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Which biomarkers to evaluate the association between psychosocial factors and neuro-cardiovascular diseases?

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Abstract

The association between psychological factors and neuro-cardiovascular diseases had been suggested. This review was performed to assess, from the results of literature, the pertinence of using new biomarkers in the occurrence of neuro-cardiovascular diseases in a psychosocial context.

We chose to consider wide-ranging descriptions of stress from psychological factors (occupational stress, financial strain, marital stress, social isolation) that may influence a physical health outcome (stroke, cardiovascular diseases) through a psychological mechanism. We addressed literature data confirming the link between new biomarkers such as cortisol, endothelial dysfunction, inflammation, and allostatic load in neuro-cardiovascular diseases related to psychological factors.

First, it was shown a link between deleterious effects of cortisol and the occurrence of neuro-cardiovascular diseases in a psychosocial context. Second, endothelial dysfunction (flow mediated dilation of the brachial artery) is observed in association with job strain and occupational category. Stressful events and distress can also substantially increase the production of pro-inflammatory cytokines. Finally, increased allostatic load is associated with higher job demands in industrial workers, lower decision latitude and job strain in healthy workers, burnout and career instability, effort-reward imbalance and exhaustion.

All the papers included in this review confirmed the link between neuro-cardiovascular diseases occurrence in a psychosocial context and these biomarkers.

Keywords

Psychosocial factors, biomarkers, cortisol, endothelial dysfunction, inflammation markers, allostatic load, neuro-cardiovascular diseases.

Abbreviations List

11β-HSD1 : 11β-hydroxysteroid dehydrogenase type 1

AMI : Acute Myocardial Infarction

AL : Allostatic Load

ATP : Adenosine Tri-Phosphate

CRP : C-Reactive Protein

CAD : Coronary Artery Disease

CAC : Coronary Artery Calcification

CHF : Congestive Heart Failure

CRH : Corticotropin-Releasing Hormone

CVD : Cardiovascular Diseases

CHD : Coronary Heart Diseases

DNA : Deoxyribo-Nucleic Acid

DHEA-S: Dehydroepiandrosterone-sulphate

EGF : Epidermal Growth Factor

ELISA : Enzyme-Linked Immunosorbent Assay

ET : Endothelin

FMD : Flow-Mediated Dilation

GR : Glucocorticoid Receptor

HPA axis : Hypothalamus-Pituitary-Adrenal axis

HOMA : Homeostasis Model Assessment of insulin resistance

HRV : Heart Rate Variability

ICH : Intra-Cerebral Hemorrhage

IMT : Intima-Media Thickness

IL : Inter-Leukin

INF-γ : Interferon-γ

OR : Odds Ratio

PSF : Psychosocial Factors

MR : Mineralocorticoid Receptor

mRNA : Messenger RNA

mtRNA : Mitochondrial RNA

MCP-1 : Monocyte Chimoattractant Protein-1

MESA : Multi-Ethnic Study of Atherosclerosis

MMSE : Mini Mental State Exam

NADH : Nicotinamide Adenine Dinucleotide Hydrate

NIHSS : National Institutes of Health Stroke Scale

NO : Nitric Oxide

NOS : Nitric Oxide Synthase

SES : Socio-Economic Status

SAH : Sub-Arachnoid Hemorrhage

RNA : Ribo-Nucleic Acid

RT-PCR : Real-Time Polymerase Chain Reaction

SNS : Sympathetic Nervous System

T2D : Type 2 Diabetes

TIA : Transient Ischemic Attack

TnT : Troponin T

TNF-α : Tumour Necrosis Factor-α

VEGF : Vascular Endothelial Growth Factor

Introduction:

Neuro-cardiovascular diseases are the major cause of death. An estimated 17.5 million people died from these diseases in 2012 worldwide representing 31% of deaths

