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Review article

Stress Evaluation through Electrophysiological Parameters: Current Research Perspectives, Legal and Ethical Aspects and Future Directions

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Abstract: Stress is an important component of current life and medical practice and also an actual research subject. It has been linked to a host of different pathologies both as a causative and as an aggravating factor. Stress affects both the hormonal and the immunological functions of the human body. Stress perception and induction were demonstrated to quantifiably modify a number of physiological parameters, which can be readily measured. These parameters evaluate the function of the cardiovascular and respiratory systems and reflect the changing skin physiology; collectively, we refer to these parameters as "stress markers". We reviewed the reliability of the heart rate, heart rate variability, pulse pressure, blood pressure, skin temperature, skin electrical conductance, electromyography, serum and saliva markers, and respiratory rate as surrogate data for stress quantification. Musculoskeletal disorders and stress often interact reciprocally, and this makes the objective evaluation of stress challenging in the context of rehabilitation therapies. Stress monitoring in rehabilitation medicine presents unique challenges due to time constraints, multiple signal interferences and specific patient compliance. We comment on the devel-opment of new technologies that might alleviate these problems in clinical settings and in research, from a technological, legal and bioethical perspective.

Keywords: stress; stress markers; heart rate; pulse rate; skin conduction; skin temperature; respiratory rate

1. Introduction

Defined as the "systemic response occurring when the human body is exposed to any stimuli capable of disrupting its physical or mental equilibrium" [1], stress has been largely reported in literature as having an extensive effect on homeostasis [2]. This is reflected in many pathologies, be they psychological [3-10] or somatic [11-28].

The general, systemic, reaction to stress may have positive values, allowing immediate adaptation to special, potentially dangerous conditions, leading to defense reactions that already belong to the classic 5Fs (fight, flight, freeze, faint, fright). But if the exposure to stress is prolonged or has a high referential intensity, then it is deleterious rather than useful.

The mechanisms through which stress creates illness are largely dependent on the malfunctioning of the hypothalamus-pituitary-adrenal axis and the autonomic nervous system [29] especially through a marked elevation of corticotropin-releasing hormone [30] and cortisol. These changes have been described as being affected not only by major life events, but also by daily hassles, such as work-related stress [31,32]. Given its ubiquity, stress exposure has been recognised by the WHO as the "health epidemic of the 21st century" [33].

In psychological terms, the effects of stress depend on its magnitude, duration, and origin (intrinsic or extrinsic). Most cognitive functions, like attention, memory, and decision-making, are influenced by stress exposure. In acute situations, for instance, cortisol enhances the cognitive functions required for an immediate response. While mild stress has positive effects on cognition, high acute stress impairs memory [34]. In case of prolonged stress, the human hypothalamus will start producing arginine and vasopressin, instead of corticotropin-releasing hormone, an initially beneficial but ultimately maladaptive response [35]. Notably, disruptions of the circadian rhythm due to stress may induce cortisol secretion abnormalities, thus affecting behavioural and emotional parameters [36] and hence the chronic response to stress. Chronically elevated levels of corticosteroid hormones can also directly affect cognition [30,37].

Somatic consequences following stress exposure also illustrate the complexity of the interplay between stress and body functioning. For example, stress-induced hyperglycaemia, despite its short-term benefits in ensuring resources for confrontation with potentially threatening stimuli, in the long term, impairs insulin sensitivity and leads to dopamine-triggered obesity [38,39]. Serotonin released following stress exposure may affect colonic motility [40], contributing to the risk of inflammatory bowel syndrome [41] or potentiating its evolution [42]. Children exposed to chronic stress may display early thyroid dysfunction and insulin resistance, because of hypercortisolism [43].

While all the aforementioned medical conditions are usually associated with psychological stress, physiological stress exposure can have the same effects. For example, exposure to heat can be the cause of increased discharge of catecholamines and cortisol; the same effects on epinephrine, norepinephrine, and cortisol levels were noticed during strenuous exercise, depending on its intensity and duration [44]. Moreover, due to the often unpredictable and transitory nature of environmental stress, subsequent research in this field will likely be focused on evaluating the impact of stress using wearable technology to capture the events and their impact on day-to-day life.

The complex manifestations of stress in its various forms and the subjective nature of stress perception represent current obstacles in standardizing an approach to correctly assess and address this condition. Acute or chronic musculoskeletal disorders (MSD) can influence the quantitative stress evaluation via physiological markers of stress; particularly the heart rate variability (HRV), plasma cortisol and electromyography are influenced due to pain-induced autonomic changes [45]. The main issue seems to be that MSD and stress often interact reciprocally: stress can exacerbate MSD symptoms (through increased muscle tension, impaired recovery and lowering pain thresholds) while MSD-related pain heightens stress reactivity and increase perceived stress, probably due to autonomic dysregulation [45]. This implies that presence of MSD pain confounds stress biomarker interpretation [46].

Physical therapy, including exercise and manual techniques, can reduce stress bias in MSD patients by alleviating pain, improving autonomic function (e.g., HRV), and lowering stress biomarkers (decrease in plasma cortisol [47] and increase of beta-endorphin [48]) while other analyses show an unstable trend of serum cortisol in long–term physical rehabilitation programs [49]. Pain from MSD lowers HRV and elevates sympathetic activity, thus biasing stress measurements toward higher perceived or physiological stress; rehabilitation therapies addresses this by enhancing parasympathetic tone, reducing inflammation and lowering HRV [50,51]. However, past studies were performed with inconsistent HRV measurements protocols, neglecting cofounding effect of stress reduction [51], hence the need for a careful assessment approach in future studies.

In this narrative review, we will endeavour to evaluate and centralize information from a variety of studies and clinical trials which focus on short-term stress induction and detection, pursuing the detection of alterations in physiological parameters, that are directly related to stress, namely the variations of cardiovascular parameters, skin temperature, skin conductance, and respiratory rate. These physiological parameters are among the most studied variables impacted by stress and have demonstrated clear and objective correlations with stress factors. Prolonged stress induction, leading to long-term stress, i.e., chronic stress [52], its effects and potential detection, while a subject of contemporary research (e.g., [53-56]), is not within the scope of this paper. For each physiological parameter discussed, we will consider several representative studies, and we will discuss their experimental findings and particularities. Finally, given the integration of new technologies in medical sensor research [57,58], we will briefly explore the current state of the relevant legal and bioethical context.

There is a vast literature regarding correlation of stress with quantifiable physiological parameters; studies are very heterogeneous in methodology. We reviewed the studies in groups related to each non-invasively measured physiological parameter, attempting to get an overview of agreed influence of stress on physiological parameters. This could be important in clinical judgement of the physiological parameters of patients under high physiological and psychological stress (such as patients with acute or chronic pain, in rehabilitation therapy or suffering from musculoskeletal disorders)

2. Materials and Methods

PubMed, Web of Science, and Google Scholar were searched for English, French, and German language studies, published from 1950 onwards, assessing the manifestations of psychological and/or physical stress, based on physiological parameters. All papers taken into consideration were peer-reviewed original research and review papers. The terms used were "physiological stress", in combination with (1) "cardiovascular parameters" and then "hypertension"; (2) "respiratory parameters" and then "respiratory rate"; (3) "skin conductance"; and (4) "skin temperature".

Inclusion criteria were: (1) use of wearable or otherwise non-invasive sensors, (2) stress induction was either physiological or psychological, (3) the whole measuring process lasted no more than 24 h.

Exclusion criteria were: (1) use of any form of invasive measurements, (2) measurement of effects of chronic stress, (3) measurement of acute stress over periods lasting more than 48 h, (4) papers not in English, French, or German. Specifically for the legal and bioethical section, specialised relevant literature and databases were consulted.

Duplicate records were removed before screening, and the remaining articles were assessed according to relevance to the review topic and eligibility criteria. This review was not registered in PROSPERO, as it was designed as a narrative review rather than a systematic review or meta-analysis. Consequently, no formal PRISMA flow diagram was prepared. For the legal and bioethical section, specialized literature, legal sources, and relevant databases were additionally consulted

The studies that yielded a clear, statistically significant association between physiological response and stress (with a large effect size, if stated by authors) were labeled as “positive”. The studies that found no significant association were labeled as “negative”. The label “ambiguous” was used for the studies that either have found a low correlation or for the studies where authors stated that the results might be due to confounding factors or insufficient sample size.

A notable methodological constraint of our narrative review should be mentioned, namely that due to heterogeneity of investigated stressors, measured outcomes and measurement protocols, a formal meta-analysis was not performed in this study.

