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**ACUTE PSYCHOPHYSIOLOGICAL RESPONSES TO
LABORATORY-INDUCED STRESS IN DIFFERENT GROUPS: AN
EXPLORATORY STUDY**

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Abstract

Occupational stress has been broadly acknowledged as a global challenge and has been the focus of concern for many researchers as stress affects workers' health and performance. Hence, the current study aimed to: a) assess psychophysiological stress among different occupational groups and b) identify differences, if any, between these groups using self-reports and heart rate variability (HRV) measures. Three different occupational groups (students; firefighters; air traffic controllers) of nine people in each group were analysed. The Trier Social Stress Test (TSST; Kirschbaum et al., 1993) was used as a stress-inducer. Linear (HRV) features from participants' electrocardiogram (ECG) were acquired using a medical-grade wearable ECG device (Vital Jacket®). Self-reports were also used before and after the stress task. Data were normalized to healthy populations, controlling for age and gender. ECG-derived normalized measures showed TSST induced stress arousal in all groups. Statistical significant differences were found in AVNN across the three groups using Kruskal-Wallis test, suggesting that the student group were more stressed compared to the other two groups. All the other HRV metrics (RMSSD, pNN50 and LF/HF), along with psychological stress results support these findings. An increase on stress self-perceptions was also found during the stress task. This study supports the idea that TSST is a gold standard stress procedure. Moreover, the use of bio-sensing platforms associated with the broad range of devices recently developed within the Internet of Things (IoT) field can be a valuable contribution for the development of "smart"; low-cost, simple and affordable quantified health technologies extended to a broad range of occupations.

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Keywords: Trier Social Stress Test, occupational health, heart rate variability", "psychophysiological stress", "bio-sensing platform"



1. Introduction

Stress has been defined as an imbalance between demands and resources. When facing a threat, the individual perceives the situation as challenging or exceeding one's individual resources and endangering well-being (Lazarus R.S., 1984). Considering that adults spent a considerable amount of time in working contexts, researchers in the area of occupational health commonly use the term "occupational stress" to categorise stress at work. Occupational stress is broadly recognized as a global challenge and has been the focus of concern for a number of researchers (Cartwright & Cooper, 1997; Ganster & Rosen, 2013; McLeod, 2011). Occupational stress refers to negative experiences caused by an imbalance between job demands and the response capability of the workers. When job demands are too high to manage, stress responses are likely to occur (Schaufeli & Enzmann, 1998).

Stress responses are characterized by an onset of body changes. Cannon (Cannon, 1935) first described these as the "fight-or-flight" response. This is our body's primitive, automatic, inborn response that prepares the body to "fight" or "flee" from perceived attack, harm or threat to our survival. Once a danger is perceived, the autonomic nervous system (ANS) is activated. The ANS has a parasympathetic (rest) and a sympathetic (activation) branch. When a threat is perceived, the parasympathetic nervous system is inhibited and the sympathetic nervous system is triggered (Gordan, Gwathmey, & Xie, 2015). Therefore, the discharge of stress-related hormones evokes several physiological responses, such as the vasoconstriction of blood vessels, increased blood pressure and breathing rate, increased muscle tension and heart rate (HR) and a decrease in heart rate variability (HRV) (Taelman, Vandeput, Spaepen, & Van Huffel, 2009).

The impact of stress in the health condition is currently well recognized. Numerous personal, organizational, and medical costs are being associated with increased stress health problems, such as heart disease, hypertension, upper respiratory tract infections, peptic ulcers, reduced immunity, migraines, alcoholism, depression, suicidal tendencies, anxiety, as well as other mental disorders (Babatunde, 2013) (Smith, Karsh, Carayon, & Conway, 2003). Particularly, the link between work-related stress and cardiovascular disease has been well established (Schnall, Dobson, & Landsbergis, 2016) and Electrocardiogram (ECG)-derived measures, such as the HRV, are being used to monitor the direct effects of stress in the cardiovascular system (Föhr et al., 2017). The HRV defines the complex variation of beat-to-beat intervals mainly controlled by ANS over the interaction of sympathetic and parasympathetic activity and it can be indexed by time- and frequency-domain parameters (Shaffer, McCraty, & Zerr, 2014). Methods in the time-domain define the intervals between successive normal QRS complexes (the Q-wave, R-wave, and S-wave). Measurements in the frequency-domain provide information of how heartbeat power (variance) distributes as a function of frequency ("Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology," 1996).