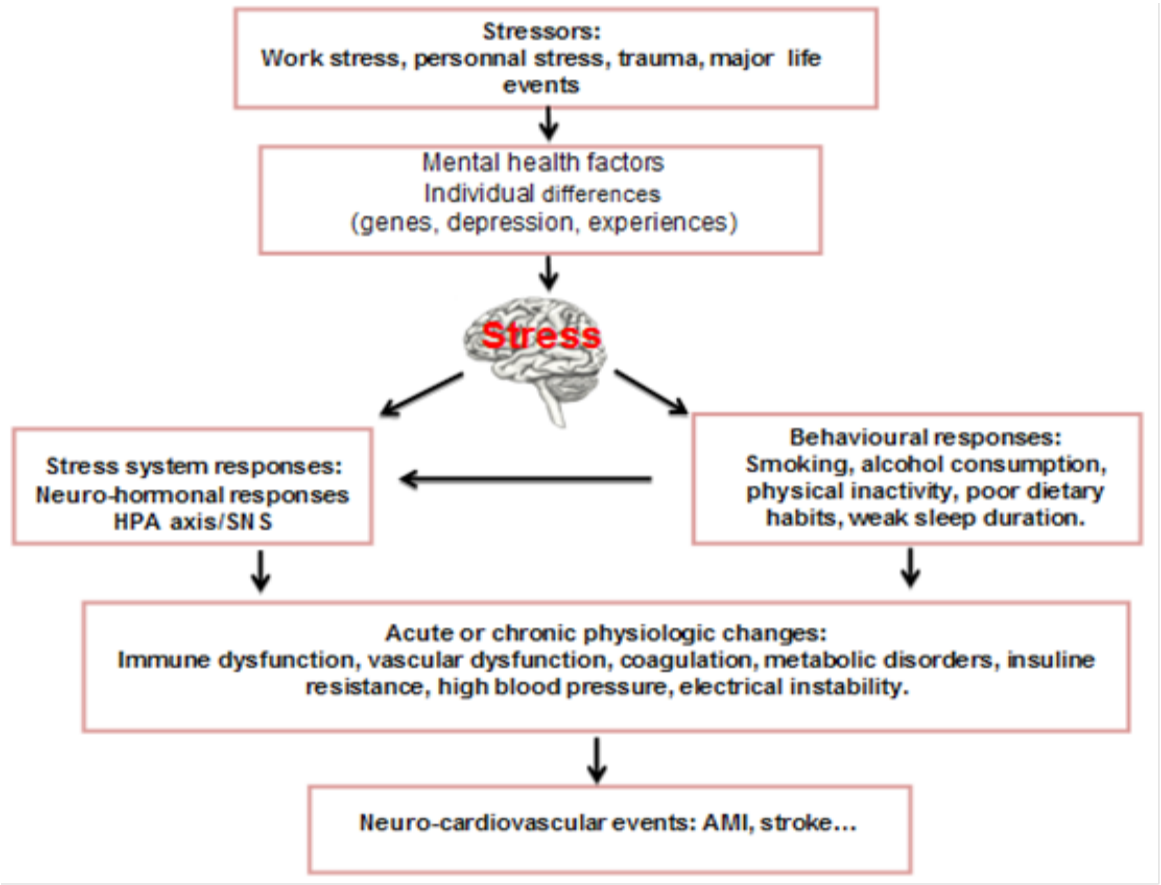

Figure 1: Different stress responses. The perceived stress is influenced by personal experiences, and genetics. Stressful experiences induce change in physiologic and behavioral responses, leading to pathophysiological modification. Chronic exposure to these mediators of stress can cause adverse effect on cardiovascular system. HPA axis: Hypothalamus-Pituitary-Adrenal axis. SNS: Sympathetic Nervous System. AMI: Acute Myocardial Infarction.

(World Health Organization, 2012). The number of fatalities is estimated to increase to over 24 million a year by 2030 and this imposes a huge burden in terms of disability and healthcare costs [1].

In addition to classic cardiovascular diseases (CVD) risk factors such as hypertension, dyslipidemia, and diabetes [2], recent works have examined the role of psychosocial factors (PSF) as a potential cause of these diseases.

PSF such as occupational stress, financial strain, marital stress, social isolation, and anxiety seem to be associated with increase risk of coronary heart disease (CHD) [3]. Socioeconomic status (SES) is inversely associated with CHD and it has been proposed that psychosocial pathways may play a mediating role [4]. The different component of PSF may act alone or combine in group and may exert effects at different stages of the life course [3,5].

The association between PSF and CHD had been suggested in a wide range of populations including young, older, men, women, socioeconomic strata, lifestyle, and conventional risk factors [INTERHEART investigators (2004),6].

There is also a clear social gradient in stroke mortality and morbidity, as lower socioeconomic groups worldwide have consistently higher rates of stroke than higher socioeconomic groups [7,8].

Two main mechanisms have been suggested to explain the link between stress exposure and neuro-cardiovascular diseases incidence or prognosis in established

disease. The first hypothesis proposes that PSF affect cardiovascular health indirectly, through the modification of lifestyle behaviors such as smoking, poor dietary habits, physical inactivity, medication nonadherence, alcohol consumption, and weak sleep duration [6,9,10]. But this hypothesis does not explain entirely the link between adverse behavioral risk profile and stress-related disorders.

The second hypothesis involves a direct pathway, through dysregulation of the sympathetic nervous system (SNS) and the hypothalamus-pituitary-adrenalaxis (HPA) stress, that could induce inflammatory, metabolic, and haemostatic changes which increase the risk of neuro-cardiovascular events [11,12].

The underling mechanisms linking PSF with neuro-cardiovascular diseases are complex. Biomarkers may identify new pathophysiological pathways, help the diagnosis and management of the disease. Moreover, biomarkers able to detecting earlier phases of disease development would facilitate targeted strategies to prevent pathological complications. Some of these strategies have prognostic significance so help to improve patient outcomes or are able to assess the risk stratification in asymptomatic individuals at higher risk [13,14].

The aim of the current review is to discuss about the role of novel biomarkers in identifying specific pathways of disease progression. A special attention will be given to cortisol, endothelial function, inflammatory markers, and allostatic load.

Cortisol: The stress hormone

The activation of the HPA axis stimulates corticotropin-releasing hormone (CRH) release from the paraventricular nucleus of the hypothalamus, which in turn stimulates the release of the adrenocorticotropin hormone (ACTH) from the pituitary. ACTH, when released into the circulation, causes an increase in cortisol release from the adrenal cortex. Glucocorticoid hormones (cortisol in humans; corticosterone in rodents) act *via* modulation of mineralocorticoid (MR) and glucocorticoid (GR-α) receptors which are classically understood to function as nuclear transcription factor. Cortisol is involved in the regulation of a large panel of physiologic functions such as glucose and lipid metabolism, body composition, and the immune system [15].

In short, cortisol is of crucial importance for adaptation to stress. It acts following a biphasic pattern in which acute and chronic stress or corticosteroid exposure have different or even opposed effects [16]. For example, moderate glucocorticoid levels improve performance on spatial memory tasks and adaptive immunity, but high, sustained levels of the steroids can impair performance.

