007). A specific analysis was conducted with the parameters from the AX-CPT (CR and TCR), which concerned only the MF condition, while performing a Friedman test on the ten blocks of the 90 min test to objectively monitor MF. The significance level was set at $p < 0.05$. Statistical analyses were performed with R statistical software (R Core Team 2016).

Results

Due to a technical problem, only 19 of the 20 subjects were considered for the analysis. Table 1 displays the values of the NASA-TLX dimension scores. Wilcoxon signed rank tests showed that MD, PD, TD, FL, EFFORT and GS dimension scores were significantly higher in the MF than in the control condition ($Z = -3.80$, $p < 0.01$, $r = 0.66$; $Z = -3.53$, $p < 0.01$, $r = 0.57$; $Z = -3.18$, $p < 0.01$, $r = 0.54$; $Z = -3.36$, $p < 0.01$, $r = 0.69$; $Z = -3.60$, $p < 0.01$, $r = 0.79$; $Z = -4.62$, $p < 0.01$, $r = 0.72$ respectively). No significant differences were observed for the PERF dimension score.

CR and TCR in the ten blocks of the AX-CPT performed in the MF condition are presented in Fig. 1. The Friedman test revealed a significant time effect on CR [X^2 (9) = 17.496, $p = 0.04$) and TCR (X^2 (9) = 18.296, $p = 0.03$] which illustrated a decrease in CR and an increase in TCR.

PRE values and the RI of all postural sway parameters are reported in Table 2. No significant differences were observed between the control and MF conditions for PRE values, whatever the postural task. In the STAT_EO postural task, the univariate Wilcoxon–Mann–Whitney tests revealed that CȮPY was significantly increased from PRE to POST in the control condition ($Z = -2.27$, $p = 0.02$, $r = 0.52$) and SampEn was significantly decreased from PRE to POST in the MF condition ($Z = -2.55$, $p = 0.01$, $r = 0.57$). In the STAT_EC postural task, COPS and CȮPY were significantly increased from PRE to POST in both control ($Z = -2.50$, $p = 0.01$, $r = 0.56$ and $Z = -2.93$, $p < 0.01$, $r = 0.65$ respectively) and MF conditions ($Z = -2.23$, $p = 0.03$, $r = 0.51$; $Z = -3.36$, $p < 0.01$, $r = 0.72$), and SampEn was significantly decreased from PRE to POST in both control and MF conditions ($Z = -3.25$, $p < 0.01$, $r = 0.70$ and $Z = -2.69$, $p < 0.01$, $r = 0.60$ respectively). CȮPX was significantly increased from PRE to POST only in the MF condition ($Z = -2.74$, $p < 0.01$, $r = 0.61$). For the UNSTAB, COPS and CȮPY were significantly increased from PRE to POST only in the control condition ($Z = -2.69$, $p < 0.01$, $r = 0.60$ and $Z = -2.36$, $p = 0.02$, $r = 0.54$ respectively), whereas SampEn was significantly decreased from PRE to POST in both control and MF conditions ($Z = -3.04$, $p < 0.01$, $r = 0.67$ and $Z = -2.55$, $p = 0.01$, $r = 0.58$ respectively). When comparing RI of postural sway parameters between the control and the MF conditions, only one significant difference was observed in the STAT_EC for the CȮPX_RI ($Z = -2.18$, $p = 0.02$, $r = 0.17$), with a higher RI in the MF condition.

Table 1 NASA-TLX dimension scores [medians (IQR)] in the different experimental conditions

Dimension	Control	MF
MD	13.8 (32.2)	71.3 (33.3)**
PD	2.9 (6.0)	21.8 (60.9)**
TD	6.3 (12.1)	51.1 (52.9)**
PERF	87.9 (27.2)	63.2 (23.6)
EFFORT	14.9 (18.4)	72.4 (27.9)**
FL	4.6 (6.9)	25.9 (41.6)**
GS	30.5 (14.0)	58.6 (13.3)**

MD mental demand, *PD* physical demand, *TD* temporal demand, *PERF* performance, *EFFORT* effort, *FL* frustration level, *GS* global score

**Significant difference between the control and MF conditions ($p < 0.01$)

Discussion

The aim of this research was to investigate the effects of MF induced by a prolonged continuous demanding cognitive task on balance control. It was hypothesized that MF would impair balance control while reducing postural sway complexity, especially in the most challenging postural contexts. Results showed that the 90 min prolonged cognitive task generated MF, which impaired balance control mainly by decreasing the value of SampEn in all the postural tasks investigated. Balance control was also impaired in the most challenging postural tasks after watching a documentary, likely due to a deleterious effect of prolonged sitting on balance control during subsequent standing.