Researchers interested in understanding the psychophysiological impact of acute stress responses in humans require a valid and reliable acute stressor that can be used under experimental conditions. The Trier Social Stress Test (TSST) has been proposed as a standard protocol for the experimental induction of stress in the field of basic and applied psychobiological research (Kudielka, Hellhammer, Kirschbaum,

Harmon-Jones, & Winkielman, 2007) (Frisch, Häusser, & Mojzisch, 2015). The TSST was created in 1993 at the University of Trier by Clemens Kirschbaum and colleagues (Kirschbaum, Pirke, & Hellhammer, 1993). Hundreds of research studies have used the TSST in order to investigate the impact of acute stress on human neurobiology (Allen et al., 2017). Overall, the TSST consists of three successive phases: 1) A preparation period for 3 minutes); 2) a free speech task in which the participants have to argue why they are the best candidate for the job they wish to apply for 5 minutes); and 3) a mental arithmetic task (e.g. count down from 1022 by 13's for five minutes). The two tasks are performed in front of a selection committee, consisted of three jury members that do not respond emotionally during the test, which makes the situation stressful for the participant (Kirschbaum et al., 1993). The TSST has been used to examine the interplay between social-evaluative threat, neurophysiological stress responses, social cognition and social behaviour. This tasks involves social evaluation, unpredictability and uncontrollability (Allen et al., 2017). The TSST tasks involve active cognitive processing and responses, and are generally associated with diverse degrees of parasympathetic withdrawal and sympathetic stimulation (Allen et al., 2017).

2. Problem Statement

As discussed previously, stress is a complex and multidimensional process, which encompasses several dimensions of analysis. In order to fully understand the stress concept, interdisciplinary research methods are needed, combining both psychological and physiological measures. However, the direct physiological measurement of stress is a complex process (Hickman, Fricas, Strom, & Pope, 2011). The consolidation of objective and reliable stress biomarkers and health has been a challenge for researchers and clinicians. One is a lack of consensus on the meaning and operationalization of the concept of stress (Rodrigues, Kaiseler, & Queirós, 2015). Another is the absence of a reliable and comprehensive background with which to investigate the way in which organisms function and adapt in a constantly changing setting (Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012). Although HRV has been proposed as a reliable and feasible measure for the quantification of stress responses, little agreement exists in the literature for the most suitable HRV-based metric for the detection and differentiation of stress events (Castaldo et al., 2015).

3. Research Questions

Taking into account the shortcomings in this area in the literature, the current study aims to provide novel findings and overcome some of the previous limitations in the assessment and quantification of stress concept. Hence, a general research question was formulated: 1) Does the Trier Social Stress Test (TSST) have the potential to evoke different psychophysiological stress reactions among different occupational groups?

4. Purpose of the Study

The current study sought to assess psychophysiological stress, by examining ECG-derived measures, using different occupational groups in order to understand if there are differences in stress

responses between groups with different work backgrounds (students, firefighters and air traffic controllers in this study). For that purpose, the TSST was used as a testing platform, since this method is a gold standard stress task for inducing and evaluating stress. A bio-sensing wearable platform integrating different biomedical systems that enable the acquisition of real time ECG signals, computation of linear Heart Rate Variability (HRV) features and collection of perceived stress levels was developed and implemented. To the best of our knowledge, this is the first study investigating and comparing psychophysiological stress responses among different occupational groups.

5. Research Methods

5.1. Participants

The current study involved a convenience sample of three different occupational groups which are exposed to different type of stressors. All the participants were Portuguese. The independent variable was “profession” and the dependent variable was “stress” (using psychophysiological metrics).

A detailed description of the participants is given in the table below (Table 01).

