Chronic stress impacts the cardiovascular system: animal models and clinical outcomes.

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Running title: Stress and cardiovascular complications.
Abstract:
Psychological stresses are associated with cardiovascular diseases to the extent that cardiovascular
diseases are amongst the most important group of psychosomatic diseases. The longstanding
association between stress and cardiovascular disease exists despite a large ambiguity about the
underlying mechanisms. An array of possibilities have been proposed including overactivity of the
autonomic nervous system and humoral changes, which then converge on endothelial dysfunction that
initiates unwanted cardiovascular consequences. We review some of the features of the two most
important stress-activated systems i.e., the humoral and nervous systems, and focus on alterations in
endothelial function that could ensue as a result of these changes. Cardiac and hematologic
consequences of stress are also addressed briefly. It is likely that activation of the inflammatory cascade
in association with oxidative imbalance represent key pathophysiologic components of stress-induced
cardiovascular changes. We also review some of the commonly used animal models of stress and discuss
the cardiovascular outcomes reported in these models of stress. The unique ability of animals for
adaptation under stressful conditions lessens the extrapolation of laboratory findings to conditions of
human stress. An animal model of chronic unpredictable stress, which appliess various stress modules in
a random fashion, might be a useful solution to this predicament. The use of stress markers as indicators
of stress intensity is also discussed in various models of animal stress and also in clinical studies.
Introduction:

Stress can be defined as a subjective perception of an adverse environmental change, which usually leads to a stress response allowing for adaptation to the new condition (159). A recent survey ranked 74 countries for levels of stress based on seven equally weighted variables: homicide rates, income inequality, corruption perception, unemployment, urban air pollution, life expectancy, purchasing-power-parity basis, and gross domestic product (GDP) per capita (36). This information was drawn from a number of monitoring agencies including the United Nations Office on Drugs and Crime, the International Monetary Fund, the Central Intelligence Agency World Fact book, Transparency International and the World Health Organization. According to this metric, the three counties with the highest levels of stress were Nigeria, South Africa and El Salvador; while, the three with the lowest level were Switzerland, Luxembourg and Norway (36). Interestingly, the three Scandinavian countries (Norway, Sweden, and Denmark) along with Finland were amongst the seven least stressed nations (36), while the USA ranked 54th and Canada placed 65th. It is estimated that nearly 75% of Canadians suffer from at least one excessive or uncomfortable stress in their lives, with those in the 18-24 old age group reportedly most affected (48). (The poll was conducted in 2400 Canadians aged 18 to 80 years, with an accuracy of 2%). A recent study scrutinized the relationship between quality of marital life and cardiovascular diseases in 1198 married individuals. The authors suggested that an unpleasant marital life could be a significant risk factor for cardiovascular disease, especially in older females (199).

Different stressors, which can be either of a physical or psychological nature, stimulate separate regions of the nervous system to elicit appropriate responses (292). Activation of the autonomic nervous system and hypothalamic-pituitary-adrenal (HPA) axis are prominent features of the stress response, which in turn affects the function of other body organs. In 1988, Sterling and Eyer forwarded the concept of “allostasis”, referring to a collection of internal mechanisms for re-establishing homeostasis during challenging conditions (223). Chronic stress exposure, or allostasis overload, leads to maladaptive responses in various body organs and activates pathophysiological mechanisms such as cardiovascular diseases (279), psychological disturbances (57), and metabolic dysregulation (199). The Interheart Study, conducted in 52 countries with a sample size of 24767 patients, examined the association of psychological stress and myocardial infarction (279). Psychological factors were arranged into four major arrays: work, home, financial, and major life events. The data confirmed that patients with myocardial infarctions showed significantly higher levels of stress in one or more of the four categories of stress (279). We survey some of the adverse effects of psychological stress on the cardiovascular system and review relevant animal models that can be used to gain mechanistic insights and propose treatment strategies.

Stress and cardiovascular adverse effects

Even though the mortality rate due to cardiovascular disease has steadily decreased during the last 30 years, it is still a major cause of death; in 2008, one third of total deaths in the U.S.A were attributed to cardiovascular diseases (275). In addition to traditional cardiovascular risk factors (hypertension,
hyperlipidemia, diabetes, etc.), mental stress also plays a major role (126). In a study by Gullette et al., mental stresses, which were defined as feelings of tension, frustration and sadness, were found to double the risk of myocardial ischemia (126). Many other epidemiologic studies confirm this relationship (366, 176, 294). For instance, for people who routinely work on a Monday to Friday schedule, the risk of adverse cardiovascular events is lower on Saturdays and increases on Monday mornings (366). In the United States, cardiac death rate also increases during the Christmas holidays, which can be a reflection of emotional stress secondary to expectations of the occasion such as gift costs, travel expenses, large-scale social hosting events, and other social events such as office parties etc. (176). Anger tantrums, death of loved ones, and work pressures are among other psychological stresses that significantly increase the rate of myocardial infarction (294). Another potentially neglected parameter in stress induced cardiovascular disease is the role of nutrition. Experimental evidence and anecdotal experiences suggest that stressed people consume more calories-condensed foods such as sweet and fatty foods, and reduced amounts of micronutrients (249, 129). This, coupled by the reduced rates of metabolism under conditions of stress (172), results in obesity and metabolic disturbances that can jeopardize the health of cardiovascular system in several ways (207, 109). A recent report by Bergmann et al. that systematically analyzed prospective cohort studies scrutinizing the association between chronic psychological stress and the metabolic syndrome concluded that psychological stress is a causal factor for development of the metabolic syndrome (27).

The sequence of chain reactions that links psychological stress to cardiovascular diseases has not been comprehensively clarified; however, activation of the autonomic nervous system is paramount. An over-reaction of the autonomic nervous system to a chronic, unexpected, or unmodifiable stressor can precipitate cardiovascular dysregulation. Over-riding of sympathetic over parasympathetic nervous system causes tachycardia, hypertension and reduced heart rate variability (22). These reactions can in turn affect brain function and cause hormonal and immunological changes that are self-perpetuating (121).

**A central role for the endothelium**

There is widespread agreement for a key role of endothelial dysfunction in the adverse effects of stress on the cardiovascular system (44,114,198, 313). Social interactions in monkeys accelerate atherosclerosis, possibly through activation of the sympathetic nervous system (317). Atherosclerosis is a chronic inflammatory state of the arterial wall associated with the accumulation of macrophages, white blood cells, low-density lipoproteins (LDL) particles, and the release of inflammatory proteins and cytokines (370). The stress response, including stimulation of the sympathetic nervous system and hypothalamus-pituitary-adrenal (HPA) associated with overactivity of the renin-angiotensin system, leads to increased levels of homocysteine and elevated cardiovascular activity accompanied by various degrees of endothelial damage. Injury to the endothelium and the subsequent inflammatory response induces production of adhesion molecules and recruitment of immune cells, which in turn perpetuates the vicious cycle of macrophage infiltration, foam cell formation, atherosclerotic plaque formation, and adverse thrombotic events (125). A recent study by Lanter et al. extends our understanding of the role of the sympathetic nervous system in stress induced thrombovascular events (191). Norepinephrine, as a stress-induced hormone, interferes with iron transporting mechanism (transferrin) and increases the
plasma level of free iron. This is important since iron is a growth limiting nutrition for propagation of bacteria within the human body. Increased free iron favors biofilm production of bacteria on the surface of atherosclerotic plaques. Overgrowth of bacteria and release of proteolytic enzymes leads to biofilm dispersion and a subsequent thromboembolic crisis (191). Short periods of stressful events induce temporary episodes of endothelial dysfunction, while frequent and repetitive stresses leads to impairment in vascular reactivity (34). In an animal study conducted by Isingrini et al., unpredictable chronic mild stress induced hormonal and depression-related physical behaviors along with decreased endothelium-dependent vasorelaxation (153). In this study, stress did not affect phenylephrine-induced vasoconstriction, while Puzserova et al. reported increased contractile responses to both electrical stimulation of perivascular nerves and exogenous norepinephrine without any discernible changes in NO-dependent vasorelaxation (268). Results from another experiment suggest that chronic mild stress increased phenylephrine-induced vasoconstriction in intact aortic ring segments, yet failed to show such effects in L-NAME incubated or endothelium denuded rings. This signifies the pivotal role of endothelium in stress-induced vascular dysfunction. Stressed animals also show morphologic vascular changes and metabolic derangements including hypertrophy of the intima and tunica media of the thoracic aorta, increased serum levels of total cholesterol, VLDL, LDL, triglycerides, and atherogenic indices (242).