The assessment of subjective mental workload from the NASA-TLX showed that participants presented higher scores in the MF condition than in the control condition, suggesting that they felt a higher subjective workload after the AX-CPT than after viewing the documentary (Smith et al. 2015; Van der Linden et al. 2006). Moreover, our results showed that the AX-CPT induced a decrease in CR as well as an increase in TCR. Taken together, these results indicated that only the execution of the AX-CPT generated a subjective and objective state of MF (Boksem et al. 2005; Marcora et al. 2009; Pageaux et al. 2013). This mental state had significant consequences on balance control, since lower SampEn values were observed in all the postural tasks following the AX-CPT. Moreover, all linear metrics of postural sway were only impaired by MF when the visual cues were lacking. These results illustrate that, although participants were still able to face the steadiness requirements when the visual cues were available following the AX-CPT, the associated MF state affected the dynamical features of postural sway.

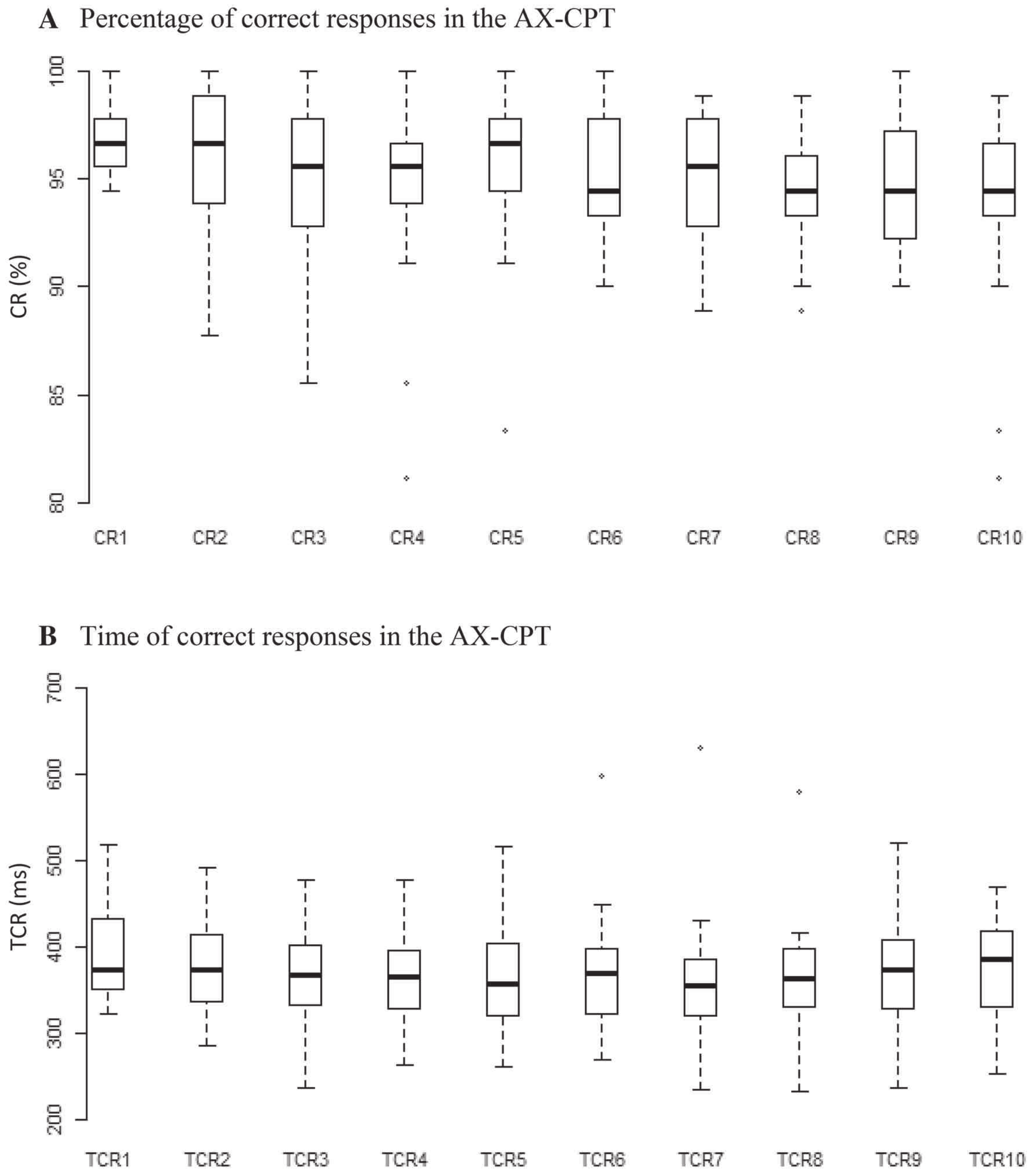

Fig. 1 Boxplot representation of the percentage (**a**) and time (**b**) of correct responses in the AX-CPT. The 90 min AX-CPT was split into ten blocks of 9 min. *CR* percentage of correct responses, *TCR* time of correct response (ms). Small circles indicate data points beyond the whiskers. Statistical analysis using the Friedman test revealed a significant time effect on CR and TCR

With MF, balance regulatory mechanisms evolved towards to a less automatic and less complex regulation mode with an increased participation of cognitive resources. Hence, contrary to our initial hypothesis, the deleterious effects of MF on balance control did not specifically concern the most challenging postural tasks since balance control was impacted in all postural contexts.

Maintaining and controlling an upright posture require a given amount of attentional resources which depend on the complexity of the postural task: the more challenging the postural task, the greater the required attentional resources (Lajoie et al. 1993; Palluel et al. 2010). Nevertheless, the instruction to sway as little as possible has constrained participants to engage full attention towards balance control during all the postural tasks to reduce postural sway as much as possible (Bonnet 2016). Hence, by affecting cortical structures involved in attentional processes such as anterior cingulate cortex and prefrontal cortical areas (Boksem