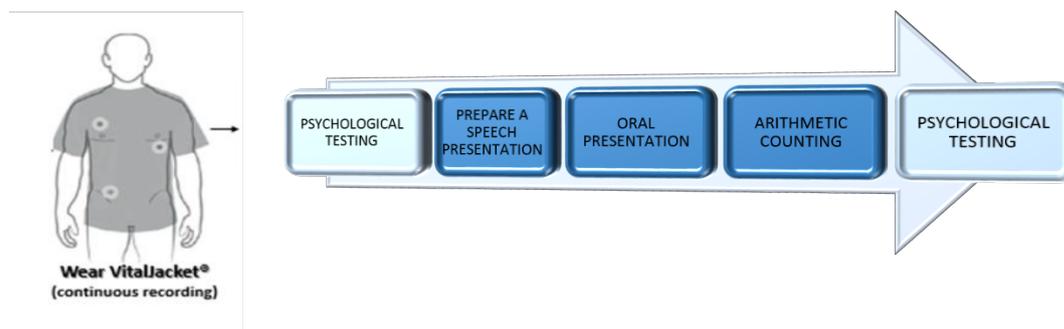


Figure 01. Study protocol workflow

Table 01. Dataset description

	Students	Firefighters	Air traffic controllers
Total (Female/Male)	9 (3/6)	9 (3/6)	9 (3/6)
Age	20 ± 3	30 ± 7	46 ± 6

5.2. Data collection

The exclusion criteria for the study were participants having a history of cardiovascular disease and/or taking prescription drugs known to affect cardiovascular function. The study was approved by the University of Porto Ethics Committee. All informants were carefully instructed about the study protocol and gave written informed consent prior to examination.

A presentation session was organized to explain the aim and the overall protocol of the study. The protocol lasted approximately 15 minutes and included a combination of tasks as shown in Figure 01.

Participants completed the demographic, medical survey and the State-Trait Anxiety Inventory (STAI 6-item short-form) (Marteau & Bekker, 1992). Emotional, physical, and cognitive aspects of stress can be quantified using this self-report assessment instrument. Considering that our goal was to evaluate the differences in state anxiety from a non-stress to a stress moment, we chose the short-form of the

STAI, that according to the authors is more sensitive to fluctuations in state anxiety (Marteau & Bekker, 1992).

Participants were equipped with Vital Jacket® (Cunha, 2012; Cunha et al., 2010). The Vital Jacket® is a wearable medical device (in form of a t-shirt) able to collect 1-lead electrocardiogram (ECG) signals in real-time, without affecting the daily activities of users. It also contains a 3-axis Accelerometer, allowing ECG signals correction for actigraphy profiles and a bluetooth to transmit real time data to another device, allowing the live visualization of data. For an offline monitoring, the data is also stored in an SD card in the Vital Jacket®. This equipment is certified according to the MDD93/42/EEC medical device directive holding the European Conformity medical device mark (Biodevices, n.a.). An android smartphone application was used for data synchronization and event marking (Figure 02). This application pairs with Vital Jacket® via Bluetooth and enables the exact annotation of events in the ECG trace, using “Radiobuttons”. This action allows for registering the events in the SD card and are synchronized with the ECG that is being acquired simultaneously. The android application stores all the information about the events in an SQL Light database warranting the synchronization between those events information and the data collected by the Vital Jacket®. The combination of the events data synchronized with the Vital Jacket® data allows to create an annotated database. Data can then be exported to be processed and analysed according to the researcher’s interests and study goals.