3. Physiological Stress and Cardiovascular Parameters

Variations of cardiovascular parameters as stress indicators have already been validated in animal studies [59,60] and in human subjects [61]. Indeed, many researchers have approached this topic from different angles (Table 1). The first research efforts towards these topics were made by Steinhaus et al. [62,63], who used a cold-hip bath and determined if the cold-induced physiological stress was sufficient to lower the pulse rate, and to increase the difference between blood pressure when standing and reclining; pulse rate and blood pressure difference were decreased and increased, respectively.

Based on current relevant literature, heart rate and heart rate variability changes during stressful events are beneficial [64-66]. Heart rate, which is controlled by both branches of the autonomic nervous system [67] in a cooperative manner [68], is considered a good reflection of some physiological parameters [69].

Table 1. Studies on the effects of stress on different cardiovascular parameters.

Parameter Studied	Study Group	Result	Year	Reference
Pulse rate measured by a blood pressure cuff on the calf	52 healthy subjects	Positive – pulse rate increases in response to stress	1954	[70]
Cardiovascular parameters measurement using various methods	22 healthy subjects	Positive – large increase in BP, PP, and pulse rate	1964	[71]
Pulse rate measured by finger electrodes	40 healthy subjects	Negative – there was no significant change in pulse rate in response to stress	1976	[72]
Continuous ECG monitoring via an autonomous device	2 surgeons of different experience levels	Positive – HR variability was increased in more stressful situations	2001	[73]
Heart rate variability measured by finger sensors	10/33/52 (depending on exp. conditions) healthy subjects	Positive – increase of HR in response to stress	2002	[74]
Heart rate variability measured by a photoplethysmography sensor in the thumb; BP with the standard method from the opposite arm	115 essential hypertension patients + 115 healthy volunteers as controls	Positive – BP and HR increase more in the hypertensive patient group	2004	[75]
PPG measurement using a pulse oximeter, during a treadmill stress test	9 healthy subjects	Ambiguous – spindle-like waveforms seem to be associated with volitional fatigue	2006	[76]
PPG measurement using a pulse oximeter, during a treadmill stress test	11 healthy subjects	Ambiguous – the low-frequency waves observed in the PPG may correlate with physical stress levels	2008	[77]

Analysis of psycho-physiological signals (including HR) by the Biofeedback Multimedia Software	85 young participants categorised by depression risk	Positive – HR increased as stress increased, although less so in depressed individuals	2011	[78]
Analysis of ECG using standard electrode placement	5 healthy subjects	Positive – the algorithmic processing of the ECG proved capable of indicating stress levels	2012	[79]
HR measurement using the Polar RS 800 heart rate monitor	2 consultant surgeons and 3 endocrine surgical fellows	Positive – HR variability increases with increased stress	2012	[80]
Analysis of ECG using standard electrode placement	30 male and 30 female healthy subjects	Positive – there were marked alterations in the ECG	2013	[81]
Analysis of ECG using standard electrode placement	17 healthy subjects	Positive – the mathematical processing of the ECG yielded stress-associated HRV	2015	[82]
Heart rate variability recording by patch	20 consultants, surgeons, and surgery residents	Positive – HR variability was higher during more stressful periods	2018	[83]
Heart rate variability recording using a Bodyguard 2 wireless heart rate monitor	8 first-year emergency medicine residents	Positive – HR variability can be used as a stress index	2019	[84]
Heart rate variability recording by an attached heart rate monitor	36 emergency medical care students	Ambiguous – HR increased during stress, but HR variability did not	2020	[85]
Heart rate variability measurement by long-term ECG monitoring	2 cardiac surgery residents and 2 senior physicians	Positive – HR variability increased with stress levels and decreased parasympathetic activity	2021	[86]
Standard ECG followed by mathematical processing of results	23 healthy subjects	Positive – ECG-derived results were able to identify different mental states with over 95% accuracy	2021	[87]
Standard EEG and HRV recordings were performed simultaneously	15 healthy subjects	Positive – it was possible to correlate HRV and EEG with different mental stress levels	2021	[88]
Heart rate variability was studied by continuous 12 h monitoring with a wrist sensor	23 intensive care unit nurses	Positive – HR variation was indicative of stress levels	2022	[89]
PPG measurements by smartphone	102 university students	Positive – smartphone-based PPG can be effectively used as a proximal stress indicator	2023	[90]

A decreased or abnormal (HRV) is commonly observed in a wide number of pathological contexts; in cardiovascular pathologies like hypertension [91], ischemic heart disease [92], and heart failure [93]; in endocrine disorders, such as diabetes mellitus [94], thyroid [95] and parathyroid disorders [96], which may also affect heart vasculature [97]; in cancer and neurodegenerative diseases [98]; and in renal pathologies, such as chronic kidney disease [99]. In addition to all these, autonomic dysfunction, which affects HRV, correlates with cardiovascular mortality, including sudden cardiac death [100] and heart failure-associated mortality [101].

Apart from the influence of heart pathologies on HRV [102], age also influences HRV [103,104], while gender and circadian rhythm are additional modifying factors [105,106]. Also, based on the findings related to autonomic dysfunction mentioned above, HRV can be used as a predictor of cardiovascular morbidity and mortality [107-109]. Moreover, both heart rate and its variability are considered good stress indicators [61]; HRV is amongst the easiest stress indicators for measurement purposes

[110]. Pulse characteristics have also been proven susceptible to stress-induced modification [111,112]. Between the central nervous system and the intrinsic cardiac nervous system the relationship is quite complex [113], and the intrinsic cardiac nervous system itself has both short-term and long-term memory functions, which are important in HRV and BP responses [114]. Recently, HRV biofeedback has been successfully used as an adjunctive treatment in patients with generalised anxiety disorder [115] opening new potential therapeutic avenues apart from treatment avenues.

Speaking about cardiovascular parameters, in the context of this review, we refer to blood pressure (BP), pulse rate (PR), pulse pressure (PP), heart rate (HR) and HRV. We have also included studies focusing on electrocardiogram (ECG), albeit most of them are used for HRV evaluation. Specifically, in the case of ECG and of experiments in controlled environments, a significant constraint is the duration of the ECG recording. It has been proposed by the European Society of Cardiology that, for proper HRV analysis in time and frequency domains, the ECG recordings have to last longer than 5 minutes [116]. Although ultra-short-term recordings (less than 5 minutes) and long-term recordings (> 24 h) are possible, the relevant physiological values usually exhibit poor correlation [117]. However, in controlled experiments, where the stressor is acute, variations in the ECG can be recorded in seconds or some minutes [118]. Finally, a few experiments listed here were performed using photoplethysmography, which is a useful tool in clinical practice [119], despite the fact that some of its aspects have not yet been fully elucidated [120].

Baker & Taylor [70], using pressure and electricity application, found that pulse rate increased in reaction to psychological stress. While cold-induced stress can also be classified as physical stress, we may assume that lower pulse rate, in the study of Steinhaus [63], may be somehow related to cold-induced vasoconstriction [121]. Keatinge et al. [71], using ice-cold showers over the chest, i.e., a physical stressor, found a large increase in both diastolic and systolic pressures, and in PP and HR. On the other hand, Boudewyns [72] did not ascertain any significant changes in PR in response to physical and psychological stress.

In 2001, Böhm [73] measured the HRV of more and less experienced surgeons, throughout 5 conventional surgeries and 5 laparoscopic surgeries per surgeon. It was found that the laparoscopic surgeries were more mentally stressful for the surgeons and generally correlated with a higher HRV than conventional surgical interventions. Moreover, in 2002, one study [74], using a virtual reality environment, concluded that HR increased in response to psychological stress [75]. In 2004, based on psychological stress induction, Kaushik et al. [75] concluded that in hypertensive patients exposed to stress, both HR and BP are higher than in non-hypertensive patients.

In 2008, Linder et al. [77] used PPG (photoplethysmography) to estimate the haemodynamic stress via detectable changes in skin blood flow, in 11 healthy subjects, to whom he induced physical stress with the Bruce Protocol treadmill test [122]. The researchers observed that the occurrence of low-frequency waves before the onset of volitional fatigue may be a suitable indicator of haemodynamic stress [77]. This experimental work was based on the earlier work of Linder et al. [76], who had used a similar methodology and achieved similar promising, but not conclusive, results.

Thanks to the study of Lin et al. [78], we have valuable information available in regard to several physiological parameters. Adjustments were made in the determination of heart rate, so that blood volume is reflected [123]. HRV was found to be affected by high levels of psychological stress, which stemmed from the teaching of surgical procedures and their actual application, as was demonstrated by Prichard et al. [80] throughout 50 endocrine surgeries. Through showing different short videos [124], each corresponding to different emotions, Bong et al. [79] demonstrated that, despite the size of the sample, through brief experimental processes, the application of certain algorithms can detect mental stress on ECG. In 2013, Karthikeyan et al. [81] used the Stroop colour word test [125], concluded that acute psychological stress can be measured in short-term ECG with over 90% accuracy. In 2015, Oh et al. [82] recorded 10 minutes of ECG signals while applying auditory stressors in 17 healthy subjects. The results from the mathematical processing of the ECG signals, regarding HRV, were quite promising.