Table 2 Postural parameters [medians (IQR)] in different postural tasks and experimental conditions

	STAT_EO		STAT_EC		UNSTAB	
	Control	MF	Control	MF	Control	MF
COPS_PRE (mm^2)	238.31 (102.61)	185.56 (165.42)	322.05 (161.82)	312.63 (151.71)	370.25 (212.87)	450.84 (265.82)
COPS_RI	0.21 (1.16)	0.23 (0.73)	0.09 (0.64)*	0.34 (0.82)*	0.31 (0.42)*	0.02 (0.4)
C$\dot{O}$PX_PRE (mm/s^2)	5.89 (1.84)	6.47 (1.12)	9.87 (2.86)	8.8 (3.14)	9.58 (2.98)	10.76 (3.75)
C$\dot{O}$PX_RI	− 0.01 (0.22)	− 0.05 (0.18)	0.02 (0.31)	0.22 (0.34)*§	0.01 (0.3)	0.04 (0.12)
C$\dot{O}$PY_PRE (mm/s^2)	4.70 (1.5)	4.64 (1.36)	8.3 (3.6)	7.88 (2.96)	8.85 (2.43)	7.78 (2.37)
C$\dot{O}$PY_RI	0.08 (0.33)*	− 0.03 (0.23)	0.2 (0.2)*	0.23 (0.35)*	0.1 (0.28)*	0.05 (0.36)
SampEN_PRE	1.48 (0.17)	1.53 (0.16)	1.41 (0.20)	1.38 (0.15)	1.29 (0.23)	1.34 (0.20)
SampEN_RI	− 0.03 (0.09)	− 0.04 (0.08)*	− 0.07 (0.08)*	− 0.08 (0.12)*	− 0.08 (0.12)*	− 0.06 (0.12)*

STAT_EO stable postural task with eyes open, *STAT_EC* stable postural task with eyes closed, *UNSTAB* unstable postural task, *COPS* COP surface area, *C$\dot{O}$PX and C$\dot{O}$PY* mediolateral and anteroposterior centre of pressure velocity respectively, *SampEN* sample entropy, *_PRE* baseline value, *_RI* value of the relative increase

*Significant relative increase

§Significant difference between control and MF conditions ($p < 0.05$)

and Tops 2008; Pires et al. 2018), one can hypothesize that the deleterious effects of MF on balance control could be attributable to attentional impairments due to MF. It can also explain why balance control was more compromised in the postural context without vision, since this deprivation increases the focus on internal body sensations and the attentional cost of balance control (Franco et al. 2018; Razon et al. 2009). The significant decrease of SampEn with MF observed in all the postural tasks suggests that participants might have been forced to engage in more cognitive resources to closely monitor and control balance in a state of MF that compromises attentional processing, thus leading to a less automatic and less complex regulation of postural sway (Roerdink et al. 2011).