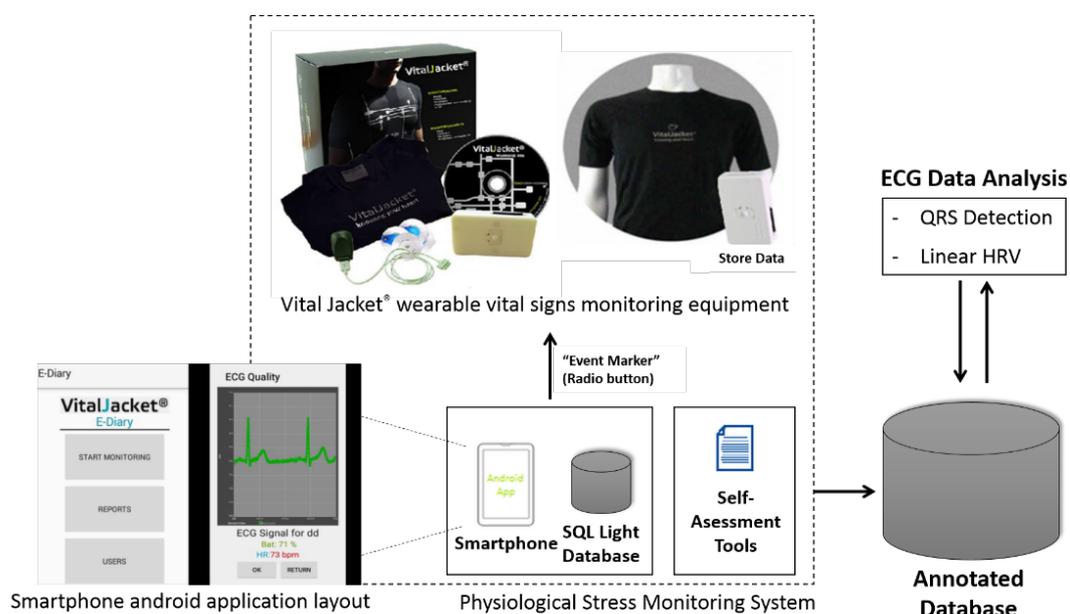


Figure 02. System block diagram explaining the bio-monitoring platform workflow

After being equipped with the Vital Jacket® equipment, participants performed the TSST. The STAI questionnaire was filled up before and just after the TSST.

5.3. Data analysis

Statistical analysis was performed using IBM SPSS AMOS (v.24) software. Taking into account the small sample number, some parameters failed in the normality test (Shapiro-Wilk Normality Test), so all parameters were analyzed using non-parametric statistical tests. Kruskal-Wallis test was the non-

parametric alternative test used to test if there were significant statistical differences on ECG-derived measures between the three groups, and the Wilcoxon signed-rank test was also used to compare STAI results from the beginning to the end of the TSST for each group (Pallant, 2013).

In order to extract heartbeat information from the ECG recordings, a software from Biodevices S.A, with an ECG analyzer was used. This software has an algorithm based on the one developed by Pan Tompkins (Pan & Tompkins, 1985) incorporating ECG physiological filters to detect the “R” points of the ECG waveform. Using this analyzer, the RR interval (time between two consecutive “R” peaks in the ECG) was extracted. A simple verification according to Clifford et al. (Clifford, Azuaje, & McSharry, 2006) was made to verify if all the RR intervals were physiologically correct. The RR intervals that have physiological validation are named normal-to-normal (NN) intervals. ECG-data were normalized using Voss ’s et al. (2015) study with a healthy population. These authors conducted a study with the largest dataset of healthy subjects (N=1906) and analyzed age and gender-related HRV differences.

Following the guidelines presented by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology ("Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology," 1996), different HRV time and spectral domains parameters were evaluated (see Table 02). For time-domain parameters, we adopted the Average of NN intervals (AVNN); the root mean square of differences between successive rhythm-to-rhythm (RR) intervals (RMSSD) and the percentage of the number of pairs of successive NNs that differ by more than 50 ms compared to the total number of NN intervals, normally referred to as pNN50 ("Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology," 1996). For extraction of spectral parameters, we adopted the Lomb Periodogram (Lomb, 1976). For frequency-domain analysis, we used both the low frequency (LF) component defined between 0.04-0.15 Hz as well as the high frequency (HF) component (0.15-0.4 Hz), respectively, and computed their ratio - LF/HF.

Table 02. Descriptions of the HRV measures analysed and their trend under stress (Castaldo et al., 2015)

Domain	Measure	Description	Features trend under stress
Time-domain	AVNN	Average of NN intervals (ms)	↓
	RMSSD	Root mean square of differences of successive NN intervals (ms)	↓
	pNN50	NN variations above 50 ms (%)	↓
Frequency-domain	LF/HF	Ratio of Low Frequency and High Frequency power band	↑

6. Findings

Regarding the ECG analysis, two blocks of 5-minutes were analysed for each participant, resulting in 54 blocks of 5-minute clinical grade-ECG. ECG-derived measures (AVNN, RMSSD, pNN50 and LF/HF) were normalized according to normative values provided by Voss et al. (Voss, Schroeder,

Heitmann, Peters, & Perz, 2015), considered the gold standard among the HRV-related studies available in literature and corresponding to each participant's gender and age interval.