Cortisol excess induces metabolic disorders

Alterations of the HPA axis enhanced cortisol release and reduced feedback sensitivity of the axis. The prolonged activation of the HPA axis, by chronic stress may result in glucocorticoid dysfunction such as elevated fasting insulin and HOMA insulin-resistance index, dyslipidemia, visceral obesity, hypertension, and arterial stiffness [17,18].

There is convincing evidence linking this endocrine disorder with high CVD risk and brain damage [18-20]. The best illustration of deleterious cortisol effects on metabolic function is provided by the Cushing's syndrome which is a long-term pathologically elevated endogenous cortisol level. The adverse effects of cortisol are also extensively described in patients using glucocorticoids medications [21].

These data were also supported by a recent study that assessed the relation between hair cortisol and presence of metabolic syndrome [15]. Participants whose hair cortisol levels fell into the third and fourth quartile had an odds ratio (OR) for having metabolic syndrome of 1.71 and 2.42, respectively, as compared to the first quartile.

Glucocorticoids influence neuropeptides in the arcuate nucleus of the hypothalamus and disrupt energy balance overall leading to type 2 diabetes (T2D) and obesity [20]. Person with T2D showed unregulated HPA axis secretion, overexpression of GR-α, and a positive correlation between HOMA insulin resistance index and 24-hour urinary free cortisol excretion [22].

Cortisol doesn't only come from the adrenal gland, but can also be regenerated in metabolically active tissues

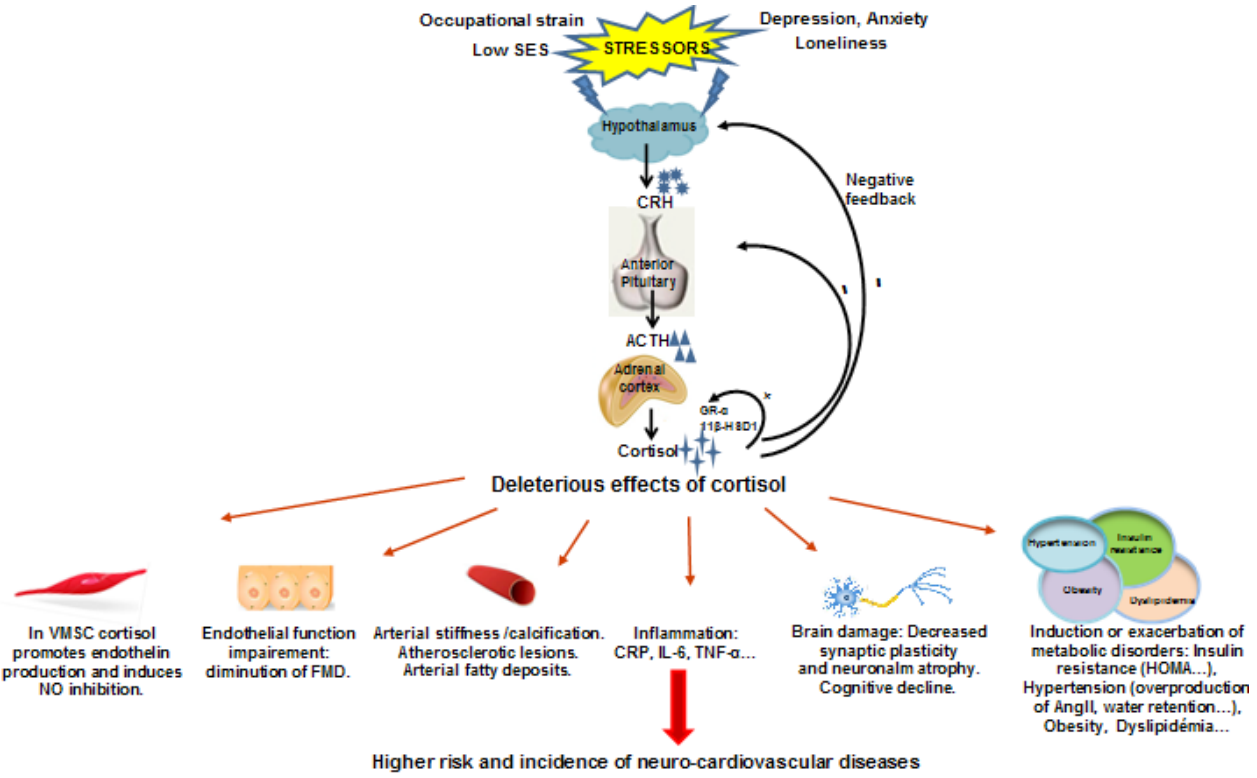

Figure 2: Schematic representation of deleterious effects of cortisol and its role in neuro-cardiovascular events. The hypotalamus responses to cortisol levels: reduces CRH if cortisol is high and increases CRH if cortisol is low. Exposure to stressors in life induces HPA-axis dysfunction: a diminished feedback regulation of cortisol secretion. On the other hand, cortisol enhances its own production (11β-HSD1) and action (GR-α). CRH: Corticotropin-Releasing Hormone. SES: Socioeconomic Statue. ACTH: Adreno-Corticitropin Hormone. 11β-HSD1: 11β-Hydroxysteroid Dehydrogenase type 1. GR-α: Glucocorticoid Receptor-α. VSMC: Vascular Smooth Muscle Cells. NO: Nitric Oxyde. FMD: Flow Mediated Dilation. CRP: C-Reactive Protein. IL-6: Interleukine-6. TNF-α: Tumor Necrosis- α. HOMA: Homeostasis Model Assessment. Ang II: Angiotensin II.

by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). Cortisol derived from tissues act in liver to influence glucose tolerance and insulin sensitivity leading to T2D. This type of cortisol can act in adipose tissue causing adipocyte hyperplasia and hypertrophy, by this way giving rise to obesity [23].