Human endothelial function is generally evaluated by one of two methods. In the first method, flow variations or changes in conduit artery diameter are measured after infusion of standard doses of an endothelium dependent vasodilator substance (e.g. acetylcholine). In the second technique, arterial diameter is assessed by shear stress-induced increase in flow (flow mediated dilation) (260). One method to increase shear stress is due to reactive hyperemia following release of an applied tourniquet or handgrip exercises.

Flow mediated dilation (FMD) is commonly used as an index of endothelial function in in vivo studies of humans and laboratory animals. Ghiadoni et al. were among the first to study the noxious effects of acute mental stress on FMD in healthy subjects. In this experiment, FMD in healthy individuals after mental stress was comparable with FMD at baseline in patients with type II diabetes (105). Acute stress raises the circulatory levels of inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (316), and also results in platelet activation (as indexed by an increased level of β-thromboglobulin) and endothelial stimulation (as indicated by an elevated level of factor VIII/von Willebrand) (236). These events can accelerate the process of atherosclerosis. In spite of this, the mechanism whereby stress leads to impaired FMD has not been comprehensively studied. We do know however that a blunted FMD is not a general response to increased sympathetic nervous activity (78) and that FMD differs according to the method used to induce hyperemia (326). Pre-existing endothelial dysfunction could act as a confounding factor in the endothelial response to stress. A standard arithmetic challenge induced vasodilation and increased brachial artery blood flow in healthy men and women (133), while the same test caused local coronary vasoconstriction at atherosclerotic sites and vasodilation in smooth segments in patients with preexisting coronary artery disease (374).
Presumptive mechanisms underlying stress-induced endothelial dysfunction

Chronic stress not only leads to activation of stress-responsive organs but also is commonly associated with riskful life-style modifications, such as decreased physical activity, obesity and smoking. All of these conditions potentially harm the cardiovascular system and aggravate stress-induced cardiovascular events (161).

The HPA axis and the autonomic nervous system are the first two responders to stressful situations (368). This suggests that stress-induced endothelial dysfunction may likely be related to responses mediated by these two systems. In other words, enhanced sympathetic activity in association with adequate hormonal changes likely alters endothelial function.

Corticotropin-releasing hormone

Corticotropin-releasing hormone (CRH) is one of the first hormones released from the hypothalamus in response to stress (365). In animal studies, intraventricular injection of CRH not only induces some stress-response behaviors such as increased arousal, but also augments heart rate and blood pressure (160). Several putative mechanisms connect stress-induced endothelial function to corticotropin release. For instance, corticotropin can increase secretion of endothelin-1 (ET-1) from cultured human endothelial cells in a time and dose dependent manner through a process that is abolished by a corticotropin receptor (CRH-R2) antagonist (160, 245). Corticotropin also causes CD14+ cells to release TNF-α while also damaging endothelial barrier function by inducing apoptosis of endothelial cells (310). Furthermore, corticotropin promotes monocyte adhesion to the endothelium (364). In support of these findings is the observation that high concentrations of corticotropin occur at several inflammatory sites, such as in the synovial fluid of patients with rheumatoid arthritis or in follicular cells of patients with autoimmune thyroiditis (69, 296). Urocortin, a CRH receptor agonist, degranulates mast cells, which in turn initiates inflammatory reactions that are coupled with increased vascular permeability (306). Urocortin also changes the antithrombotic property of endothelial cells through stimulation of the proinflammatory mediators IL-1β and IL-6 (178). In addition, urocortin also reduces the synthesis of PGI₂ (96) and negatively impacts the growth of endothelial cells (19). In spite of these findings, there is some evidence supporting an anti-inflammatory and protective effect of corticotropin in vascular tissue. For example, Inada et al. reported that selective blockade of CRH-R1 on human aortic endothelial cells promotes TNF-α induced expression of vascular adhesion molecule-1. In this experiment, pitavastatin up-regulated expression of CRH-R1 mRNA, suggesting a role for corticotropin receptors in the vasoprotective effect of this drug (148). The corticotropin receptor agonist urocortin improves the vasodilatory effect of acetylcholine after ischemic-reperfusion injury (102, 192). Increased levels of corticotropin have also been reported after beneficial physical activities (305). So, it can be assumed that increases in this hormone are a component, albeit minor, of stress-induced cardiovascular diseases.

Additional work is needed to clarify the role of corticotropin in the pathogenesis of stress-induced vasculopathy. To add to this complexity, it should be reiterated that while increased blood levels of corticotropin occurs after physical stressors and following psychosocial stresses during pregnancy, some
studies could not show increased levels of corticotrophin in the systemic circulation after mental stress. This obstacle may be due to fast uptake, quick metabolism, or accelerated tissue binding (260).

**Cortisol**

As discussed earlier, stress activates the HPA axis and so leads to increased secretion of adrenal glucocorticoids including cortisol. Levels of cortisol can increase up to ten fold under intensely stressful situations (276). There is ample evidence supporting a role for cortisol in stress-induced endothelial dysfunction, some of which is discussed below.

First, cortisol decreases membrane adenylyl cyclase activity and cAMP levels (39). The ubiquitous second messenger cAMP has an important role in maintaining endothelial function and prevention of atherosclerosis in addition to decreasing endothelial permeability and promoting cadherin-dependent cell adhesion and inhibiting hyper permeability evoked by inflammatory reactions (99). An important additional role of cAMP is in mediating re-endothelialization of the damaged vascular intima (87). Several anti-inflammatory functions have also been described for cAMP. For instance, it diminishes high glucose induced-inhibition of nuclear factor kappa B Kinase (IKK-β) activity and TNF-α induced increase in IKK-β, which results in the inhibition of the production of proinflammatory cytokines. Endothelial cAMP content also reduces hyperglycemia induced reactive oxygen species (ROS) production and has an inverse relationship with oxidized LDL levels. Agents that increase cAMP levels also decrease plasma triglycerides and raises HDL and apolipoprotein A1 levels (88). All of these actions signify beneficiary effects of cAMP that can be reduced or reversed by cortisol.