In one pilot study conducted for 2-3 days, HRV was recorded in consultants, surgeons, junior, and senior residents during surgeries [83]. The researchers found that HRV increases during the most stressful intervals, which corresponded to surgical interventions. Therefore, it is certainly demonstrated that surgeries are associated with high occupational stress levels, and HRV [126-128].

In 2019, Mefford et al., [84], using emergency medicine residents performing intubations as their sample, found out that occupational stress is negatively correlated with HRV, the authors considered this a manifestation of increased autonomic arousal. The study of Stein et al. [85], on emergency medical care students participating in scheduled simulated assessments, revealed decreased HRV variability and parasympathetic withdrawal; however, the differences between the test period and the control/rest period were not significant. There was also no linear relation detected between HRV variability and anxiety levels [85].

Using the Montreal pressure experiment and ECG monitoring, Zhou et al. [87] assessed whether it is possible to perform a biometric analysis of the ECG under different psychological pressure states; the accuracy of ECG-based detection of stress levels proved to be at 95% [87]. In the same year, Awad et al. [86] analysed stress levels in cardiac surgery residents as compared with stress levels in their senior counterparts, using long-term ECG monitoring. He concluded that the higher stress level is obtained in surgery residents; this is correlated with a lower parasympathetic activity [86].

One study [88] demonstrated five significant relationships between electroencephalogram (EEG) and HRV features associated with psychological stress, which represent useful findings in terms of future perspectives in monitoring stress levels and their fluctuations. The experimental conditions included three different states: rest, stress induction, and meditation. The study of Ahmadi et al. [89], concluded that HRV was indicative of occupational stress, and could effectively be used as a stress marker.

In 2023, a different approach was adopted by Nelson et al. [90], using a smartphone-based PPG, to eliminate the need for a study in a laboratory setting. They used the Trier Social Stress Test [129], coupled with self-reported stress and anxiety levels. They determined that PPG provides a proximal measure of psychological stress and anxiety.

4. Physiological Stress and Skin Temperature Variation

Thermoregulation in general is defined as the homeostatic mechanism of the human body to maintain the level of internal body temperature as constant as possible, despite environmental changes [130]. Generally, thermoregulation is effective under conditions of physical stress and can maintain body temperature within safe limits [131]. In general, thermoregulation is believed to be performed by the brain [132,133], whilst newer theories dispute this [134,135]. Regardless, it seems that there is a degree of reversible adaptation of thermoregulatory mechanisms, at least to prolonged physical workload and therefore stress [136].

Several factors influence skin thermoregulation, such as body mass, so that an increased muscle mass will aid in heat preservation [137]. The variations of body temperature as a reaction to stress and anxiety have been the subject of numerous studies since the 1950s [138-140]. Anyway, the phenomenon itself has been known since the early 1910s [141-143].

Stress is predominantly associated with an increase in central arterial blood flow and a decrease in venous blood flow, in a phenomenon known as stress hyperthermia [144]. In the extremities, the opposite happens as there is peripheral vasoconstriction with a decrease in temperature [145,146]. Interestingly, studies on animals also reveal an association between stress and skin temperature. For example, skin temperature variations have been documented as a chronic stress marker in laying hens (*Gallus gallus domesticus*), by Herborn et al. [147]. In humans, some studies have investigated the link between skin temperature and stress (Table 2). Historically, the first study was conducted by Mittelmann & Wolff [148], who specifically focused on the comparison between patients with Raynaud disease and subjects not afflicted by this pathology [149,150]. They found that both healthy subjects and those suffering from Raynaud disease experienced drops in finger temperature in response to affective stress, although in the second case, these were accompanied by pain and cyanosis [148]. Notably, most studies were successful in documenting a stress-related temperature variation, although the correlation was not always positive.

Baker & Taylor [70] found that skin temperature increased in response to emotional stress. The latter experiment of Boudewyns [72], using three different experimental protocols, found that finger skin temperature is negatively correlated with both physical and emotional stress, thus confirming the results of Mittelmann & Wolff [148].

The study of Meehan et al. [74], already referred to previously in the cardiovascular parameters section, demonstrated that the increase in psychological stress is correlated with a skin temperature drop in the fingertips. The opposite result was obtained by Kaushik et al. [75], where skin temperature increase was higher in psychologically stressed hypertensive patients compared to healthy individuals, the authors concluding that it is possible to correlate skin temperature with sympathetic system activity. Moreover, based on the study of Lin et al. [78], we may conclude that psychological stress decreased finger temperature, even more so in depressed individuals, compared to relatively psychologically healthy individuals, further highlighting the immediate effects of stress on the sympathetic, rather than on the parasympathetic nervous system.

Vinkers et al. [151] measured the intestinal core temperature, significantly reduced in response to psychological stress, such as the skin temperature. There was a fluctuation between the significance of these latter changes, based on sex and measurement location, but overall, the authors observed a general decrease of skin temperature in distal sites. Curiously, the measured decrease in intestinal core temperature seems to be at odds with experimental data from animal studies [152-154]. However, the mesenteric blood flow, and consequently temperature, has also been found to be decreased in a previous stress-induction experiment [155] and this may be consistent with the diversion of blood flow from the gastrointestinal tract during the initiation of the fight-or-flight response [156]. We would like to note at this point, the fact that all female participants took oral contraceptives may have affected the experimental results, as there is ongoing research linking such drugs with an abnormal stress response [157,158].

The study of Pérez-Guarner et al. [159] on marathon runners indicated that, while there was a skin temperature variation on the posterior upper limb and knee, correlated with creatine kinase levels and pain perception, respectively, basal skin temperature in general is not a reliable method to gauge physical stress; the results of this study are therefore ambiguous. On the other hand, Ahmadi et al. [89] reported, based on 12 h continuous monitoring of the wrist skin temperature, a positive correlation between occupational stress and skin temperature levels.

Finally, the study of Gioia et al. [160] concluded that nose temperature was reduced during the psychological stress, although no significant variations were detected in other facial areas. Moreover, the study had several limitations as discussed by the authors. Crucially, it seems that the reproducibility of skin temperature variations, at least based on some stimuli, is feasible, based on the experiments of Priego-Quesada et al. [161].

Table 2. Studies on the effects of stress on skin temperature variation.

Parameter Studied/Study Methodology	Study Group	Result	Year	Reference
Finger temperature measured by a thermistor unit	52 subjects	Positive – finger skin temperature was increased during stress	1954	[70]
Finger temperature measurement with surface electrodes	20/40/133 (depending on exp. conditions) healthy subjects	Positive – finger skin temperature was found to decrease with increased stress	1976	[72]
Skin temperature measured by finger sensors	10/33/52 (depending on exp. conditions) healthy subjects	Positive – finger skin temperature was found to decrease noticeably in individuals exposed to stress	2002	[74]
Peripheral temperature measured in the middle finger by photoplethysmography	115 essential hypertension patients + 115 healthy volunteers as controls	Positive – finger skin temperature of hypertensive patients was higher when stressed, compared to healthy volunteers	2004	[75]
Analysis of psychophysiological signals (including finger temperature) by the Biofeedback Multimedia Software	85 young participants categorised by depression risk	Positive – finger skin temperature decreased with stress induction; this phenomenon was even more pronounced in depressed individuals	2011	[78]
Skin temperature measured with different methods at different sites	22/24 (depending on exp. conditions) healthy subjects – all female participants on oral contraceptive therapy	Positive – a gradient-like temperature response to stress was recorded, with the greater temperature decrease associated with the more distal locations	2013	[151]
Skin temperature of the posterior upper limb and knee + individual blood markers	17 healthy marathon runners	Ambiguous – while there was some temperature variation correlated with pain and creatine kinase perception, temperature variation was a poor predictor of physiological stress	2019	[159]
Skin temperature recovery following the cold stress test	14 physically active participants	Positive – good reproducibility of the results was shown for the posterior leg region	2021	[161]
12 hr continuous skin temperature monitoring on the wrist	23 intensive care unit nurses	Positive – positive correlation between skin temperature and stress levels was identified	2022	[89]
Thermal imaging of various facial regions and cross-mapping	30 healthy Caucasian volunteers	Ambiguous – there was a negative correlation between nose temperature and physiological stress, but not any other significant variation in other facial areas	2023	[160]

5. Physiological Stress and Skin Conductance Variation

Skin conductance is characterised by both a phasic and a tonic component [162,163]. The tonic component is a slow-changing conductance response to non-specific stimuli – this is greatly affected by personality traits [164-167]. Phasic components reflect rapid variations in response to specific stimuli. This second component is also influenced by perspiration [165,166]; when sweat is eliminated and the ducts are closed, this phasic component will largely subside. The activation of the 'fight or flight' system causes the filling of the palmar and plantar sweat glands and the increase of skin conductance before the sweat is removed with a following decrease of sweat and skin conductance [168]. Therefore, an increase in the number of skin conductance fluctuations and the amplitude of skin conductance fluctuations can be utilised for the evaluation of the sympathetic nervous system [168,169] and, by implication, of the stress levels of an individual. When discussing skin conductance measurements, it is important to take into account the multitude of potential confounding factors [170]. Skin conductance with induced stress levels is one of the most well-researched physiological parameters (Table 3).