In animal research, an elegant study of Masuzaki H *et al*, showed that mice with 11β-HSD1 gene overexpressed in lipid-storing tissue induces many metabolic disorders. This gene is encoding for the enzyme converting inactive cortisone into active cortisol. The transgenic animals display salt-sensitive hypertension, insulin resistance, obesity, as well as a higher GR-α expression in visceral fat [23]. Conversely, the pharmacological inhibition of 11β-HSD1 activity in ApoE knockout mice, an experimental model of atherosclerosis, has a beneficial effect on insulin sensitivity and reduces the development of atherosclerotic lesions [24]. All these data approve the strong link between metabolic disorders, atherosclerosis development, cortisol and consequently results of CHD.

Additionally, cortisol excess can induce stimulating proinflammatory processes in the endothelium [25]. As a reminder, inflammation is the main mechanism leading to metabolic disorders and cardiovascular diseases.

Glucocorticoids strongly stimulate angiotensino-

gen (AGT) and angiotensin type 1 receptor (AT1R) mRNA expression in several cell types such as hepatocytes, adipocytes, cardiomyocytes and vascular smooth muscle cells (VSMC) [26,27]. Cortisol stimulates Angiotensin II (Ang II) action. Ang II, the main acting peptide produced by AGT, is well known for its properties on water and salt retention, as well as for its proatherogenic effect, and for inducing sympathetic nervous system stimulation.

PSF modulate cortisol secretion and thereby cause metabolic disorders

Some literature data confirming that cortisol is associated with metabolic disorders in relation with PSF. Low SES is associated with both visceral obesity and perturbed cortisol secretion, with elevated stress-related cortisol secretion, as well as cortisol escape from dexamethasone suppression [28,29].

It also was identified a strong association between visceral obesity and blunted dexamethasone response in persons with symptoms of anxiety and depression [28].

Furthermore, in cynomolgus monkeys, *Macaca fascicularis*, exposure to psychosocial stress was followed by a diminished feedback regulation of cortisol secretion, suppression of the reproductive axis, and depressive behavior. To induce this social stress, social status was manipulated such that half of the previously subordinate females (experimental phase I) became dominant (experimental phase II) and half of the previously dominant females (experimental phase I) became subordinate (experimental phase II). During the second experimental phase, this social stress was associated with visceral obesity, insulin resistance, dyslipidemia, hypertension, and coronary artery arteriosclerosis [30].

A recent systematic review of 40 research articles demonstrated that cortisol was the biomarker used most frequently and was positively associated with PFS [31], including job strain, low socioeconomic status, and environmental factors. In this key paper, psychosocial stress was associated with CVD risks such as vascular pathology (hypertension, blood pressure fluctuation, and carotid artery plaque) as well as metabolic factors such as high blood glucose, dyslipidemia, and elevated cardiac enzymes.

PSF, deleterious effects of cortisol, and CVD

In this field there is convincing evidence linking cortisol excess with high neuro-cardiovascular diseases. Incidence and prognosis impact of cortisol on neuro-cardiovascular diseases related to stress were widely studied [18-20].

Cortisol is associated with coronary atherosclerosis and may enhance structural and functional cardiac disease. In order to understand the association between cortisol response to mental stress and high-sensitivity cardiac troponin T (hs-cTnT) in healthy older individuals, a cross-sectional study was conducted involving 508 men and women aged from 53 to 76 years drawn from the Whitehall II epidemiological cohort [32]. Salivary cortisol response to standardized mental stress tests and hs-cTnT plasma concentration using a high-sensitivity assay were measured. Coronary calcification was measured using electron-beam dual-source computed tomography and Agatston scores.
The study found that heightened cortisol response to mental stress was associated with detectable plasma levels of cTnT using high-sensitivity assays in healthy participants, independently of coronary atherosclerosis. A robust association between cortisol response and detectable hs-cTnT (OR: 3.98; p=0.003) were found even after adjustment for demographic and clinical variables associated with CVD as well as for inflammatory factors. The association remained even when participants without coronary calcification were included (n=222; OR: 4.77; p=0.025) or when data were adjusted for coronary calcification in participants with positive Agatston scores (n=286; OR: 7.39; p=0.001).

Progression of chronic congestive heart failure (CHF) was associated with activation of neuro-endocrine stress response systems including the HPA axis that modulates the production and secretion of glucocorticoids including cortisol from the adrenal cortex. The study of Güder G *et al* demonstrated that higher serum levels of cortisol were independent predictors of increased mortality risk

[33]

However, it is possible that the single serum cortisol measurement may be influenced by the physical stress due to the actuate illness or the emotional stress associated with the hospital admission. So, measurements of only one time point may yield inconclusive results. Especially when we know that some other studies, using serum or saliva specimen collection, have not shown associations between cortisol and cardiovascular risk factors [34,35] or reported that low cortisol levels were associated with cardiovascular risk factors [36]. In fact, cortisol is a HPA axis-related hormone with a robust circadian rhythm where levels typically peak in the morning hours and decline across the day [37]. So, it is possible that the single serum cortisol measurement may be biased by confounding factors. Scalp hair is a novel matrix that allows for measurement of hormones over a period of several months [38]. Hair cortisol level is easy to assess using an enzyme-linked immunosorbent assay (ELISA) methods.