Second, cortisol decreases nitric oxide (NO) synthesis or bioavailability. The endothelium has a primary role in adjusting vascular function by producing NO and other biologically active vasodilator materials (224) that decrease vascular resistance, inhibit platelet adhesion and aggregation, and decrease vascular smooth muscle cell proliferation. Alterations in the control of these processes, a feature of endothelial dysfunction, often leads to atherosclerosis and other vascular disorders (38, 162) that are accompanied by a proinflammatory, proliferative, and procoagulatory state (95). NO is produced by the catalytic activity of NO synthase (NOS), which transforms L-arginine to L-citrulline. All isoforms of NOS require five cofactors/prosthetic groups: flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin (BH4), and Ca^{2+}/calmodulin. Calcium is required for the activation of neuronal NOS (nNOS) and endothelial NOS (eNOS), but not for the activity of inducible NOS (iNOS) (110). If eNOS lacks its substrate L-arginine or one of its cofactors, the enzyme will produce superoxide instead of NO manifesting from an uncoupled state of NOS (375). Cortisol inhibits eNOS and decreases NO production. Incubation of human umbilical endothelial cells with cortisol decreased NO production by 60-70%, a phenomenon that can be blocked by mifepristone, a glucocorticoid receptor antagonist (355). Recently, Liu et al. reported the presence of a glucocorticoid response element in the eNOS gene promoter region. Relatively small increases in cortisol (30 to 100 nmol/L) decrease the expression of eNOS in human endothelial cells. Endogenous agents such as 11-beta hydroxysteroid dehydrogenases 1 and 2 modulate catalyze the interconversion of active and inactive glucocorticoids; suppression of these enzymes augmented the inhibitory effects of glucocorticoids on eNOS expression (203). Importantly, cortisol disturbs NO availability by increasing ROS production. The mitochondrial electron transport chain (NADPH oxidase) and xanthine oxidase system are largely responsible for ROS production (155).
Superoxide anions reduce NO availability directly by forming peroxynitrite and indirectly by oxidizing BH4, which leads to NO uncoupling and further ROS production. Accordingly, exposing human umbilical vein endothelial cells to dexamethasone (10^{-7} \text{ mol/L}) raised hydrogen peroxide production and increased peroxynitrite formation (155).

The third mechanism by which cortisol affects vascular function is via enhancement of ET-1 secretion from vascular cells (245). This effect can be a direct result of cortisol or may be the consequence of glucocorticoid-induced oxidative stress in vascular tissue (290). In a study of healthy human subjects, 3 minutes of mental stress elicited endothelial dysfunction as assessed by FMD. The endothelin A receptor antagonists (BQ-123) prevented functional impairment, signifying the role of endothelin in vascular dysfunction during events related to mental stress (313).

Some metabolic changes induced by cortisol can have detrimental effects on vascular function. Cortisol is a as a counter-regulatory hormone to insulin action and so increases blood sugar levels. Stress-induced glucose dysregulation, called stress hyperglycemia, has been studied after both physical and psychological stresses (83, 103). This phenomenon was considered a part of the physiologic response to stress. However, further studies revealed that better control of hyperglycemia reduces morbidity and mortality in critically ill patients (343, 342, 347). From a mechanistic point of view, insulin resistance can occur at different levels of glucose metabolism including insulin receptor substrate (IRS)-1, phosphatidylinositol 3-kinase, protein kinase B (Akt), and glucose transporter (GLUT 4) function (341). The resultant hyperglycemia exerts its detrimental effects on endothelial function through different pathways, most of which result in impaired NO production (108). A laboratory study in rabbit reports that even 3 hours of high blood glucose can injure the macro- and microvasculature, as measured by endothelium dependent vasodilation (112). Chronic psychological stress can also lead to intracellular structural changes, which in turn aggravate hyperglycemia. Swollen mitochondria with loss of cristae and reduced matrix in association with reduced Na^{+}-K^{+} ATPase, Ca^{2+}-ATPase, and increased vascular permeability occurs in masticatory muscles of rats following five weeks of psychological stress (79). In addition to impaired NO-dependent vasorelaxation, hyperglycemia also stimulates expression and secretion of inflammatory mediators possibly through an oxidative mechanism (86). Importantly, even hyperglycemia induced in healthy individuals increases blood levels of inflammatory mediators. This effect is more prominent in patients with underlying glucose metabolism disturbances and can be reversed by glutathione administration, suggesting a role for oxidative stress in the process. Secreted cytokines (TNF-α, IL-6) in turn escalate insulin resistance, oxidative stress, and promote the instability of atherosclerotic plaques (IL-18) and adverse cardiovascular events (85).

**Autonomic nervous system activity**

Even though there is little doubt about the increased activity of the autonomic nervous system during stressful situations, it remains unclear how this eventually manifests in endothelial dysfunction. In a study of sixteen healthy volunteers, sympathetic nervous system overactivity elicited by lower body negative pressure reduced FMD. Intra-arterial phentolamine abolished this effect and so signified the role of alpha-adrenergic receptors (141). In another experiment with thirty-one healthy young adults, the negative effect of mental stress on endothelial function in resistance vessels was confirmed; however, this impairment was inhibited by propranolol and neurogenic blockade but not by
Beneficial effects of beta-receptor antagonists in preventing atherosclerosis and improving endothelial function in stressed animals reiterate the importance of the sympathetic nervous system in stress-induced endothelial dysfunction (34, 212, 166). The experimental protocol for activation of the sympathetic nervous system and the vascular bed studied (conduit vs. resistance vessels) are likely to affect the outcome of some studies. Evidence for this is provided by the data of Dyson et al. who studied four different methods of sympathetic nervous system stimulation (lower body suction, cold pressor test, mental arithmetic task and activation of muscle chemoreflex) under conditions that produced similar levels of peak shear stress; they reported variations in basal vascular tone and importantly, where reductions in FMD were not a universal response (78).

Hypersensitivity of the sympathetic nervous system occurs during chronically stressful conditions. Animals exposed to chronic repetitive (“habituated”) stress are able to synthesize and store higher amounts of catecholamines, although catecholamine release at rest and the surge after an episode of habituated stress is lower in stressed animals. However, stimulation by a novel stress elicited an exaggerated sympathetic response in these animals (123), suggesting increased hyper-responsiveness of the autonomic nervous system and that the catecholamine surge may be regulated differently when stimulated by habitual versus novel stress. Hyperactivity of the sympathetic nervous system, especially on a chronic basis, can harm the cardiovascular system in different ways. First, the continuous pressor effects of released catecholamines may counteract vasodilatory effects of NO and can also accelerate the atherosclerotic process. Importantly, Spieker et al. reported that intra-arterial infusion of norepinephrine, unlike mental stress, failed to decrease FMD (313). The time course of stress-induced decrease in FMD is also different from the time course of heart rate and blood pressure changes (105). Furthermore, in vitro studies on human endothelial cells show that norepinephrine increases eNOS activity under acute conditions (301). On the other hand, NO appears to be involved in triggering catecholamine surges in stressed animals, as L-NAME administration decreased stress-related responses (340). Collectively, it seems unlikely that stress-induced increases in catecholamines are directly responsible for endothelial dysfunction (334).

Another putative mechanism is activation of the renin-angiotensin-aldosterone system (RAAS) resulting from sympathetic overactivity. The activity of the RAAS in a study of 1743 individuals was higher in depressed people and those who lived alone (129). Some investigators suggest aldosterone as a marker of depression (234). Central activation of mineralocorticoid receptors leads to sympathetic overactivity and increased secretion of proinflammatory cytokines both in central and peripheral tissues (113). Increased plasma renin activity and angiotensin II also occurs after intense physical activity (189), heat exposure (184), and mental stress (186). Rats stressed by a new environment (open field) or predator exposure show increased RAAS activity, which was attenuated by beta antagonist (propranolol) administration (64). Stress induced sympathetic activation of the juxtaglomerular apparatus in the kidneys escalates RAAS activity (123). ACE inhibitors improve despair behavior in rodents during swim tests (215) and mice lacking ACE demonstrate fewer signs of depression (248). Adjusting to a new sodium balance during stressful conditions could activate the juxtaglomerular apparatus. Patients with a decreased sense of well-being and chronic fatigue responded favorably to beta antagonists or mineralocorticoid administration along with increased dietary sodium (41). Furthermore, several animal
studies report that reduced body sodium is associated with anhedonia (inability to derive pleasure from normally enjoyable activities), anxiety, decreased physical activity and abnormal cardiovascular parameters such as increased resting heart rate, reduced heart rate variability, and exaggerated cardiovascular response to stressors (115, 195, 118).