Starting with the research of Baker & Taylor [70], skin conductance was found to be increased during psychologically stressful periods. Later on, Meehan et al. [74] used a virtual reality setting and determined that a psychological stress-associated variation of skin conductance occurred, but did not fulfill all the necessary aspects to be considered a reliable stress indicator. Such a change, along with the change in heart rate, was attributed to the height reaction [171,172] induced in the virtual reality environment.

Skin conductance changes in response to perioperative stress were studied by Storm et al. [173]. It was determined that such changes might indeed be a useful tool in studying perioperative stress. Interestingly, the variations of skin conductance were positively correlated with BP and blood epinephrine levels [173]. Based on the research of Kaushik et al. [75], skin conductance did not increase as much in hypertensive patients, potentially due to a neuromuscular or some other dysfunction.

Another approach, using picture perception, was adopted by Najström & Jansson [174], who determined that the variations of skin conductance in response to psychological stress can be used as a predictor for future emotional responses to stress. On a relevant note, the already presented study of Lin et al. [78] determined that skin conductance increases in response to psychological stress, a phenomenon more pronounced in depressed individuals.

Another interesting experiment, conducted by Memar & Mokaribolhassan [175], evaluated skin conductance as a response to stress, in drivers during real-time conditions. The data were collected from the MIT-BIH database [176]. The study revealed an increase in skin conductance. Interestingly, it is noted that the effect of temperature on skin conductance in such experiments is quantifiable enough to potentially skew some values [177]. The recent research of Scavone et al. [178], including an emotional stressor task [179,180] and mindfulness induction [181], noticed that skin conductance, which correlates with sympathetic stimulation, depends on stress perception and on the psychological state of the individual.

Bari et al. [167] measured skin conductance variations on university students, both during mental stress and relaxation in two phases with a degree of interdependence between. Finally, the study of Ahmadi et al. [89] found that skin conductance, evaluated through electrodermal activity, did not effectively correlate with psychological stress levels.

Table 3. Studies on the effects of stress on skin conductance variation.

Parameter Studied/Study Methodology	Study Group	Result	Year	Reference
Electrodes are attached to the middle finger	52 subjects	Positive – skin resistance decreases, therefore skin conduction increases during stress	1954	[70]
Skin conductivity measured by finger sensors	10/33/52 (depending on exp. conditions) healthy subjects	Ambiguous – while there was a variation in skin conductance, it was not uniform across the different experimental conditions	2002	[74]
Skin conductivity measured by electrodes on the dorsal and palmar side of the hands	11 patients scheduled for elective laparoscopic cholecystectomy	Positive – skin conductance was found to vary between conscious and sedate patients	2002	[173]
Skin conductance measured by electrodermography	115 essential hypertension patients + 115	Ambiguous – skin conductance increased in proportion to stress, but less compared to healthy individuals	2004	[75]

	healthy volunteers as controls			
Skin conductivity measured by electrodes on the index and second fingers of the non-dominant hand	73 police officers recruited to enter active duty	Positive – skin conductance changes may be used to predict future individual responses to stress	2007	[174]
Analysis of psychophysiological signals (including skin conductance) by the Biofeedback Multimedia Software	85 young participants categorised by depression risk	Positive – skin conductance increased in response to stress, and even more so in depressed individuals	2011	[78]
Measurement of skin conductance response with two stainless steel finger plates	54 undergraduate students	Positive – skin conductance was dependent upon stress and the psychological state of individuals	2020	[178]
Measurement of skin conductance with EDA electrodes	40 healthy university students	Ambiguous – skin conductance, measured in two phases; it was realised that there may be a degree of interdependency between these two phases	2021	[167]
Skin conductance evaluated via the MIT-BIH database	17 drivers in real-time conditions; 8 selected for final data analysis	Positive – skin conduction (termed galvanic skin response) is a reliable indicator of stress	2021	[175]
Skin conductance was evaluated via electrodermal activity, using 12 hr continuous monitoring on the wrist	23 intensive care unit nurses	Negative – not sufficient correlation between skin conductance and stress levels	2022	[89]

6. Physiological Stress and Respiratory Rate

Respiratory rate (RR), also known as respiratory frequency [182], is generally considered an important clinical marker for the physiological and psychological status of an individual [183-185]. Normal RR range is between 12 and 18 respirations per minute [186]. An elevated RR over 20, termed tachypnoea, is often indicative of severe pathological situations, such as sepsis, shock, heart failure, and respiratory insufficiency [186-189]. The opposite condition, bradypnoea, when the RR is lower than 8 respirations per minute, is frequently observed in drug-induced narcosis or sedation [190,191].

Regarding the control of respiration, the electrical stimulation of the amygdala can change breathing patterns [192,193]. The amygdala has a key place in anxiety and stress responses, influencing the RR alongside other related parameters such as pCO2 and respiratory depth [194-197].

Furthermore, respiratory control is also exerted by the sympathetic system [75] and may be modulated by the anterior cingulate cortex and brainstem [198-200]. Because RR is a fundamental physical process, which can be both conscious and unconscious, i.e., non-modifiable and modifiable, it is considered a good biomarker for well-being [201]. In all the research groups included in our study, the subjects were healthy from a respiratory system point of view, as RR and other respiratory parameters are usually affected in patients suffering from asthma [202,203], obstructive pulmonary pathologies, either occupationally- or environmentally-induced or otherwise [204-208].

An early trial, conducted by Keatinge et al. [71], also mentioned in the cardiovascular section, determined that ice-cold showers over the chest were sufficient to increase the respiratory rate. One clinical trial conducted by Masaoka & Homma [209], analysed respiratory patterns during mental stress and physical exposure to stressors. The study`s results showed that participants' anxiety levels were correlated with an increased respiratory rate. Interestingly, the authors discussed the possibility that the respiratory responses of different test subjects may reflect different personality traits or psychological and physiological disorders [210-212].

In another study, again using exposure to both mental and physical stress, respiratory frequency was found to increase more during physical than mental stress, although respiratory depth and pCO_2 increased only during physical stress [213]. The study concluded that there is a difference in vagal modulation between physical and emotional stress states. It is highly likely that the feedback from active muscles under conditions of physical stress [214-216] is enough to cause a difference in the psychological stress state to the extent where only the brain exerts an influence on respiration. The modulation is expected to be parasympathetic in origin, at least as far as the initial stages of exercise are concerned [216,217].

The study of Kaushik et al. [75], while evaluating the stress-inducing potential of everyday actions, found that RR increased notably in hypertensive individuals, compared to non-hypertensive individuals. Conversely, the study of Lin et al. [78] failed to detect any significant effects of stress or depression on respiratory rate.

Table 4. Studies on the effects of stress on respiratory rate variation.

Parameter Studied/Study Methodology	Study Group	Result	Year	Reference
Respiratory rate measured using a Tissot spirometer	22 healthy subjects	Positive – there was a measurable respiratory rate increase	1964	[71]
Analysis of breathing patterns using a respiratory pattern gas analyser	10 healthy males	Positive – a positive correlation between stress and respiratory rate was observed	1997	[209]
Analysis of breathing parameters with the VU-AMS version 4.3 system	22 healthy subjects	Positive – respiratory rate increased more during physical exercise and less during mental exercise	2002	[213]
Respiratory rate measurement with a respiratory belt and sensor applied to the upper part of the chest	115 essential hypertension patients + 115 healthy volunteers as controls	Positive – there was a marked increase in respiratory rate in hypertensive patients, compared to healthy volunteers, during stress	2004	[75]
Analysis of psychophysiological signals (including respiratory rate) by the Biofeedback Multimedia Software	85 young participants categorised by depression risk	Negative – The respiratory rate was not significantly affected by stress induction	2011	[78]

7. Legal and Ethical Challenges

Nowadays, the integration of new technologies in medicine [218-223], in general, and in sensors [224,225] in particular, has raised a host of legal and bioethical challenges [226]. The integration of artificial intelligence (AI), into stress monitoring and evaluation [227,228], has further complicated matters. The recognised problems and challenges have driven the development of new legislation and, while the necessity of protection of patients’ rights in a bioethical context necessitates novel approaches.