The obtained values are suggestive of stress in all groups, when compared to normative values (Figure 03). Statistical significant differences were found in AVNN across the three groups using Kruskal-Wallis test $\chi^2(2, n = 27) = 6.099, p = 0.047$. Additionally, there is a common tendency for more stress in the student group, considering that AVNN, RMSSD and pNN50 are below the average for normative values and LF/HF values are above (Figure 03).

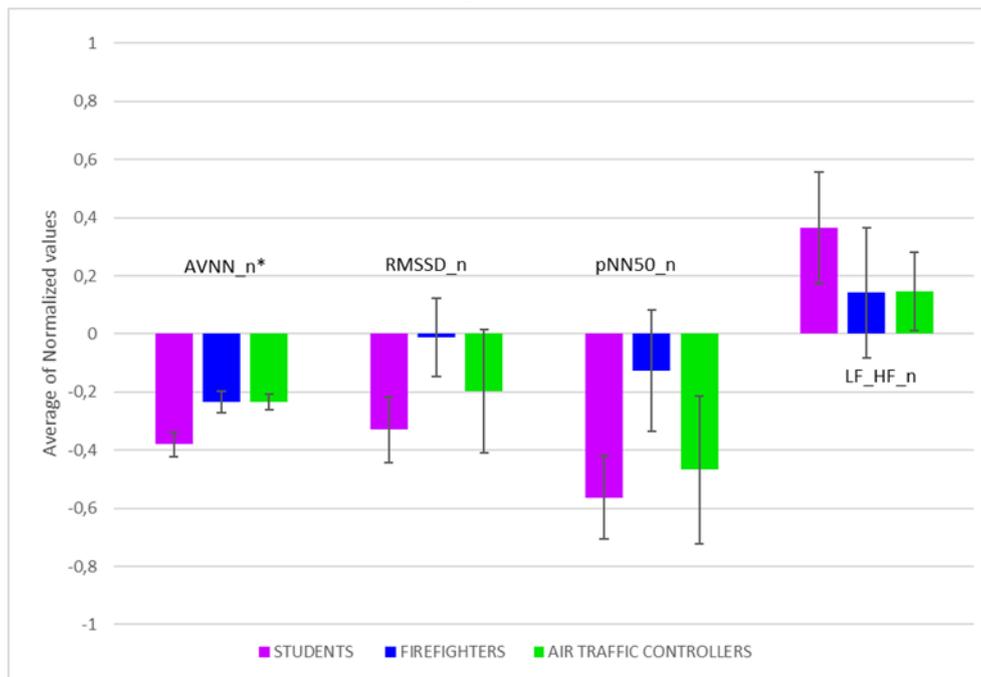


Figure 03. Normalized mean values for ECG-derived measures using Voss 's et al. study with health population. Values of AVNN, RMSSD and pNN50 below dashed line are suggestive of stress. Values of LF/HF above the dashed line could also be suggestive of stress. *Statistical significant changes were found for AVNN using Kruskal Wallis test, $p < 0.05$.

Below is an example of how ECG-derived measures (AVNN and LF/HF) change during the stress task. (Figure 04). As can be seen, there is an increase on LF/HF and a decrease in AVNN just after starting the speech phase.