Unexpectedly, our results showed that balance control was also impaired after watching a documentary, especially when the postural context was challenged in the absence of visual cues or by a decreased relevancy of proprioceptive cues from the ankle joints due to the instability of the support (Ivanenko et al. 2000). In these challenging postural tasks, participants displayed a higher amount of postural sway and lower sway complexity after watching the documentary. Although it cannot be ruled out that watching the documentary may have induced MF, the fulfilment of the AX-CPT generated a higher subjective state of MF than the control condition. The impairment of balance control in the control condition might be attributed to a deleterious effect of prolonged sitting on balance control during subsequent standing. Indeed, prolonged sitting can induce musculoskeletal discomfort in the lower back region, due to sustained low level activation and loading of passive tissues (Baker et al. 2018). The sustained loading of passive tissues can induce residual deformation in the viscoelastic passive tissues of the lower back, thus resulting in soft tissue creep and decreased passive stiffness of the spine (Howarth et al. 2013; Kastelic et al. 2018). Creep of the passive structures induced by prolonged sitting can induce alterations in neuromuscular control and acutely compromise kinesthetic sense of the trunk by delaying reflex muscle activation of the back muscles (Dolan and Green 2006; Kastelic et al. 2018). Hence, prolonged sitting increases the challenge of controlling spine motion (Dolan and Green 2006; Kastelic et al. 2018) and it is likely to adversely affect activities such as standing balance performed following extended bouts of sitting. Triglav et al. (2019) also reported that prolonged sitting had an impact on skin sensitivity by significantly increasing cutaneous sensitivity thresholds, which represents another potentially disruptive element of prolonged sitting on standing balance control. These negative impacts of prolonged sitting produced limited effects on subsequent balance control when participants stood on stable ground with the eyes open, likely due to the availability of information from different sensory systems, which enabled the preservation of an adaptive postural behaviour. In contrast, the adverse effects of prolonged sitting on subsequent standing balance control would be more pronounced with poorer sensory contexts, when vision is suppressed or when proprioceptive cues from the lower limbs are getting less relevant when standing on an unstable support.

Participants who took part in the present study were subjected to two experimental treatments that involved a 90 min period of sitting. Hence, prolonged sitting could have produced adverse effects on the control of standing balance in both experimental conditions. Although we could think post factum that participants were constrained to adopt an upright sitting posture to perform the AX-CPT whereas they may have adopted a more relaxed sitting posture when watching the documentary, no kinematic nor electromyographic analyses were performed in this study to characterise the sitting postures adopted by the participants. Therefore, future research should include such analyses to accurately examine the influence of different prolonged sitting postures on balance control. Motion and electromyography analyses could also gain insights into the multi-joint coordination and the associated muscle activation patterns when subjects had to maintain standing balance under MF. Another limitation of this study was the lack of data about the physiological responses to the experimental conditions. Performing a prolonged continuous demanding cognitive task which induces MF can also cause an acute stress (Blons et al. 2019), which is likely to affect balance control (Doumas et al. 2018). Since an acute stress have cardiovascular consequences that can be assessed through heart rate variability (Blons et al. 2019), future studies about the effects of MF on balance control should include an assessment of heart rate variability to specify the impact of MF-related stress on balance control.

Conclusions

We provided evidence that both a 90-min continuous demanding cognitive task and watching a 90-min documentary impaired balance control. The continuous demanding cognitive task have produced MF, which may have induced changes in brain functioning and affected balance control by compromising the attentional processing. The influence of watching a documentary on balance control could stem from an adverse effect of prolonged sitting on balance control during subsequent standing. This last and unexpected result should be investigated in greater depth.

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Author contributions Conceptualization: BH, FN, HC; methodology: BH, FN, HC; data curation: BH; formal analysis: BH, BT; software: BT; writing-original draft preparation: BH; writing-review and editing: FN, TP, HC; supervision: FN, TP.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

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