7.1. Legal Considerations

The potential integration of wearable, non-invasive biosensors paired with the use of artificial intelligence (AI) in stress monitoring and evaluation raises a series of complex legal challenges, which must be addressed to ensure compliance with the European regulatory framework.

First and foremost, it is critical to highlight that the processing of biometric and physiological data, such as heart rate variability, skin conductivity, and body temperature, falls within the scope of the General Data Protection Regulation (GDPR) [229], as the aforementioned ones constitute special category personal data (Art. 9 GDPR) classified as sensitive. As such, it is imperative to acquire the explicit informed consent of the individuals concerned. The collection and processing activities of sensitive data are also accompanied by strict purpose limitation, data minimisation and robust security measures.

A concrete example that illustrates the risks of deploying such devices in research or healthcare settings is the Royal Free Hospital and Google DeepMind case (UK, 2015–2017) [230]. In that instance, the Royal Free NHS Foundation Trust shared health records of approximately 1.6 million patients with DeepMind. The access was used for monitoring software for mobile devices, called Streams, which promises to improve clinicians' ability to support patients with Acute Kidney Injury (AKI). The UK Information Commissioner's Office (ICO) found that patients had not been adequately informed about the use of their sensitive medical data, and that the data sharing arrangement lacked a clear legal basis under data protection law. The case highlighted the dangers of "function creep" [231], where data collected for one medical purpose is repurposed for AI research without sufficient transparency or safeguards.

Furthermore, the EU AI Act [232], enforced as of February 2, 2025, classifies systems that infer emotions or stress from biometric signals in workplaces or educational settings as unlawful in most contexts, unless applied solely for medical or safety reasons, thereby potentially constraining emotion inference algorithms in stress monitoring devices depending on the respective specific context [233]. At the same time, organisations developing AI systems classified as high-risk must conduct fundamental rights impact assessments and ensure compliance with the obligations deriving from the AI Act (Art.16, Art. 60, etc.), always aligning with pre-existing regulations such as GDPR (Art. 2.7 AI Act) mentioned above.

In parallel, the increasing reliance on connected wearable devices necessitates adherence to the Network and Information Systems Directive (NIS2) [234], in force since late 2023, which imposes cybersecurity and incident reporting obligations on operators of essential and important digital services (e.g. healthcare providers or medical device manufacturers). Moreover, it mandates proportionate risk management, breach reporting, supply chain security, and personal accountability for senior executives [234].

A concrete example that illustrates the risks at stake comes from the 2017 disclosure of severe vulnerabilities in Medtronic's implantable cardiac devices [235], where researchers demonstrated that hackers could exploit unencrypted communications between pacemakers and external monitors to manipulate settings or drain batteries. Although Medtronic later patched the flaws, the case attracted global media coverage and regulatory scrutiny, highlighting how inadequate cybersecurity in connected medical devices can endanger patient safety and erode trust in digital health technologies. In the context of stress-monitoring wearables, a similar breach could expose highly sensitive biometric data or allow malicious manipulation of device outputs, jeopardising both the privacy and physical well-being of research participants in multiple ways.

Finally, when big data analytics are employed for pattern recognition or behavioral profiling, transparency, fairness, and accountability requirements must be observed, ensuring individuals are not subject to opaque or discriminatory automated decision-making processes (Art. 22 GDPR). This is concerning, as multiple physiological data collected by stress monitoring devices (ECG, plethismography, EEG, etc) could be effectively used as biometrical identification and/or profiling [236,237]. Conclusively, legal compliance calls for embedding data protection, cybersecurity and governance by design into the entire technological architecture for stress monitoring systems.

7.2. Bioethical Considerations

From a bioethical perspective, deploying wearable stress sensors and AI-driven analytics must be grounded in the core ethical principles of autonomy, beneficence, non-maleficence, and justice [238]. In the context of this article, autonomy translates to the requirement that individuals provide fully informed consent, understanding not only the immediate data collection but also potential future inferences and uses. Beneficence entails ensuring that the measurement of physiological signals genuinely benefits individuals without introducing unnecessary anxiety or misinterpretation of stress levels. Specifically about this matter, current literature highlights risks of over estimation of stress metrics and "false positives" that may inadvertently harm well-being [239]. Non maleficence similarly demands that continuous stress monitoring avoids psychological harm, stigmatisation, or misuse; especially in employment or caregiving settings. Finally, justice requires that such technologies do not disproportionately burden or exclude vulnerable populations, such as older adults or individuals with mental health vulnerabilities. The potential for misapplication, such as using stress data for performance evaluation or surveillance, raises concerns about ethical misuse of medicalised metrics in non-medical contexts [240]. Accordingly, interdisciplinary oversight by legal experts, clinicians, ethicists, and technologists is essential to ensure that innovations in stress monitoring respect individual rights and promote health rather than unintended harm.

8. Discussion

From the aforementioned, it is obvious that, in the overwhelming majority of instances, there is a notable variability regarding the interpretation of physiological parameters in response to stress, mainly because different researchers have used different methods and have sometimes reached different conclusions. First of all, regarding cardiovascular parameter variability in response to stress, from the 22 studies mentioned, only one study, that of Boudewyns [72], had a distinctly negative result in respect to a notable change in cardiovascular parameters in response to stress, and other three studies [76,77,85] had ambiguous results. The remained studies demonstrated a significant and quantifiable variability of some cardiovascular parameters in response to physical and mental stress.

Regarding skin temperature, out of the 11 studies mentioned, 7 studies were concerned with skin temperature fluctuations at the extremities; 5 studies reported a decrease in finger skin temperature in response to stress [72,74,78,148,151], while two reported the opposite result [70,75]. The research of Vinkers et al. [151] specifically mentioned that the temperature decrease was greater in more distal body locations. Regardless, all the above-mentioned 7 studies are considered to have a positive result, in order that stress level was actually correlated with a variation in skin temperature. Other studies, such as that conducted by Priego-Quesada et al. [161] and Ahmadi et al. [89] confirmed this relationship. Anyway, for different reasons, we have classified the results of Priego-Quesada et al. [161] and Gioia et al. [160] as ambiguous.

In the case of skin conductance, out of the total of 10 studies mentioned, only one, that of Ahmadi et al. [89], had a decidedly negative outcome, i.e, it did not find any quantifiable correlation between stress and skin conductance. Two other studies had ambiguous results- Meehan et al. [74] reported that the was not a uniform variation of skin conductance in response to stress, and Kaushik et al. [75] mentioned that hypertensive patients did not exhibit such a pronounced conductance increase compared to healthy individuals. The remaining 7 studies [70,78,173-175,178] all demonstrated a positive result, meaning that there was a quantifiable fluctuation of skin conductance in response to stress.

Last but not least, in the case of respiratory rate, out of the 5 studies mentioned, four had a positive correlation between stress exposure and RR [71,75,209,213].

8.1. Comparative Results for Stress Detection

Multiple studies have documented the fluctuations of more than one of the parameters heretofore discussed, in response to different types of stress, emotional or physical, real or perceived. For example, Atanassov [241] reported changes in respiration, pulse rate, blood pressure, and body temperature, among other parameters, in subjects under catharsis as a psychotherapeutic treatment. Physical stress, in the form of mild exercise, affects dermal blood flow and sweating, and, consequently, skin temperature and conductance respectively [242]. In one study, carried out in the UK, the thermal stress, evaluated through the increase of core body temperature, was found to increase heart rate and respiratory rate [243]. From the studies presented herein, a notable number have measured parameters of different nature [70,71,74,75,78,89,173] (Table 5).

Early results from the research by Baker & Taylor [70], who induced stress by an electrical spark and induced muscular tension, revealed that there was a positive correlation between stress, skin temperature, skin conductance, and pulse rate. A subsequent experiment [71], represented by a cold water shower, concluded that it was sufficient to induce measurable changes in both cardiovascular and respiratory parameters, i.e., represented by an increase in pulse pressure, pulse rate, blood pressure, and respiratory rate.

The experiment of Meehan et al. [74] demonstrated that stress induction in a virtual environment designed to induce, among others, a height reaction, i.e., the placement of subjects in a stress-inducing virtual height situation, was enough to cause a clear HR increase and a decrease of finger skin temperature. Notably, the same study showed that skin conductance varied but not consistently relative to different stress levels. The study of Storm et al. [173], on patients at a perioperative period, found that skin conductance dropped significantly during anaesthesia, and sudden rises in sedated patients may signal a decrease in the depth of anaesthesia. The same study showed a positive correlation between BP and skin conductance changes, although this last variable was used, along with hormonal levels, to gauge the general physiological state of the patient. Anyway, we have not included this study in the cardiovascular parameters section.