In community 283 dwelling elderly patients demonstrated that higher hair cortisol levels were associated with a history of CVD (including CHD, stroke, and peripheral arterial disease) [39]. This study found that participants in the highest hair cortisol quartile had a 2.7 times increased risk of CVD compared to the lowest quartile and an increased risk of type 2 diabetes mellitus (OR: 3.2). Importantly, this risk was similar to the effect of traditional cardiovascular risk factors such as hypertension (OR: 2.5), abdominal obesity (OR: 2.2), and dyslipidemia (OR: 3.3), suggesting that long-term elevated cortisol may also be a relevant risk factor. Importantly, no associations were found between long-term cortisol levels and chronic non-cardiovascular diseases.

It also remains to be determined whether hair cortisol levels are predictors of the main clinical endpoints especially in the onset of acute myocardial infarction (AMI). A recent report has demonstrated that hair cortisol was increased in patients admitted with AMI as compared to levels in control subjects, indicating that systemic cortisol exposure within the three months before admission to the emergency department was higher in patients who developed an AMI than who were admitted for other reasons such as non-myocardial chest pain, infections, and syncope [40].

The study of Manenschijn L *et al* is cross-sectional and can therefore only suggest an association between hair cortisol levels and a history of CVD. However, Pereg's study showed that high cortisol levels are present before the onset of a cardiovascular event. Taken together, these findings suggest that this association may reflect a causative link.

In order to determine the potential mechanism, a recent study examined the association between cortisol responses to acute laboratory-induced mental stress test, consisting of Stroop task and a 5-min mirror tracing task, and the progression of coronary artery calcification (CAC). The authors included healthy men and women participants without history or objective signs of CHD, drawn from the Whitehall II epidemiological cohort [41]. Of note, CAC was measured at baseline and at 3 years follow up using electron beam computed tomography. This study reported an association between cortisol reactivity and CAC progression, with a 27% increase in the odds of progression *per* SD change in cortisol responsivity. These associations were largely independent of conventional risk factors. This relationship was most evident in participants without detectable CAC at baseline, which further supports the notion that heightened cortisol reactivity might be important in the aetiology of atherosclerosis and is not a simple marker of disease progression. The authors showed a prospective association between cortisol stress reactivity and progression of sub-clinical atherosclerosis. These findings provide support for the hypothesis that hyper-reactivity of the HPA axis is one of the mechanisms through which psychosocial stress may influence the risk of CHD. Obviously, we cannot, rule out the role of unmeasured confounding risk factors or genetic influences that might account for cortisol response patterns and CHD risk (Van den Akker EL *et al*, 2008) such as GR polymorphism which may be related to higher pro-inflammatory activity and greater risk of CHD. Interestingly, blocking cortisol production with metyrapone in healthy participants prevented adverse clinical effects such as men-

tal stress-induced endothelial dysfunction (*i.e.* flow-mediated dilation: FMD).

Stress alters many brain areas, including the hippocampus, medial prefrontal cortex, and amygdala. The medial prefrontal cortex is highly sensitive to stress and plays an important role in the regulation of emotions and coping with environmental challenges [42].

Hippocampal damage, loss of dendritic plasticity, and memory impairments are present in patients with hypercortisolism syndrome. Impairments of executive functions, language skills, motor functions, and information processing speed were also reported [20]. Interestingly, it was showed that corticosterone-synthesis inhibitor, metyrapone, was able to prevent ischemia-induced loss of synaptic function in the hippocampus of rat [43].

The study showed that Chinese patients with a poor outcome and nonsurvivors had significantly increased serum cortisol levels on admission (643 [IQR, 456-786] *vs* 441 [IQR, 367-511] nmol/L; $p<0.0001$); IQR: Interquartile ranges. There was a positive correlation between levels of cortisol and the National Institutes of Health Stroke Scale (NIHSS), glucose levels, and infarct volume. Cortisol can be seen as an independent short-term prognostic marker of functional outcome and death patients with acute ischemic stroke, even after adjustment for confounding factors. Combined model with cortisol can add significant additional predictive information to the clinical score of the NIHSS.

In order to evaluate the prognostic value of cortisol in patients with intracerebral hemorrhage (ICH), a small prospective observational study was conducted including 61 consecutive patients with ICH. The authors have assessed serum cortisol concentration with regard to their accuracy to predict functional outcome and death within 90 days. They found that cortisol level at 8 am is an independent prognostic marker of functional outcome in patients with ICH. This observed result was comparable to the NIHSS or ICH score, and is of especially prognostic value of death in patients with ICH. The higher the level of 8 am cortisol is, the more the risk of death in patients with ICH increases. The report indicated that cortisol levels increased with hematoma volume and neurological deficit (assessed by the NIHSS), reflecting the severity of the ICH. This study suggested that 8 am serum cortisol level may be used as an additional prognostic factor in ICH patients [44].

Some studies have found that elevated plasma or urinary cortisol concentrations in acute ischemic stroke are related to greater stroke severity, larger infarct volume and/or unfavorable outcome, including death [45,46]. Patients with stroke and high cortisol levels are more prone to suffer from adverse cardiac events, which may lead to higher mortality rates. Moreover, these patients had a worse prognosis after stroke, characterized by the development of infectious disease, related to an immune dysregulation resulting from neuroendocrine disturbance and immunosuppressive properties of cortisol [47]. In accordance with this context, a significant correlation was present between IL-6 and cortisol levels the first two days after stroke ($p<0.05$). Median IL-6 levels were correlated to severity of paresis on days 1 and 7 and to the cognitive function assessed by the Mini Mental State Exam (MMSE) ($p<0.05$) [48].