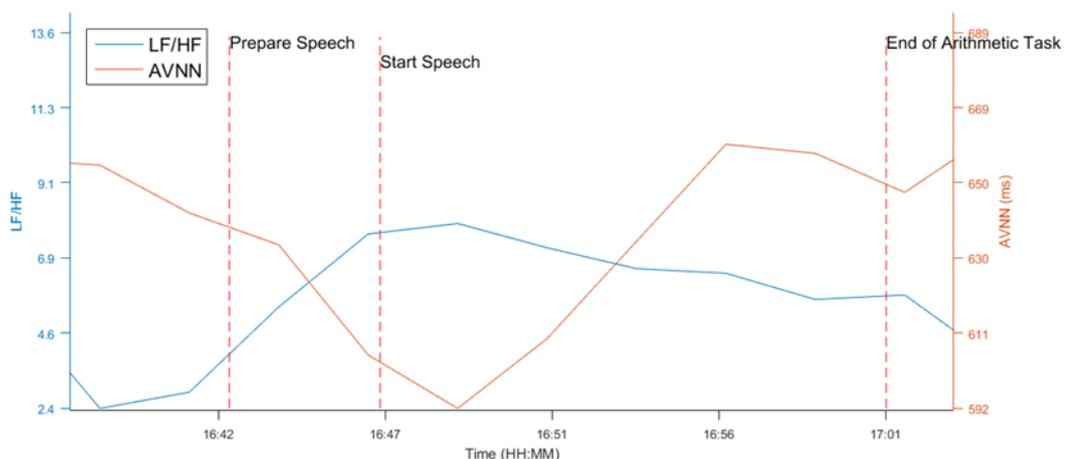


Figure 04. ECG analysis based on AVNN and LF/HF for a participant during the TSST.

STAI scores showed that participants had higher levels of stress during the stress experiment (1.93 ± 0.51) compared to the non-stress condition, before the TSST (1.80 ± 0.56). However, Wilcoxon test does not confirm the significant difference in the two stages.

7. Discussion

This study aimed to assess psychophysiological stress among different occupational groups and to understand if there are differences between these groups, using self-reports and HRV measures. Physiological results found support for the importance of using the TSST as a gold standard procedure designed to produce a psychophysiological stress response. ECG-derived measures were indicative of stress, when compared to normative values (Voss et al., 2015). Lower values of AVNN during the TSST suggest an overall increase in heart rate or cardiac sympatho-excitation, typical in stress situations (Tharion, Parthasarathy, & Neelakantan, 2009). The AVNN is an indicator of the ratio of the cardiac sympathetic to parasympathetic tones. A similar study conducted with students using TSST, showed that AVNN metric was the most reliable metric to recognize mental stress (Pereira, Almeida, Cunha, & Aguiar, 2017). The trend of AVNN proves its efficacy in distinguishing mental stress among different occupational groups. Changes in the RMSSD and pNN50 are both reported in the literature to reflect parasympathetic modulation ("Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology," 1996). Hence, decreases of RMSSD and pNN50 during the stress condition reflected a depressed HRV, typical during stress (Castaldo et al., 2015). Within the frequency-domain, a significant increase in the LF/HF ratio during the stress condition was also found, also indicating the effect of stress on sympathovagal balance. Higher values reflect the domination of the sympathetic system over the parasympathetic one; typical of stress responses (Kaur, Bhalla, Bajaj, Sanya, & Babbar, 2013). These results were concordant with an elevation of self-perception measures of stress (STAI 6-item) in the stress condition.

The significant reduced AVNN in the student group along with other ECG-derived measures showed a consistent behaviour, suggesting more stress in the student group. A possible explanation of these results could be related with the fact that these groups have different work experience backgrounds. Firefighting and air traffic control are known for being very stressful occupations (Jou, 2013) (Perrin et al., 2007). Therefore, these professionals would be more used to dealing with stress on a daily basis, particularly with the unpredictability and uncontrollability of daily encounters, and these components are part of the TSST. Another possible explanation could be the age difference. Students in this sample were young compared to the other two groups. Being still young could have caused the stress levels to be higher compared to older people.

This study has limitations, particularly the small sample size and the simplistic protocol of the study. Despite that, this study shows that the TSST is a useful tool for the assessment of stress in different groups. Possible implications of this study could be the design of protocols whereas is important to assess the effects of stress on individuals, using experimental settings (ex., examining the effects on working memory and attention (Olver, Pinney, Maruff, & Norman, 2015)). Findings confirm the negative impact of stress in a broad range of cognitive functions. Additionally, the TSST can be used to investigate

biological pathways between stress and cardiovascular disease (Birkett, 2011). Finally, the use of this type of bio-sensing platforms, in association with a wide range of wearable devices recently developed within the Internet of Things (IoT) can be a valuable contribution for the development of “smart”, low-cost, simple and affordable quantified health technologies extended to a broad range of occupations.

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