Stress induction, via the simulation of everyday aversive, demanding or difficult activities, such as mental arithmetic, was sufficient to cause a greater increase in skin temperature, respiratory rate, and cardiovascular parameters, compared to healthy patients; interestingly, skin conductance increased more in healthy patients [75]. In the study of Lin et al. [78], where stress was induced by mental arithmetic testing, skin temperature dropped, but skin conductance and heart rate increased, with no changes in RR. These responses were blunted in depressed individuals. Finally, in a rather different setting, analysing physiological parameters in nurses after 12-hour shifts [89], leads to the conclusion that stress was found to increase skin temperature and heart rate, without any effect on skin conductance.

Table 5. Comparative presentation of stress-induced variation of different parameters.

Study	Year	Skin Temperature	Skin Conductance	Respiratory Rate	CV Parameters	Reference
Stress response to physical stimuli (electrical spark & induced muscular tension)	1954	Skin temperature increases in response to stress	Decrease of skin resistance, i.e., increase of skin conductance, during stress	n/a	Pulse rate increases in response to stress	[70]
Stress induction by a cold-water shower on the chest	1964	n/a	n/a	Pulse rate increased	Large increase in BP, PP, and pulse rate	[71]

Stress induction in a VR environment	2002	Noticeable decrease in finger skin temperature	Stress-induced variation	n/a	HR increases along with stress	[74]
Operation-related stress evaluation	2002	n/a	The fall of skin conductance positively correlated with the depth of anaesthesia	n/a	BP positive correlation with skin conductance fluctuation	[173]
Stress induction by simulated everyday activities	2004	Increase in skin temperature commensurate with stress	Low positive correlation with increased stress	Positive correlation with stress levels	HR increase, BP increase	[75]
Stress induction by mental arithmetic testing	2011	Skin temperature drops during stress; the phenomenon is more evident in depressed individuals	Skin conductance increases during stress; the phenomenon is more evident in depressed individuals	No significant change in respiratory rate in response to stress	HR increases, although less so in depressed individuals	[78]
Stress evaluation in nurses on 12 hr shifts	2022	Positive correlation between skin temperature and stress	No definite association with stress	n/a	HR increases commensurate with stress	[89]

From the comparative presentation above, it is obvious that in individuals under stress, whether mental or physical, more than one physiological parameter is affected. This is only natural, considering the interplay between different physiological parameters, even in the absence of any stressor. For example, let's consider the influence of respiration on heart rate; respiration and heart activity are linked, so that inspiration causes an increase in heart rate, and expiration has the opposite effect. Breathing with a respiratory rate of 6 respirations per minute will cause a considerable oscillation of heart frequency [244]. Moreover, the outputs from the heart and breathing influence brain areas involved in emotion regulation [245]. At this respiratory frequency, heart activity, brain processing (consciousness, emotions, reasoning), endocrine and immune activity are in harmony, a phenomenon named coherence. Cardiac coherence, i.e., the coordination of heart and cerebral activity, is observed in various spiritual practices [246]. In particular, breathing with a frequency of 6 breaths/minute leads to a resonance frequency which is associated with a better autonomic nervous system activity and a better physiological functioning, overall, an improvement in well-being [247,248].

The clinical implications of the alterations of physiological markers of stress response may be significant, considering that the parameters discussed are regulated by both sympathetic and parasympathetic components of the autonomic nervous system. As indicated by Lin et al. [78], depression may initially affect the sympathetic division of the nervous system, followed by the parasympathetic one. The current classification of depression severity is still largely based on questionnaires [249-251]. We propose that by using physiological stress markers, such as those discussed heretofore, it might be possible to grade a subject's depression severity and adjust the therapy for an early-stage sympathetic to a late-stage parasympathetic one. A potential problem in that regard would be the inconsistencies, specifically in terms of skin conductance variation, depending on the individual's state of mindfulness as evidenced by a couple of studies [252,253]. Regardless, based on a comparative study between different machine learning algorithms, it was determined that an accuracy of over 90% is possible when trying to detect stress based on skin conductance [254]. On a related note, stress is known to alter the inflammatory processes at the level of the skin [255,256], and will presumably affect some of the physiological stress parameters. More specifically, it has been proven that the application of capsaicin, a natural compound with a lot of beneficial properties [257-261], at the level of the skin, will result in a higher degree of inflammation, in stressed organisms [262-264].

The effects of pre-existing stress and depression on these markers has also been discussed by Rabavilas et al [265]. The emotional state affects such markers even in animals, as demonstrated by a number of authors [266-269].

8.2. Electromyography (EMG) Use in Stress Detection

Ancillary electrophysiological measurements that might be adapted to wearable sensors in the future include electromyography (EMG), a technique which already has numerous applications [270-272]. The investigations for its use on stress detection started towards the end of the 20th century and are based on the premise that stress induces muscular tension, in a similar fashion to physical workload [273,274]. As physical and emotional stress conditions result in the release of hormones and mediators, the muscles are subsequently affected [275,276].

The research of Lundberg et al. [277], using as a sample 62 women of various ages, performing different stress tests determined there was a significant increase in the tension of the trapezius muscle during mental stress. Using a similarly large sample of 70 subjects, Luijcks et al. [278] performed EMG in the trapezius muscle, and determined that it is possible to use muscle activity to quantify individual susceptibility to stress.

Another research effort, that of Van Galen et al. [279], used a sample of 10 participants with a high trait anxiety score and 10 participants with a low trait anxiety scores; their scores were determined using the Spielberger's Trait and State Anxiety test [280]. Cognitive stress was induced using a computer task; the test revealed increased neuromotor activation during times of mental stress.

The recent research of Pourmohammadi et al. [281] studied at the same time the EMG and ECG results of 34 healthy students; for mental stress induction, a variety of methods were used, comprising mental arithmetic, the Stroop colour test, time pressure, and a perceived stressful environment. It was thus demonstrated, by comparison to the ECG, that EMG can be used for multilevel stress detection.

It has been proposed that the signal of the EMG is more accurate in prediction stress, compared to pulmonary parameters [282]. Aside from the oft-used trapezius muscles, other muscles may be studied through EMG for stress detection purposes [283]. Even so, the use of EMG at this moment is not as widespread and as highly regarded as other indicators, most prominently cardiovascular parameters. Part of the problem is that, at the moment, most researchers using EMG to detect stress, aim at a binary outcome, i.e., stress/no-stress detection, while it may be more fruitful to go for a multi-level stress detection approach [281].

8.3. Stress and Hormonal Fluctuations

The evaluation of a number of stress-related biomarkers, such as those present in saliva, may also contribute towards a more complete image of stress levels evaluation [284]. Objective markers, such as serum cortisol levels, have also been successfully evaluated in that regard [285-288], but their interpretation still entails a degree of bias [61,289,290]. It is still unclear how and why cortisol levels, especially when fluctuating in response to stress, are related to the pathological manifestations of stress [291]; on one hand, cortisol elevation during stress is related to a certain immune dysfunction, but on the other stress also leads to exacerbation of autoimmune diseases [292-294].

Although cortisol is one of the most studied stress markers, there are other hormones which can, at least to a degree, reflect stress levels. For example, thyroid-stimulating hormone (TSH), as well as T3 and T4 levels were found to be influenced by stress levels, albeit in thyroidectomy patients [295]; pending similar experiments in more diverse samples, the results are nonetheless encouraging. A decrease in T3, T4 and TSH levels has been confirmed in the serum of male adult rats [296]. It is already well-established that both acute and chronic stress have an adverse effect on the thyroid gland [297,298].

Psychological stress may also lead to the development of insulin resistance, especially in genetically predisposed individuals [299]. Severe abnormalities in the release and response to insulin have already been confirmed in rat models [300,301]. Therefore, the correlation of insulin resistance in stressed individuals may be an interesting research perspective.

Chronic stress, in roughly the same way as chronic medication, can result in disruption of the hormonal appetite pathways. Glucocorticoid excess, either stress-induced or medication-induced, can result in overeating and obesity [302]. Indeed, the metabolic effects of glucocorticoid drugs are well-documented [303]. Regardless, when stress leads to obesity, obesity itself may result in psychological stress [304-307] in a never-ending vicious circle. In the same vein, it is also documented that stress can induce or exacerbate skin disorders [308,309], which in turn may be a cause for psychological stress [310,311] resulting in a vicious circle like obesity.