More recently, a study using gene expression within carotid endarterectomy samples demonstrated that patients with clinical history of stroke or transient ischemic attack (TIA) had higher GR-α mRNA level in vascular tissue and higher plasma cortisol levels as well compared to asymptomatic patients without any clinical history of stroke/AIT who underwent surgery for primary prevention [27,49]. This study corroborates also the association between heightened cortisol level and stroke/AIT onset.

The knowledge regarding mechanisms of stroke development involving cortisol remains limited. A recent study of Hunter G et al. provided some elements of a response to this issue. They demonstrated an association between GR-α and stress-dependent transcriptional regulation. This work has established that, in the brain of in adult male rats, the GR-α is translocated from the cytosol to the mitochondria and that stress and corticosteroids have a direct influence on mitochondrial DNA transcription and mitochondrial physiology. Animals were exposed to both acute and chronic immobilization stress, and then mitochondrial

RNA (mtRNA) expression using was examined quantitative RT-PCR. It was found that acute stress had a main effect on mitochondrial genome *in vivo* (mtRNA expression) and that expression of NADH dehydrogenase 1, 3, and 6 and ATP synthase 6 genes were significantly down-regulated. Chronic stress induced a significant up-regulation of NADH dehydrogenase 6 expression. Adrenalectomy abolished acute stress-induced mtRNA regulation, demonstrating glucocorticoid dependence [50].

Endothelial dysfunction and neuro-cardiovascular diseases related to stress

Endothelial dysfunction is observed in the early stages of atherosclerosis and is associated with increased plaque rupture in many adverse outcomes [51].

Animal models, particularly non-human primates, showed that psychosocial stress causes endothelial damage and accelerates atherosclerosis in females. Monkeys under social stress develop endothelial injury: this effect is blocked by propranolol, the β-adrenergic receptor antagonist, indicating that norepinephrine is implicated [30].

FMD of the brachial artery is the reference technique to measure arterial tonometry. In community-based sample of employed individuals, Charles LE *et al*, examined the association of work hours, job demands, job control, job strain, and occupational category with brachial artery FMD. Occupational category was significantly associated with FMD: female blue-collar workers had the lowest mean FMD value ($p<0.001$) and those in the management/professional and services categories had the highest mean values. These results suggest that alterations of endothelial function may be one of the pathways linking occupational categories to FMD [52]. There are several mechanisms through which occupational category may be associated with endothelial function, and they include psychological stress and unhealthy lifestyle behaviors [53].

Similarly, a previous study demonstrated that participants perceiving themselves to be of lower social status in their communities exhibited reduced endothelial function (lower FMD) [54].

In addition to stress, depression, which was recently considered as a new risk factor for poor prognosis after an acute coronary syndrome, is characterized by an endothelial dysfunction and may contribute to the development of coronary artery disease (CAD) [55]. This information was supported by Chen H et al. who showed that stress score was an independent powerful predictor for decreased brachial FMD [11].

Social isolation and perceived loneliness are also major PSF [56,57]. Using a new model system of social stressors, an interesting report showed that prairie vole, Microtus ochrogaster, that is a highly social rodent species, developed an impairment of vascular endothelial function after experimental isolation. This impaired endothelium-dependent vasodilation was not observed in wild animals [58]. These finding confirm clearly that PSF-related stress may play a role in endothelial dysfunction.

Carotid intima-media thickness (IMT) and ankle-brachial index are used to estimate the burden of atherosclerosis. In a recent study conducted on the Multi-Ethnic Study of Atherosclerosis (MESA) participants, significant positive associations were observed between work hours and common carotid IMT among women, after adjustment for age, race/ethnicity, education, annual household income, and CVD-related risk factors. In addition, longer hours of work were significantly associated with lower levels of ankle-brachial index, among men [59]. There is evidence for robust link between CVD and endothelial dysfunction.

Inflammation markers

As a reminder, inflammation may be the starting point of the atherosclerotic process that results ultimately in a host of clinical complications, including ischemia, acute coronary syndrome, and stroke [2].

Stressful events and distress can also substantially increase the production of pro-inflammatory cytokines that are associated with a spectrum of age-related diseases such as heart diseases and stroke. Accordingly, stress-related immune dysregulation might be one core mechanism behind a diverse set of health risks [60].

Asberg M *et al*, conducted a study by including 195 women on long-term sick-leave for a work stress-related disorder (burnout) and healthy women. Participants, mainly white-collar workers, were recruited from one of the major Swedish insurance companies. The study design has involved only one single time point observation during the course of a prolonged stress condition and data were collected from an almost complete two-year follow up. Prolonged psychosocial stress was assessed through self-reports. Of note, this study used the Oldenburg Burnout Inventory which measures degree of professional burnout, participants who scored above the 75th percentile on this questionnaire were included. A large panel of interleukins (IL) were measured such as IL1-α, IL1-β, IL-6, IL-8, interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), monocyte chemotactic protein-1 (MCP-1), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF). Only increased levels of MCP-1, VEGF and EGF were found in women exposed to prolonged psychosocial stress. MCP-1 levels were more than twice as high in the sick leave group compared to the healthy controls. VEGF levels were three times as high in the sick leave group, and EGF levels were more than twice as high, compared to the healthy group [61].

An interesting review provided argument in favor of positive association between workplace stressors and inflammatory markers such as CRP and TNF-α [60].