Angiotensin II, a potent vasoconstrictor, can harm endothelial cells independently of its pressor effect (3), for example by increasing cellular lipid peroxidation (170) and reducing cholesterol efflux (167) in macrophages and scavenger cells; both actions lead to accelerated foam cell formation and a hastened atherosclerotic process. Angiotensin II stimulates uptake of oxidized LDL by macrophages, possibly mediated by IL-6 (169). Margination, adhesion, and translocation of leukocytes, all prerequisites for atherosclerosis, are enhanced by angiotensin II type I receptors (AGT1R) (218, 219, 259,380). Angiotensin II also promotes the expression and production of adhesive and proinflammatory molecules (vascular cell adhesion protein 1, intercellular adhesion molecule 1, monocyte chemo-attractant protein-1, macrophage inflammatory protein 1 alpha, IL-8) on the endothelial and vascular smooth muscle cells (55, 145, 200, 267, 282, 325). Angiotensin II affects vascular smooth muscle signal transduction and several growth factors (transforming growth factor-β, platelet derived growth factor, and basic fibroblast growth factor) that stimulate smooth muscle proliferation (165). Deletion of the AGT1R gene in Apo-E deficient mice reduces the development and progression of atherosclerosis (358). Atherosclerotic plaques are also more stable and contain fewer numbers of macrophages in AGT1R deficient mice (98). Central and peripheral blockade of AGT1R with candesartan provides cardiovascular benefits in addition to modifying responses to psychological stress (254). In addition, candesartan abolished hormonal and sympathetic responses mediated by the hypothalamic-pituitary-adrenal axis in rodents undergoing isolation-induced stress, while also reducing anxiety and protects against gastric ulceration caused by cold-restraint stress (293).

In spite of the untoward consequences of activation of AT1R by angiotensin II, there is another counterregulatory cascade of reactions that is triggered by activation AT2/Mas receptors and which has beneficial effects during stressful situations. The heptapeptide angiotensin 1-7, the product of ACEII interacting with angiotensinogen, is the principle agonist for AT2/Mas receptors. The peptide fragment angiotensin 1-7, was long considered an inactive byproduct of the RAS. Later, Schiavon et al (47) examined the effect of this substance in releasing vasopressin from the hypothalamo-neurohypophysial system. This was a foundation of a series of interesting experiments that changed our understanding of the physiologic role of this substance (286, 287, 289, 56). By acting preferentially on AT2/Mas receptors, angiotensin 1-7 has beneficial effects not only during the stress response but also in several other pathophysiological conditions including cardiovascular, renal, immunological, and neurological diseases (197, 288). A report by Walther et al. was amongst the first to suggest that Mas receptor-deficient mice show increased anxiety in behavioral tests (356). Furthermore, a functional interaction between Mas and AT1 receptors occurs in the amygdala, an important nucleus in the limbic system regulating many psychological responses (348). Intravenous injections of an AT2/Mas receptor agonist or an ACEII activator reduced stress-induced tachycardia (217). Intra-ventricular injection of these substances also diminished isoproterenol-induced tachycardia and renal sympathetic activation. Therefore, one can concluded that activation of AT2 receptors modifies sympathetic nervous activity during stressful
conditions. The cardioprotective effects of angiotensin 1-7 have been shown in several studies (288, 122, 26).

Role of proinflammatory mediators and oxidative stress in stress-induced endothelial dysfunction.

There is much evidence supporting a role for proinflammatory cytokines in stress induced cardiovascular diseases. Bernberg et al. reported increased levels of proinflammatory mediators, IL-6 and chemokine (C-X-C motif) ligand 1 (CXCL1) in Apo E -/- mice following psychological stress (28). Increased peripheral levels of IL-6, soluble intercellular adhesion molecule 1 (sICAM-1), and C-reactive protein (CRP) also occur in a rabbit model of atherosclerosis following mental stress (196). There are increased circulating levels of tumor necrosis factor-α (TNF-α) and interleukin-1 beta (IL-1β) in a rat model of anhedonia induced by unpredictable chronic mild stress (117). Increased levels of proinflammatory cytokines have consistently been reported in several human studies; in a multi-ethnic study of atherosclerosis, cynical distrust causing psychological stress produced higher levels of IL-6 and CRP (272). Another investigation revealed that middle age women with psychological symptoms have higher levels of IL-6, IL-8, TNF-α, and IL-2 compared to those without symptoms (373). Higher levels of proinflammatory cytokines and the ratio of proinflammatory/anti-inflammatory cytokines occur in healthy individuals who are under a greater degree of psychological stress (350). Several meta-analyses confirm dysregulated levels of inflammatory cytokines in major depression (76, 144, 142, 202). The role and importance of inflammatory cytokines in inducing aberrant endothelial function was examined in several studies (255, 297, 235, 94). For instance, TNF-α and IL-1 decrease eNOS activity along with reducing eNOS mRNA and protein content (379) while at the same time also reducing vasodilatory response to bradykinin and arachidonic acid in human cutaneous vessels. Other suggested mechanisms include alteration in calcium channel expression and activity (333), upregulation of Rho-kinase expression and function (143), increased ROS production (378), and enhanced COX expression (229).

Inflammation and oxidative stress are closely related phenomena, as ROS is produced during immune reactions form an important part of the body’s natural defense or repair mechanisms. Meanwhile, reactive radicals attract more immune cells and further exacerbate inflammatory reactions. Oxidative stress increases the rate of lipid peroxidation, which accelerates the atherosclerotic process and impairs vascular tone (257, 320). Nuclear factor-κB (NF-κB) is thought to link psychological stress and oxidative stress- induced inflammatory organ dysfunction, including impaired cardiovascular regulation (32). The NF-κB pathway is a critical component of the inflammatory processes that is activated by oxidative stress (372, 62) and has a role as a ubiquitous transcription factor having multiple roles such as mediating inflammatory responses to a variety of signals, regulating immune function, causing endothelial cell activation, and controlling cell growth (18, 20, 106). NF-κB is normally present in an inactive form in the cytoplasm by virtue of binding to a family of inhibitory NF-κB (IκB) proteins. Stimulation by a variety of stimuli activates IκK-α and IκK-β (also known as IκK-1 and IκK-2), resulting in the phosphorylation of IκB and its proteasomal degradation. IκB degradation liberates NF-κB, allowing it to translocate to the nucleus and induce gene expression of proinflammatory cytokines such as IL-1β, IL-6, TNF-α, COX-2, lipoxygenase, iNOS, and adhesion molecules (VCAM-1, ICAM-1, PCAM, E-selectin).
Increased activity of NF-κB occurs in peripheral blood mononuclear cells of healthy volunteers undergoing a brief laboratory test (33). This increment was proportional to the levels of catecholamines and cortisol secretion. The Trier Social Stress Test, used as a form of mental stress that includes a speech and an arithmetic test in the presence of an audience, also increased NF-κB in participants (174) (fig 1).