8.4. Stress and Immunity

Let's consider for a moment the role of stress in immunity. It has been established that stress affects immune function when chronic [312]. Even if the focus of our review has been acute stress, prolonged exposure to the same acute stressors inevitably leads to chronic stress; chronic stress exposure, whether conscious or not, can induce changes at a genomic level [313]. The reverse is also true, i.e., relaxation methods can also lead to quantifiable gene expression changes [314-317]. Another question then arises: is stress-associated immune mediation clinically relevant? This is a very complicated question, whose elucidation is fraught with methodological and investigative challenges [291].

A number of studies have nevertheless attempted to tackle this conundrum. The first such study was performed by Cohen et al. [318] in 1991. The authors found that there was a positive correlation between stress levels and the risk of developing a cold. The widespread suppression of the immune system and its association with disease have been examined in detail by other authors [319]. In 2015, Cohen et al. [320], reported that people with a higher degree of interpersonal tension were more likely to catch a disease. On a related note, there is even evidence to suggest that low stress levels in individuals may increase the effectiveness of vaccines and, conversely, vaccines are less effective in stressed individuals [321,322].

According to recent evidence, the endogenous levels of somatostatin have an important role in stress perception and other neurological disorders [323]. Somatostatin receptors have also been implicated in stress response in rodents [324]. It was speculated that stressed individuals appear to have a higher cancer incidence, and this might also be related to serum somatostatin levels [325,326]. Given that somatostatin and its analogues are used in the treatment of some types of cancers [327-329], the psychological effect of the administration of such drugs merits further investigation. Additionally, more than 1/3 of oncologic patients have significant distress [330,331], which may adversely affect their prognosis, given the stimulatory effects of stress on cancer [332-334]. Stress may also modulate various other signalling pathways therefore increasing the risk for developing certain cancers [335-341].

Given that there is a considerable number of stress signs and symptoms, which can vary between individuals [342,343], this is the reason why a more objective form of stress evaluation is necessary. Interestingly, changes in stress symptoms may be related to variations in musculoskeletal disease indices, based on the research of Leino et al [344].

8.5. Stress and Morbidity

A brief deliberation, specifically on cardiovascular pathologies, is warranted, given their prominent association with stress [345-349]. The socioeconomic impact and burden of disease of cardiovascular diseases (CVDs), especially in the population of Western countries, is well-known. Both the incidence and prevalence of cardiovascular disease have been steadily increasing over the last years [350-352]. Even though the CVD-associated mortality may be somewhat decreased, the negative effects of these conditions are still important enough [353,354]. Despite the connection between certain workplace environments and CVDs having been verified, the mechanisms behind it remain unclear. Regardless of the particular type of work environment, there exist two prevalent theories on how such an environment, with its stress-inducing capacity, can increase the risk for cardiovascular stress. One theory refers to the demand-control or job strain model [355] later adapted by Backé et al. [356], and the other is based on the effort-reward imbalance (ERI) model [357,358], latter updated to include overcommitment [359]. Perhaps by using stress markers, such as the ones already mentioned, it will be possible to examine the validity of these models and how they can be better exploited for diagnostic purposes.

But it is not just cardiovascular diseases that are associated with stress. Stress, especially mental stress, has been proven to affect the organs of the gastrointestinal tract [42,360-363], especially in the cases of indigestion [364-366], irritable bowel syndrome [41,367-369], and perhaps motility disorders and gastroesophageal reflux disease (GERD) [370]. A demonstrable association between asthma [371-374], dermatological conditions [375-378], autoimmune [26,28,379], endocrine conditions [380-382] and stress is mentioned in medical literature.

8.6. Stress and Occupational Parameters

We would also like to remark that there is an obvious connection between stress and occupation [383,384]. Undeniably, the increased stress, evident in modern times, is linked more to occupation-related rather than environmental stressors [385,386], especially important for the medical staff. Indeed, stress is evident in medical professionals [387-389] even since their student years [390-393]. It is logical to consider the increased prevalence of stress in medical professionals, compared to other professionals [394-396], given the presence of specific psychosocial stressors [397]. Not least, burnout in healthcare professionals is a relatively common occurrence [398-400]. Such burnout, apart from being detrimental to them, can severely hamper their performance [401].

Besides the reference to the university-induced stress in the case of medical students, we would like to remark that, oftentimes, the educational system is stress-inducing even for high school [402,403] or elementary school students [404-407], although most researchers note that other factors are also at play. The COVID19 pandemic [408] added yet other stress-inducing factors to this, for several children [409]. To combat the stress-inducing effects of the educational system, at various levels, novel learning approaches are required (e.g., [410-416]) and strategies both for students and educators alike (e.g. [417-419]) at all educational levels are required.

8.7. Applications in Rehabilitation Medicine

The stress monitoring devices are usually multi-parametric recording (for instance combining plethysmography, electrocardiogram, electrical impedance evaluation or blood pressure measurements, etc). When treating rehabilitation patients (such as recovering from vascular accidents, major trauma, neurological impairments, etc.), monitoring multiple physiological signals is useful yet frequently compromised by several barriers: time, signal interferences and patient compliance.

The time barrier: deploying multiple devices for multi-parametric monitoring is a time-consuming, error-prone task that systematically adds burden to daily charting [420]. Use of specifically built devices that can be used without interfering with braces, casts, etc. would improve the daily schedule of both patients and caregivers.

The signal interferences are multi-modal in a rehab practice: movement from active physical therapy introduces severe kinetic artifacts in continuous electrical recording, especially EEG / EKG [421]. Also, electrical noise can be greater due to proximity to various rehab-specific devices (such as high frequency field generators, motors from active movement machines, etc).

Patient compliance is often a serious problem in rehabilitation procedures, as frequently the patients face physical limitations, cognitive impairment, post-stroke fatigue. Addition of multiple monitoring devices (each one with its own cables, set of placement rules, etc.) increase the chance of dropping-out [422]. Presence of multiple tracking wires or metrics frequently increases the patient stress ("white-coat effect") [423]. Because of all these limitations, it seems that patients in rehabilitation would greatly benefit from custom-built sensors; we discuss below the main research done in both theoretical research of stress detection (subsection 8.8) and in improving physiological stress-detecting sensors (subsection 8.9).

8.8. Future Research Perspectives and Integration of New Technologies

Regarding recommendations for future research, an aspect that requires further investigation is the effect of stress on nerve conduction speed. One interesting research finding centres around the potential healing mechanisms of yoga [424]. Several authors have proposed that yoga reduces sympathetic activity, increases GABA secretion [425], regulates the hypothalamic-pituitary axis [426], reduces stress in general [427], and provides an anxiolytic effect [428].

It has been documented that an increase of even 1° C in body temperature will increase nerve conduction velocity, which is also affected by the circadian rhythm [429,430]. It would be reasonable then to assume that in most studies documenting a variation in skin temperature in response to stress [70,72,74,75,78,89,148,151,159,161], a measurable difference in nerve conduction velocity would have been observed.

A further factor supporting our proposal of altered nerve conduction properties in response to stress is the association between various immune system cells, such as mast cells, neutrophils, macrophages, and T cells, with neuronal factors [431,432] in stress-induced inflammatory processes. Specifically at the level of the skin, the most important association with nerve fibres seems to be exhibited by mast cells [433,434]. Indeed, mast cells, when activated by stress, have been proven to modulate the properties of the integumentary system and its response to inflammation [255]. This, in our opinion, could be part of the explanation of the different responses of stressed individuals and also of the exacerbation of skin inflammations in stressed individuals.

Some electrophysiological parameters such as HRV, surface EMG, galvanic skin response, or cortical evoked potentials can be implemented to describe and assess physiological load and recovery dynamics in sports medicine and in patients undergoing hip or knee arthroplasty [435,436]. These parameters can supplement morphological investigations and provide essential data on the neurophysiological adaptation to surgical trauma, rehabilitation, and biomechanical alignment of the lower limbs [436-441]. Some electrophysiological stress signatures can provide contextual information for outcomes reported in planning accuracy or revision etiologies, and to understand differences induced by various surgical approaches [442-446].

It is also well-established that the level of human body activity does fluctuate during the day based on circadian hormone secretion [447]. Mental performance also exhibits a circadian rhythmicity [448,449] and we may hypothesise that stress perception is also affected accordingly.

Another aspect that must be considered is that stress perception between different individuals differs based on their previous stress exposure; repeated prior stress exposure will most likely result in decreased stress responsiveness [450-452].