It is well documented that high levels of C-reactive protein (CRP) predict CHD [62]. The analyses performed by Emeny R et al. [63,64] provide new evidence for CRP association with stress. In this study, work stress was measured by the Karasek job strain index. A strong association between job strain and CRP was observed in age and sex adjusted models, as well as in models adjusted for classic CHD risk factors (β-estimate=0.39, $p<=0.05$ and β-estimate=0.27, $p<0.05$, respectively).

Moreover, another study showed that CRP levels were increased in high stressed group of young drivers [65].

A cross-sectional study was used in order to describe relationships among stress work place and inflammation at a fixed point in time [66]. The urinary IL-8 levels of nurses working in acute care department, who reported a higher level of professional stress, were almost twice higher than the levels of unstressed employed nurses working in other department ($p<0.01$). Curiously, mean urinary IL-8 and cortisol levels were positively correlated ($p<0.05$) in women who lived stressful work experiences, confirming thus the link between HPA axis dysregulation and inflammatory biomarkers.

More recently, in a population-based sample of 869 adults from the MESA Stress Study, DeSantis AS *et al*, found that HPA axis activity may mediate the associations between psychosocial stressors and inflammatory processes (IL-6, IL-10, TNF-α). This study, together with Fukuda's study, provides argument in favor with associations of cortisol activity and inflammatory markers [67].

The IL-6, is an important inducer of CRP, and the combination of the two molecules is important in the process that leads to the development of CVD [68].

Negative emotions, such as depression and anxiety, increase the production of IL-6 [69]. A longitudinal study highlighted the deleterious longer-term immunological consequences of chronic stress: the average annual rate of increase in serum IL-6 was about fourfold higher in men and women who were chronically stressed by caring for a spouse with dementia than in similar individuals who did not have caring responsibilities [70].

The study of Epel ES *et al* supports the hypothesis that chronic stress might be associated with premature ageing of immune cells. Telomerase activity and telomere length (which are two cellular markers indicating cell ageing) were measured in peripheral-blood mononuclear cells obtained from mothers caring for a chronically ill child, as well as from mothers of healthy children. Carers reported greater stress than controls. Higher level of stress was associated with lower telomerase activity and shorter telomere length. Reports of high stress levels were also associated with higher oxidative-stress activity, as measured by levels of F2-isoprostanes [71].

It is important to note that some PSF have cause lasting damage. For example, in a prospective study, mal-

treated children showed higher inflammation twenty years later, which persisted after accounting for other childhood exposures and health behaviors [72].

Another pathway through which work stress is hypothesized to impact CHD is by increasing blood coagulability. Several studies have found a positive relation between stress at work and hemostatic factors: blood coagulation factor VII and VIII [73] and increased perivascular inflammation: CRP and IMT [74].

Inflammatory processes are linked to a hypercoagulable state and to endothelial dysfunction, which are also mechanisms for CHD. Accumulating data have demonstrated a causal link between stress and these systems [75].

Examining inter-relationship among cortisol, inflammatory markers, and endothelial function would be of a great interest to identify new biomarkers. In fact, based on the theoretical background, glucocorticoids may have a proinflammatory effect [16,76], so it is hardly surprising that the hormone stress is linked to the inflammatory mechanisms.

On the other hand, glucocorticoids stimulate the production and release of endothelin (ET), a potent vasoconstrictor, by VSMC and seem to exercise this effect by enhancing transcription of prepro-ET mRNA [77].

Another emerging strand of evidence links cortisol and the nitric oxide (NO) system, a powerful vasodilator and the key factor involved in the phenomenon of flow-mediated dilation (FMD). In fact, glucocorticoids are known to inhibit the NO synthase (NOS) [78], both directly by decreasing their production [79] and indirectly by reducing production of the essential NOS [80], thereby impairing endothelial function in cell and tissue models [81].

NO and ET systems are functionally antagonistic in their effects on endothelial function. Glucocorticoids have been shown to inhibit the NO and induce ET. Therefore, it is no wonder that cortisol mediates mental stress-induced impairment of endothelial function in humans. A strong link between cortisol and endothelial dysfunction/FMD is absolutely plausible.

PSF can affect allostatic load and induce neuro-cardiovascular diseases

As we show above, stress is known to lead to adverse changes in multiple biological systems, including endocrine, metabolic, and immune systems, which may eventually cause neuro-cardiovascular diseases [82]. A large body of literature on stress and physiological functioning has focused on single biological markers such as cortisol and interleukin level. However emerging research on stress argues for the importance of simultaneously considering multiple processes rather than a single underling mechanism [83].

Allostatic load (AL), is a multisystem indicator of physiological changes resulting from stress, which is computed using biological markers of multiple biological systems simultaneously [82]. The aim of AL is to summarize levels of physiological activity across range of regulatory systems related to stress response. The original formulation focused on different markers including cardiovascular risk factors, HPA-axis activity, SNS activity and biomarkers obtained from fasting blood [84]. Due to this multidimensionality, AL is thought to be a more comprehensive and sensitive measure of the effects of chronic stress on the body than any single biomarker [82,85]. In fact, even when the changes in each one of these systems are modest and not predictive of health outcomes, the cluster of changes across different multiple physiological systems present a health risk [86]. There is growing evidence that stress-related wear and tear of the body can be measured by the AL [87,88].