**Adverse effect of stress on the heart**

The vascular consequences of chronic psychological stress have been addressed above; these mechanisms are likely to have secondary effects on cardiac function. Some of the pathophysiologic mechanisms that target cardiac performance more directly are discussed below.

**Stress-induced cardiomyopathy**

Takotsubo syndrome, stress-induced cardiomyopathy, and broken heart syndrome all refer to a transient left ventricular dysfunctional syndrome wherein stress (physical or emotional) has a major role in its pathogenesis (256, 238). Even though many of its clinical and laboratory signs (such as chest pain, shortness of breath, electrocardiographic and angiographic features) resemble acute coronary symptoms, atherosclerosis has no role in the pathophysiology (331). This syndrome first was described in 1990 in Japan (6). Clinical presentation mimics acute coronary syndrome or myocardial infarction; however, during angiography, ventricular apical ballooning without significant coronary obstruction is evident. Perimenopausal women have an extra susceptibility to this syndrome, probably due to the role of estrogen deficiency as a participating factor in the pathogenesis. Other suggested mechanisms include acute catecholamine surges, abnormal coronary vessel reactivity that reduces myocardial microperfusion, impaired myocardial metabolism, viral infection, and endothelial dysfunction. Sympathovagal perturbation resulting in autonomic imbalance has received much attention because early post-stress measurements of plasma catecholamines reveal overactivity of the sympathetic nervous system in afflicted patients. Evidence for a therapeutic and prophylactic role of adrenoceptor blockade also highlights the role of autonomic imbalance (256). High levels of catecholamines reduce coronary flow in the microcirculation leading to myocardial stunning (303, 188). A higher density of adrenergic receptors in apical regions of the heart has been suggested to account in part for ventricular dysfunction and apical ballooning (233). Immobilization stress in rats provides a useful animal model to mimic key features of this syndrome (339). A bolus intravenous injection of epinephrine in rats produces a reversible myocardial dysfunction with apical hypokinesia and basal hypercontractility and is considered a model of stress-induced cardiomyopathy (253).

**Stress-induced cardiac ischemia and arrhythmias**

Psychological stress results in reduced coronary blood flow in freely moving dogs, as measured by inserting a probe in the left circumflex artery to demonstrate that narrowing of coronary vessels, along with evidence of electrocardiographic findings of myocardial ischemia, following an episode of anger (344). Feeling of tension, frustration, and sadness doubles the risk of myocardial infarction in patients with coronary artery disease (126). In 98 young patients (age 38-60) with myocardial infarction who underwent 3 SPECT (single-photon emission computed tomography) imaging at rest, those with higher levels of anger, either in reaction to an environmental stimulus or as a chronic personality character,
were more prone to myocardial ischemia when exposed to mental stress (258). Similar findings were made in an earlier 14 year study of 1328 patients with coronary heart disease patients where there was an inverse relationship between the degree of hostility (measured by the Cook-Medley Hostility Scale) and survival rates in subjects below 61 years of age (42).

In addition to ischemia, which can trigger cardiac arrhythmias, psychological stress per se destabilizes the myocyte membrane (273). In the study by Verrier et al. (344) discussed above, cardiac fibrillatory threshold potentials were reduced by 40% in stressed dogs. A more recent experiment in stressed rodents also reported decreased effective refractory periods, an impaired conduction of electrical activities, and ventricular fibrosis (50). Autonomic imbalance and catecholamine surges are implicated in the arrhythmogenic effects of emotional disturbance, as shown by Lampert et al. who reported that increases in catecholamine levels during human emotional turmoil are correlated with changes in heart rate, systolic blood pressure and repolarization indices (T-wave amplitude, T-wave area, and T-wave alternans, which is a periodic beat-to-beat variation in the amplitude or shape of the ECG, and is an indicator of those at high risk of developing potentially fatal cardiac arrhythmias.) (189, 190).

Ventricular parasympathetic nerves are known to have protective effect against ventricular arrhythmias, and it is likely that decreased parasympathetic activity during psychological stresses would facilitate ventricular arrhythmias (328), although it should be pointed out that the situation is likely more complicated that merely considering the balance of the sympathetic and parasympathetic neural regulation of cardiac function and electrical activity—for example the role of temporal and spatial activation of various components of this system is unclear. Emotions result from complex responses to external (or internal) stimuli, which activates various loci in the brain asymmetrically (130, 7), resulting in impulses that are transferred ipsilaterally and stimulate autonomic ganglions in a spatially inhomogeneous manner to elicit appropriate responses (327). This brain-cardiovascular cross talk with its anatomical and physiological variations could partial account for the range of responses to stress in different people.

Hematologic consequences of stress

Disturbances in hemostasis are an important consideration in the pathogenesis of stress-induced cardiovascular disease. The balance between procoagulant and anticoagulant factor is a constantly changing process that is often skewed in many pathologic conditions. Enhanced prothrombotic states have long been described in the fight-or-flight response (49). This primitive response protects animals from hemorrhage in cases of accidental trauma. However, repetitive and prolonged stress in association with endothelial dysfunction outweighs the counteracting anticoagulant system and can potentially lead to thrombus formation (352, 317). It is important to consider some of the most important mechanisms that link heightened coagulation to stress-induced cardiovascular adverse.

The brain’s perception and interpretation of a stressful situation likely initiate hyper coagulopathy. Early studies by Gunn and Hampton reported that stimulation of specific areas of the dog brain that is related to autonomic integrative functions is associated with transient increases in factor VIII levels (127). Hemodynamic parameters, catecholamine levels and D-dimer changes all correlate with stress appraisal in a study of 47 males; moreover, those who anticipated a higher amount of stress or a more challenging situation showed greater levels of fibrin turnover (368). The relationship between stress perception and
coagulation cascade, as a risk factor for cardiovascular disease, has also been confirmed by studies showing higher serotonin sensitivity of platelets in depressed and anxious individuals (304, 376). Interestingly, Lesch et al. reported that both human platelet serotonin uptake sites and brain serotonin transporter proteins originate from the same gene (194), suggesting an active interplay between the brain and blood coagulation system in which serotonin could have a cross-talk mediator role (25). Age, sex, integrity of cardiovascular system, and socioeconomic status are participating factors in this scenario, as the elderly (369), males (157), those with lower socioeconomic status (157), and also those with a history of coronary heart disease or hypertension (351, 350) are at increased risk for having a higher procoagulant activity for extended periods of time. Inversely, a state of happiness blunts the stress-induced fibrinogen response, irrespective of age, gender, socioeconomic status, body mass, and smoking (319).

Another explanation for a heightened coagulatory status could be sympathetic nervous system activation (266). There is a solid body of evidence that support this notion. For instance, infusion of adrenaline increases blood levels of factor VIII, von Willebrand factor (VWF) antigen, tissue type plasminogen activator, and platelet levels within 15-40 minutes. Platelets and endothelial adrenoceptors have essential roles in these reactions (349). Stimulation of endothelial beta-receptors releases factor VIII, VWF, and tissue plasminogen activator. Catecholamines also adjust coagulation by affecting hepatic clearance of these factors (349, 353). Chemical sympathectomy with 6-hydroxydopamine prevents stress-induced prothrombotic enhancement in an animal model of physical-restraint induced stress (314).

Hemoconcentration (increased ratio of blood cells and macromolecules to plasma volume) is another potential participant that hastens stress-induced procoagulatory status (8). The resultant hyper viscosity increases shear stress and raises the probability of rupturing of atherosclerotic plaques (8). Activation of the sympathetic nervous system is once again implicated as major cause, since the increased systolic and diastolic blood pressure following sympathetic overactivity causes plasma to move from the intravascular space, while there is retention of blood cells and proteins in the vascular lumen (14). This phenomenon could occur during stress-induced hyper coagulopathy, although de novo synthesis or activation of coagulation factors cannot be ruled out (14).