Various interventions, such as acupuncture, appear to play a role in altering stress perception or stress induction [453]. Research on animal models has determined the positive anti-stress effects of acupuncture [454,455]. The research on humans has also produced some encouraging results [456-462]. These anxiolytic effects could be studied in comparison with yoga, another traditional healing method discussed above. After the 1950s, a number of research attempts have also been made, using different detection system, to determine the nature and exact location of acupuncture points [463]. Still on the subject of alternative medical approaches, we would venture to suggest that the variability of cardiovascular parameters in response to stress, should be studied in patients in whom phytochemicals derived from ethnomedical formulations for relevant cardiovascular pathologies have been administered; examples of such phytochemicals are pinosylvin [464], and piperine [465,466].

It seems reasonable to take into account, in future experiments, the physical and mental state of the individuals participating in the experiments and measurements, and to specifically design experimental protocols aiming to determine the differential response to the same stressors of individuals suffering from different conditions. The use of continuous real-time stress monitoring during several physiological human activities has been proposed by Tang et al. [467]. Time-domain and frequency domain parameters for the HRV may also be used towards that end [468-470].

The role of cardiovascular stress markers has already been mentioned, and the role of stress in cardiovascular disease has been described. Given that the cardiovascular system is readily affected by and promptly reflects stress levels, it is fitting to discuss the role of electric heart activity recordings, namely electrocardiogram (ECG), in stress detection. Indeed, some studies have used various sensor modalities, including ECG recordings, to determine mental stress levels [471-476]. Depending on the precise methodology used and the limitations of each experimental process, various degrees of accuracy were achieved [477].

Taking into account the current advances in neuroscience regarding the role of the brain in perceiving and evaluating stress [478], the electroencephalogram (EEG) [479] may be added to the proposed battery of tests for stress detection. Some relevant studies have been performed [478,480-483], each with its advantages and limitations [484]. Many other modalities, such as electrodermal activity (EDA) [485,486], accelerometer (ACC) [486], and pulse oximetry [487] have been tried as stress assessment methods.

Electrophysiological stress profiling has also expanded into other medical disciplines where the neurophysiological load interplays with metabolic pathology. Evidence from ophtalmologic surgery reveals interferences of systemic stress responses and neuroregulatory pathways translating into alteration of disease progression and surgical outcomes [488-491]. Neuro-metabolic research has clarified mechanistic gaps between morphology and function, translating peripheral-central metabolic pathways and revealing their interaction in the stress response patterns [492-494].

8.9. New technologies and future rehabilitation monitoring

As discussed in the relevant subsection, the compliance of rehabilitation patient might be reduced if the monitoring technology is cumbersome to wear. Newer wearable sensors are designed to address this problem by being smaller, easier to use, with a hypoallergeninc profile; a lot of reasearch is being put into reducing the signal interferences discussed above.

In the last decade, the rapid development of the 3D printing industry and its application in medicine [495,496] has come about, with many advantages for patients, including interventional precision and specificity, reduced risks of infection and medical errors, and overall improvements in the quality of medical services [497]. Many biomaterials were developed with broad properties, with a focus on applications for humans. Moreover, novel manufacturing techniques like custom 3D printing might have the potential to address the variable reliability of the sensors. Increased biocompatibility, reduced risk of inflammation, enhanced precision, and the low cost of production are some of the qualities of 3D printed constructions that can also be seen in the field of physiological measurements [498,499].

Many categories of wearable (non-invasive) sensors (electroencephalograph, glucose, blood pressure, tactile, respiratory, sweat, or oxygen saturation biosensors) were designed and manufactured from biomaterials such as nanocomposites, hydrogels, or biopolymers [500,501]. Regarding biomaterials-based sensors, they are usually used on sensors applied to the skin. Due to their properties, skin irritation or allergic reaction is greatly reduced, and given the 3D printing technologies, any shapes, densities, or consistency can be achieved for a perfect match with each patient's needs [500]. Hossain et. al managed to create electrodes with enhanced flexibility for measuring cortisol levels. Natural cellulose-based materials were used, that was integrated in a mixed biosensor that simultaneously measured the muscle activity [502].

Compatible materials exist that can produce chemical responses to different measurable levels of hormones or other natural/artificial substances (for example, graphene mixed with polylactic acid is great for the electrochemical response of epinephrine [503]). Similar to Hossain's work [502], other multimodal biosensors can be created to highlight very specific correlations between various measurements (for example, associating cellulose-based sensors for cortisol with commercial/artificial biosensors that detect pulse or respiratory rate-related measurements).

3D printing sensors, besides the advantages of using biomaterials, can be shaped in any form and strictly controlled to create high-precision wearables for different types of signals, especially stress-related. Schmitz et al. created 3D-printed electrodes for electrodermal activity (EDA) using a filament made of polylactic acid mixed with graphene [504]. Thus, with high precision and specificity, the sensors were easily included in wearables without coarse structures and tested on 6 human patients for stress-related measurements [504]. Besides working as well as commercial sensors, the opportunities for minimalist designs and increased adaptability are great characteristics that could play a huge role in the future.

Chest movements can be easily used as a reference for the respiratory rate if optimized methods to attach and keep the sensors for long periods can be developed [505]. Tavares et. al managed to include a sensor made with Fiber Bragg Grating (FBG) technology for heart rate and respiratory rate measurements, that was integrated in a 3D printed flexible capsule [505]. Many approaches to integrating this type of sensor in "cushions" were tried, but using 3D printing has the advantage of specific positioning and enhanced control over the mechanical characteristics of the biosensor surroundings [505,506].

Hydrogel sensors used for stress-related measurements, such as skin temperature combined with muscle deformation (strain), were also designed [507]. Using a 3D printable hydrogel mix based on polystyrene sulfonate and conductive polymers, a sensor with outstanding performance and properties was obtained [507]. With increased stretchability (a relevant characteristic for strain measurements) and its supersoft structure, the bioprinted sensor outperformed the commercial electrodes [507].

These results show the potential of customizable sensors for future measurements and research. The main advantages lie in the possibility of creating complex structures for specific morphologies and in the strictly controlled mechanical properties, which greatly affect the biosensors' capacities to record.

Finally, new methods, or variations of existing ones, have been proposed for more comprehensive stress level assessments [508]. For example, using biometric sensors, it was possible to examine the stress-reducing effects of certain environments in older adults [509]. In another case, brief structure respiration practices were found to reduce stress and physiological arousal [510]. Interestingly, it has been proposed that physiological stress parameters can be used to discriminate between beginners and experienced professionals, at least in certain domains [511]. Whatever the case, an individual assessment for each subject is needed to document the baseline stress levels on a case-by-case basis [512], to be better able to evaluate the fluctuations of physiological parameters as stress indicators. The availability of stress-monitoring systems with higher accuracy [386] and wearable biosensors [513] will certainly improve matters, along with the potential applications of artificial intelligence [514-518] and big data and deep learning [519-522], always respecting any bioethical considerations [523].

9. Conclusions

The importance of stress for the physical and psychological health of individuals has been increasingly evident. However, due to the diversity of stress-related symptoms, it is not always easy to clinically determine the stress levels of an individual. On one hand, alternative stress evaluation methods, such as hormonal levels, must be introduced as objective physiological stress markers. On the other hand, such methods are usually invasive to some degree and more difficult to perform, compared with the more traditional methods discussed in the context of this paper. We therefore consider that this research direction is more important for future research, and that more experiments, but with a higher degree of standardisation, internal and external validity, should be performed, examining, whenever possible, more than one marker in both healthy and non-healthy subjects. Regarding the bioethical and legal aspect, it is apparent that the integration of AI-powered stress monitoring through wearable biosensors or other similar and equivalent means offers significant research potential yet is accompanied by complex risks under the existing regulatory framework, mainly, of EU. The legal risks of advanced stress monitoring techniques are mainly related to unregulated collection of massive amounts of personal physiological data, regulatory classification of 3D-printed biosensors as medical devices, liability for manufacturing defects, and informed consent for custom-printed sensors. Lessons from high-profile cases mentioned above underscore that only through strict legal compliance, scientific validation, ethical integrity and robust cybersecurity can such technologies be deployed responsibly, safeguarding both fundamental rights and patient safety. Finally, novel 3D printing technologies offer new possibilities on such research but, at the same time, give rise to legal and bioethical challenges.

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Abbreviations

The following abbreviations are used in this manuscript:

ACC	Accelerometer
AKI	Acute kidney injury
BP	Blood pressure
CVD	Cardiovascular diseases
ECG	Electrocardiogram
EDA	Electrodermal activity
EEG	Electroencephalogram
EMG	Electromyography
GDPR	General Data Protection Regulation
HR	Heart rate
HRV	Heart rate variability
ICO	UK Information Commissioner's Office (ICO)
PP	Pulse pressure
PPG	Photoplethesmography
PR	Pulse rate
RR	Respiratory rate
SC	Skin conductance
TSH	Thyroid-stimulating hormone

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