The traditional way of calculating an AL index has focused on the distribution of biomarkers within a given sample and then counting the number of dysregulated biomarkers for each individual [84]. AL index is constructed based on predefined cut-off values of many clinical biomarkers. For example, Juster RP *et al*, used cortisol, dehydroepiandrosterone-sulphate (DHEA-S), CRP, fibrinogen, insulin, glycosylated haemoglobin, albumin, creatinine, pancreatic amylase, total cholesterol, high density lipoprotein cholesterol (HDL-cholesterol), and triglycerides. Systolic and diastolic blood pressure values based on three

resting oscillometric recordings were included and waist-hip ratio measurement to assess truncal adisposity. Since all biomarker values were obtained, individuals' values were coded with respect to clinical reference ranges and aggregated into an AL index as follows: quartiles were calculated and values falling within the highest 75th percentile were scored as 1 while those falling normally below the 75th percentile were scored as 0 and summed to obtain an AL index for all biomarkers. The exceptions to this formulation were DHEA-S, albumin, and HDL-cholesterol, whereby the lowest 25th percentile correspond to the highest risk and were scored as 1. AL indices ranged from 0 to 15 [89].

Increased AL is associated with higher job demands in industrial workers in Germany [90], lower decision latitude and job strain in healthy Montreal workers [91], burnout [89] and career instability, effort-reward imbalance and exhaustion [92,88].

Of note the study of Mauss D et al, published in 2016 replicates former results published in 2015 in a large sample of German industrial employees using a short form AL. The revised form of AL [88] included diastolic blood pressure, waist circumference, glycosylated hemoglobin, low-density lipoprotein, and heart rate variability. Based on predefined subclinical cutoff values, a 5-variable AL was calculated. This short form of AL corroborates data obtained from the original one, which comprised 15 parameters. Taken together, these studies confirmed the pertinence of AL index in the assessment of the cumulative burden exerted on the body through variation to adapt to life's strain [87,88].

Recent evidence suggests an interactive effect of job strain and informal caregiving on AL. The study provides some evidence for adverse effects of stress at work combined with high caregiving burden on physiological functioning [12].

Other studies found no effect of job strain on AL [93,94]. Number and type of biomarkers vary by study and this may explain this difference.

Strengthen and weaknesses of the new biomarkers

In this review we chose to consider wide-ranging descriptions of stress from PSF and any type of exposure that may influence a physical health outcome through a psychological mechanism. We addressed literature data confirming the link between cortisol, endothelial dysfunction, and inflammation in neuro-cardiovascular diseases related to PSF. In this review we choose to collect data from a wide range of neuro-cardiovascular diseases in order to have more information on the effect of PSF.

From the key papers of this review the data about the PSF impact on CVD are reliable. These evaluations used rigorous methodology and then lead us to draw firm conclusions. This review highlights the potential of new biomarkers to reveal neuro-cardiovascular diseases occurrence in a psychosocial context.

Alterations in neuro-hormonal stress response systems (catecholamines) happen quickly following exposure to stress and cannot be used as a biomarker to traduce underling chronic psychosocial stress exposure [95,96].

Although there has been much recent progress to identify neuro-cardiovascular diseases risk biomarkers, the emergence of each new biomarker or group of biomarker raises questions of mechanistic relevance. In other words, are the target molecules a biomarker, or are they related in a causal way to the disease pathogenesis? How much do these new markers worth? But if we make our own mind:

(1) Will the biomarkers help clinicians to improve patient outcomes?

(2) How should clinicians incorporate these new biomarkers into clinical practice/ standard care?

(3) What is the overall improvement of diagnostic offered by these new biomarkers?

(4) Several differences exist between women and men in the incidence, clinical course, outcome, and comorbidities, so more attention should be given to these differences in order to counteract these confounding factors [97,98].

(5) The potential role of genetics in these complex relationships is unknown. It is possible that individuals who have special variants of the polymorphisms associated with in-

creased production of cortisol show worse immunological dysregulation when confronted to stressful events?

(6) We should keep in mind that overall the most powerful indexes are based on a combination of data, including clinical, electrocardiographic and biological measurements [13,99,100]. Data replication in larger studies is necessary to reveal the concrete significance of these biomarkers in the development or prognosis of neuro-cardiovascular diseases.

Clinical perspectives

Psychosocial stress is theoretically modifiable. It is currently the subject of increased attention through interventions based on stress reduction.

There are no studies addressing the role of workplace stress prevention in the neuro-cardiovascular disease incidence in comparison with lifestyle risk factors and standard risk factors. We think that working time arrangements or changement of management method of employees (especially by improving social interactions) might be the main strategies to avoid negative effects of occupational stress.

Most behavioral interventions, based on health educational program designed to attenuate the stress or music therapy [101,102], and pharmacological tests, based on randomized controlled trials of anti-depressant treatment [103], to reduce psychosocial stress in primary and secondary prevention of cardiovascular disease have not shown a real benefit.

However, mind-body interventions such as yoga and tai chi seem very promising in this field [104,105]. Regular yoga practice tends to facilitate autonomic flexibility, enhance self-regulation and induce a response characterized by parasympathetic dominance, reduced sympathetic activation and increased heart rate variability (HRV). Yoga can lead to improvement in sympatho-vagal balance [106], cognitive performance, and with better oxygen saturation [104].

These interventions have generally produced positive immune and endocrine changes (cortisol) [105,107-109]. Although it is not yet clear the all significance of immunological changes, the preliminary evidence seems to be promising. Only future studies including higher number of participants and investigating the biological and behavioral pathway through which PSF act on these diseases can reveal the true relevance of this practice.

Conflicts of Interest

None.

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