Stress biomarkers

Biomarkers are measurable indicators of a specific physiologic condition or a disease state, and can be used to evaluate the reproducibility of an experimental method and/or response to a therapeutic intervention. Biomarkers can also be used to predict the occurrence of a disease. Using biomarkers as “endpoint surrogates” has several advantages including, economic, rapidity, and ease of performance benefits (13). In a multicenter study conducted in Switzerland, plasma levels of stress markers in patients presenting to the emergency department with non-specific symptoms were strongly correlated...
with 30 days survival rate, causing the authors to suggest that this evaluation might be helpful in stratification of risk assessment (244). There is a close relationship between our understanding of disease pathophysiology and/or the therapeutic effects of an intervention and identifying pathognomonic markers. In psychological conditions, the diversity of stressors and heterogeneity of personal responses (aside from the involvement of many body organs and presence of confounding factors) have hindered the discovery of reliable biomarkers. In this section, we review some commonly used stress biomarkers that are used in clinical and basic science research.

**Indicators of HPA axis activity**

Cortisol levels are the most reliable indicators of HPA activity and have been measured in urine, saliva, serum, and hair (241). There is an abrupt surge in the plasma cortisol levels following acute stress, which then returns to baseline values after removal of stress (310). Intense chronic stressful conditions abolish physiological diurnal variations of cortisol secretion and leads to a high steady state value (227). A state of hypo-glucocorticoid, which is associated with increased vulnerability to autoimmune disease, may follow chronic overactivity of adrenal glands (251). Even though determination of plasma cortisol is the most widely used method, there are several downsides to this. For instance, venipuncture is a potentially stressful procedure that has the potential to affect the measurement (1). Physiological diurnal variation is another important issue to consider in interpreting the results. Plasma cortisol is a measurement of total plasma cortisol; any medication or pathologic condition that affects transcortin or albumin levels may falsely affects the readings (225). Assessment of salivary cortisol is a useful method to address most of these shortcomings since it represents 70% of free (unbound form) plasma cortisol levels and is not influenced by salivary volume and flow (261). Sampling of saliva does not induce pain or stress and due to the stability of cortisol in saliva, samples can be kept at room temperature for up to four weeks (43). Transfer of cortisol from blood to saliva is a fast process (2-3 minutes); therefore, this method can detect acute variations in blood levels and circadian rhythm (43). Hair cortisol levels have been used to monitor long-term stress levels. Since growth of human scalp hair is approximately 1 cm/month, hair cortisol mirror stress levels over a period of several months (331). This method has several advantages over serum or salivary measurements; importantly, it is a noninvasive method and does not induce acute situational stress at the time of sampling. Diurnal variations are not applicable to this procedure and cortisol levels are very stable in hair samples (226). On the other hand, there are some drawbacks; for example, the effects of short-lived stressors are difficult to detect using this technique. A wide variety of stresses including physical, metabolic, and psychological can affect hair cortisol levels. Another important issue is that even though a growing amount of data points to the blood as a major source of hair cortisol, there is some controversy about this as Ito et al. showed that human hair follicles have steroidogenic enzymes (154). When placed in a culture medium, hair follicles respond to CRH by up-regulating proopiomelanocortin transcription. Adrenocorticotropin hormone (ACTH) also increases immune-reactivity of cortisol in hair follicles. Therefore, there is some doubt about the local production of cortisol in hair follicles and the physiological importance of this phenomenon is unclear.
Salivary α-amylase

From the time that Gilman et al. (107) reported increased salivary alpha-amylase (sAA) level in response to physical activity and its association with blood cortisol level, many investigations have established the role of sAA as a stress marker. Alpha amylase is a digestive enzyme, which metabolizes starch to glucose and maltose (377). Since salivary production is controlled by the autonomic nervous system, the amount and composition of saliva is expected to change during the course of psychological disturbances. Indeed, increased sAA levels occur after several diverse stressful conditions ranging from laboratory settings to natural life situations. A comprehensive list of these investigations can be found in reviews by Nater and Rohleder (240, 277). Even though clinical and animal studies suggest that sAA may represent a promise as a reliable stress marker, more scrutiny is needed to standardize this test based on stressor type and chronicity (40).

Immunological markers

Inflammatory cytokines such as IL-6, TNF-α, and CRP have been used as nonspecific markers of stress in epidemiologic studies. Many adverse effects of psychological disorders, including endothelial dysfunction, altered course of infectious diseases, autoimmune/inflammatory overactivity, allergic and neoplastic syndromes have been related to altered levels of cytokines (80, 232). For instance, early childhood abuse in females increases the risk of diabetes and cardiovascular diseases, possibly through immune and chronic inflammatory dysregulation (30). Increased levels of erythrocyte sedimentation ratio (ESR) and CRP, as non-specific markers of systemic inflammation, also occur in depressed patients (58). In another study of patients with rheumatic arthritis, those who showed signs of depression had higher levels of ESR compared to non-depressed persons (2). Treating depression normalizes or at least alleviates cytokine dysregulation. By way of example, in the study of Hannestad et al. selective serotonin reuptake inhibitors (SSRIs) lowered IL-6 and TNF-α level (131). Electroconvulsive therapy of depressed patients also shows some promising results in reducing TNF-α (139). In a study of 199 women at different stages of breast cancer, ten weeks of cognitive-behavioral stress management down-regulated anxiety-induced expression of proinflammatory cytokines in circulating leukocytes (12). However, treatment of depression is not always accompanied by reduced levels of proinflammatory cytokines (361,210). In an attempt to show causal relationship between stress-induced cytokine changes and cardiovascular complications, Lu et al. (204) evaluated the effect of an unpredictable chronic mild stress (UCMS) protocol on vascular inflammation in rabbits. In this experiment, UCMS induced hypertension (without changes in lipid profiles) along with depression-like behavior. Pro-inflammatory markers including CRP, monocyte chemoattractant protein-1 (MCP-1), macrophage migration inhibitory factor, intercellular adhesion molecule-1 (ICAM-1) and vascular expression of TNF-α, were all increased. UCMA also decreased expression of endothelial NOS and caused atherosclerosis to appear 16 weeks later. Treatment of cultured vascular smooth muscle with plasma from stressed animals resulted in up-regulation of MCP-1 and ICAM-1 expression and also increased phosphorylation of p38 and c-Jun N-terminal kinase. Administration of TNF-α neutralizing antibody or P38 and JNK inhibitors decreased these changes (204).

The exact mechanism(s) and source(s) of cytokine production during stressful situations remain to be elucidated. One proposed mechanism is suppression or dysregulation of the parasympathetic nervous
system. Stimulation of the efferent branch of the vagus nerve diminishes cytokine production from macrophages and other immune cells via stimulation of nicotinic receptors. This anti-inflammatory effect of acetylcholine may be blunted in stressed people who have sympathetic overactivity (205). Another putative mechanism is the stimulatory role of angiotensin II on immune cells leading to the production of inflammatory mediators (308, 337). Some evidence reiterates the critical role of adipose tissue in the induction of inflammation; this raises the possible role of adipose tissue as an important source of proinflammatory cytokines in depression (205).

Further to the effects of stress on the cytokine profile, stress-induced hormones also affect other elements of the immune system. Increased levels of glucocorticoids induce neutrophilia associated with lympho- and monocytopenia (90, 89). There are also altered ratios of immune cell subpopulations (67), decreases in natural killer cell performance and impaired T- and B-cell responses (90).

**Indicators of oxidative imbalance**

As mental stress boosts the proinflammatory state, one can expect a skew in the oxidative-antioxidant balance that favors the generation of free radicals. For instance, increased levels of homocysteine and CRP (two markers of inflammation) during psychological stresses escalate the production of reactive oxygen species (ROS) (338, 132). Psychological stress also activates NF-κB, which in turn induces a pro-oxidative state (143). Mental stress activated NF-κB within 10 minutes in a group of healthy volunteers; this stimulation was proportional to the levels of stress hormones (cortisol, catecholamines, etc.) released and returned to basal levels within 60 minutes after removal of stress (33). A study of medical students showed increased oxidative stress, in terms of single strand breaks of DNA in lymphocytes, augmented sensitivity to lipid oxidation and elevated plasma antioxidant status on exam day compared with non-exam days (307). Rats with stress induced by conditioned taste aversion had levels of 8-hydroxydeoxyguanosine (8-OH-dG) levels, a sensitive indicator of DNA damage (149). Similarly, levels of 8-OH-dG positively correlate with depression score in humans (150). The presence of 8-OH-dG is not only linked to DNA repair mechanisms and increased risk of cancer but also with endothelial dysfunction and atherosclerosis pathophysiology (371). Increased immune-reactivity of 8-OH-dG occurs in all cell types of human atherosclerotic plaques (216). In contrast, improved antioxidant mechanisms are related to a reduction of stress symptoms. For instance, whisker removal in mice induces increased spontaneous locomotor activity that is associated with granulocytosis, increased plasma peroxynitrite levels, and protein carbonyl formation/lipid peroxidation in brain, heart, liver, and spleen (357). Under these conditions, the applied stress is accompanied by a minimum amount of physical abuse. Administration of two herbal formulas containing substantial amounts of antioxidants found in two traditional Chinese medicines (Shengmai San is composed of three herbal components and used to treat cardiac diseases, while Ling Gui Zhu Gan Tag has four herbal components and used to treat edema) not only prevented biochemical changes but also stopped stress-induced behavioral consequences. In another animal study of depressive-like behavior in rats, the increased immobility time in forced swimming tests after acute restrain stress was alleviated by vitamin C administration. This effect was comparable to the effect of fluoxetine. Vitamin C also reduced some of the biochemical disturbances such as lipid peroxidation, while increasing the activity of superoxide dismutase, glutathione reductase,
and glutathione peroxidase (245). Anti-depressive and anti-oxidative effects of ascorbic acid have also been reported after a chronic, unpredictable stress protocol (232). Antidepressant properties have been described for other antioxidants as well (247, 285, 201, 278, 211). A randomized double blind placebo controlled pilot study in humans showed that the addition of vitamin C to antidepressant therapy significantly improved clinical outcomes (10). Addition of N-acetylcysteine to a tranylcypromine regimen also increased the response rate of patients with major depression who were resistant to conventional depression therapy (53).

Disease modeling

In considering an animal model of a human disease, strict criteria should be defined to establish the validity and utility of that particular model. Relative resemblance in signs and symptoms (face validity), pathophysiologic mechanisms (construct validity), and therapeutic responses (predictive validity) are three basic criteria that strengthen the applications and importance of a model (367). Of course, construct validity relies on our existing knowledge of the pathophysiology of the disease of interest; however, gaps in our understanding of underlying mechanisms hinder production of an ideal model with adequate construct validity. Additionally, an animal model should be easy to use (utility) and show consistent phenotypic changes, in terms of molecular and biochemical variations (reliability). Reproducibility of data by other scientists is of paramount importance in favoring acceptance of a disease model (263).

Studies in laboratory animals allow for mechanistic insights into the association between stress and cardiovascular. Ideally, animal models should imitate the natural course of diseases and induce similar stress responses as seen in humans; unfortunately each model of stress suffers from its own limitations. Many animal models feature physical stress and neurohumoral variations, while other models are based on psychological stress and secondary interactive changes. Animal models of stress can conveniently be divided to “physical stress models” and “psychological stress models.” It should also be mentioned that some symptoms of psychiatric disease are extremely unlikely to be modeled in animals; by way of example, some features of human depressive mood disorders, such as feeling of guilt or suicidal thoughts, are not possible to recreate in animals. Other examples would be the inability to cope with financial distress or loss of a loved one. Hence, behavioral responses such as loss of motivation or anhedonia are considered as signs of depression in animal models but have questionable validity to extrapolate to human experiences (291).

Animal models of physical stress

A) Thermal stress

Several animal modes of physical stress depend on acute reduction of body temperature, which result in the stimulation of the hypothalamic thermoregulatory center and subsequent activation of the HPA axis. As a result, blood levels of ACTH blood levels increase and mimic stressful conditions.
Cold water
The cold-water test has been used in different forms. In one method, rats are placed in containers filled with cold water (15-20°C) and allowed to swim or stand on their feet and keep their head above the water level for 15 minutes (274, 309). Some investigators used 4°C water and shortened the swimming time to 5 minutes (309, 193). Animals that are unable to swim or those that sink are excluded from the experiment. The cold-water immersion test is used as a model of both acute and chronic stress. In the case of acute stress, animals are sacrificed 30 minutes after swimming time; while, in modeling of chronic stress, animals are exposed to the stressor for longer periods (10-20 days) and sacrificed one hour after the last test session. Like other laboratory models, this method has its own positive and negative aspects; for example, in cases of acute stress, the effects can be measured within a short period of time, while fast adaptation can complicate the outcomes of chronic stress modeling.

Cold environment
This method requires that animals be placed in refrigerators (4°C) for 15 minutes once to induce acute stress; repeating this procedure for 7-10 days produces a model of chronic aversive conditioning (270). A variation that has been reported places animals in the cold (4°C) for 30 min (346); in another version the temperature was reduced to -8°C and the test period increased to 4 hours (92). Sudden changes in ambient temperature elicit stress responses and corresponding hormonal changes. In an attempt for innovation of a more ethically acceptable laboratory method, which may also better resemble human usual life experiences, some investigators have applied a local cold-induced stress to the sole of the feet (164). Local cold stress is applied by using cages designed with a 0°C ice pad on the floor. This maintains constant contact between animal’s paws and the cold source. Increased activity of sympathetic nervous system, endothelial dysfunction, hypertension, proteinuria, hypercoagulation, and reduced hepatic/renal blood flow has all been reported using this method (164, 171).

A model of cold stress called SART (specific alteration of rhythm in temperature) stress has been described as a model of autonomic dysautonomia (135). In this method animals are kept in an environment where the temperature fluctuates from 24 to 4°C every hour for a total of 7 hours and is then maintained at 4°C for the next 17 hours. This procedure is continued for 7 consecutive days (134). Many cardiovascular changes, including altered blood flow (137), electrocardiographic changes (267), and hematologic variations (134), have been reported with this method.

B) Immobilization stress
Immobilization is extensively used as a physical method of stress induction (364-366). This method can be applied in one of two ways. In the first method, movements are restricted by placing animals in an appropriately sized animal holder (121). The second technique requires animals to be immobilized by stretching and fixing their limbs to an underlying board using adhesive tape; a metal loop coil restricts head movements (77). Different time periods have been used to model acute or chronic stresses (187, 75, 68). Immobilization mimics a stressful and inescapable life situation in which adaptation is less likely to modify the physiological responses (31). Immobilization tests have been used in the study of neurodegenerative and post-traumatic stress disorders (312); it also has immunological consequences.
as it decreases the number of natural killer cells and reduces concanavalin A-induced lymphocyte proliferation (323). From a cardiovascular point of view, restraining (120 min/day, 14 days) increased systolic blood pressure and negatively affected endothelium dependent induced vasorelaxation; increased activity of the ACE and augmented angiotensin II levels coupled with high levels of malondialdehyde and oxidized products of NO occur, along with increased expression of gp91 (phox) and Rho-associated kinase-1 in arterial walls (63).

**Immobilization in a cold environment**

This method is a combination of thermal and immobilization stresses and is thought to intensify stress responses. After restraining the animals in a supine position, animals are placed in a cold environment (5±1 °C) for 3 hours (270, 209) or immersing in cold water (22 °C) for 1 hour (175). This stressor provokes sympathetic activation and activates the HPA axis. As a result, stress ulcers develop in less than 3 hours.

C) **Electrical shock stress**

Another way to induce psychological stress in animals is to subject them to weak electrical currents. Rodents are particularly sensitive to the neurological effects of electrical shock. This method places animals in specialized cages that have electrified floors to ensure conduction of the electrical current to the animal's feet. Protocols that differ in terms of intensity, frequency, and duration of electrical shock have been used to induce various degrees of stress (274, 330, 82, 359). Animal behaviors such as predictability or avoidability can influence the magnitude of stress response (360, 221, 37, 237). In case of acute stress, animals are sacrificed 15 minutes after electrical shock, while chronic stress experiments continue for a period of 10-14 days and the animals are studied 1 hour after the last treatment session.

In an early experiment conducted by Friedman and Iwai (97), an approach-avoidance conflict (animals had to press a lever for food that deliver an electrical shock to them) induced hypertension in genetically susceptible animals. This hypertension remained throughout the 26 weeks of the experiment. Further to hypertension, unpredictable electrical shock also results in reduced body weight gain, diminished locomotor activity, and lessened social interaction time, states which are compatible with signs of depression in human (37).

**D) Behavioral despair stress**

In this test, animals are forced to swim in a standard container filled with water. This method provides a life threatening and inescapable situation which acts as a psychological stress that provokes stress responses. There are several modifications, in terms of water temperature and swimming time, for this test (309, 93, 300, 322). This method has been extensively used for the screening of antidepressant drugs and stress induced nociception (70, 262, 54, 264, 163, 173). Antidepressants decrease passive floating time after repeated episodes of forced swimming, which has been interpreted as a sign of hopelessness. However, some have questioned the relevance of this behavior (floating), as it may be an acquired lifesaving behavior for preserving energy (292, 362). Atherosclerosis-promoting effects of forced swimming haves been shown in a stress protocol consisting of restrain and forced swimming in rats fed a normal diet (73). This chronic stress model enhanced different aspects of atherosclerosis including increases in cholesterol and triglycerides blood levels, decreased HDL, increased oxidative stress, reduction of vascular elastic fibers, and boosted foam cell formation. Hypercholesterolemic and
oxidative effects of stress remained 20 weeks after cessation of the stress protocol. The deleterious effects of forced swimming on cardiac function have also been shown in this experimental setting (290).

E) Psychosocial stress

Social defeat stress

Exposing the test animal to a dominant and aggressive counterpart for known periods of time is thought to mimic psychological stress in humans. The social defeat test is mostly used in mice and rats. To promote aggressive behavior, the dominant animal may be housed with a female prior to social defeat test to establish a sense of territorial authority and possession. As with other behavioral tests, there are some modifications in terms of frequency and length of exposure and measured responses (336, 11, 29, 111). Defeat stress reduces social interaction and enhances anxiety-originated behavior in the submissive animal. Similar to stress-induced psychopathology in human, the animal’s behavioral disturbances respond to chronic (not acute) treatment with antidepressant drugs. This resemblance in the chronology of response is considered strength of this test, which not only models depression-related psychological disorders but may also represent social phobia and post-traumatic stress disorders (263).

In a mouse model of chronic social defeat stress, a combination of stress and a high fat diet resulted in dysregulation of lipid profile including increased plasma levels of non-HDL cholesterol and intrahepatic accumulation of triglycerides (60). These events were associated with changes in the expression of hepatic genes involved in lipid metabolism. In rats, the social defeat test is associated with sympathetic over-activity and tachyarrhythmia. Interestingly, the intensity of the cardiac autonomic response is related to the degree of the emotional perturbation (302). The social defeat test can also induce a pro-arrhythmogenic state in the myocardium, possibly through diminishing myocardial refractoriness and impaired conduction (50).

Communication box-induced stress

Ogawa and Kuwahara mostly used rodents in this test that was originally described in 1966 (247). In one example, psychological stress develops in test animals after they witness paw electrical shock treatment in another group of animals and hearing their vocalized stress responses. The two groups of animals (shocked and non-shocked) are placed in two compartments of a cage separated by a transparent plastic dividing wall. As with other behavioral tests, there are modifications in various aspects of this method in terms of cage size, duration of stress, and time of stress application. In the communication box method all the somatic responses are secondary to pure psychological stress as there is no physical harm to the observer animals (247, 151, 146, 281). Significant increases in body temperature, elevations of plasma corticosterone (81, 329), and prolongation of ethanol induced sleeping time (328) have been reported following administration of this protocol. There are several reports about reductions of plasma zinc levels after the communication box-induced stress test (329, 332). This is relevant to the strong association between human depressive disorders and the low plasma zinc levels (329, 246). Even though the direct role of reduced zinc level in stress-induced cardiovascular events remains to be determined, the importance of zinc in maintenance and normal function of cardiovascular system is important to also note (51).
**Neonatal-maternal separation stress test**

This test evaluates the effects of early life psychological stresses on subsequent life events by separating pups from their mothers for a fixed period of time each day. Separation periods usually start on the second day of life and each pup is kept individually in a cage with controlled heat and humidity. A white noise is applied to prevent pups from hearing each other (177, 180). This stress has both structural and behavioral consequences. The stress of isolation activates the HPA axis and causes morphological and functional changes in the hypothalamus and other regions of the brain (168). The relationships between neonatal isolation stress with adulthood addiction (181), response to psycho-stimulants (182, 183), nociceptive stimuli (66), learning, memory (35), locomotor (222), and exploratory activities (24) have all been investigated using methods based on this model. Blood pressure in adult males, subjected to separation from their mothers, is 20% higher than controls, an effect that has been related to increased hypothalamus-pituitary stimulation resulting in higher sympathetic activity. The exact mechanism for this sex specific effect remained to be explained. Meanwhile, sex specific differences in blood pressure have also been reported for spontaneously hypertensive rats, as male rates have higher blood pressure elevations than age-matched females using this test (104).

**Predatory stress test**

A predatory stress test requires exposure of test animals to their natural predators or their odors. For instance, mice are confronted to cats or exposed to cat odor. This situation results in acute sympathetic stimulation and elicits all stress-related hormonal and neurological reactions (5, 214). This model is extensively used to study anxiety as well as for screening of anxiolytic substances (21). This test also has several characteristics such as uncontrollability, unpredictability, and adequate intensity, making it useful in the study of post-traumatic, stress disorders (79). Even though the predatory test is considered a model of acute stress response (156), habituation to predator exposure can occur and this may be related to the long-lasting anxiogenic behaviors that have been reported (79). Diazepam, buspirone, substance P, and the selective serotonin 5-HT$_{A1}$ receptor antagonist (WAY 100635) all reduced fear-induced reactions and increased exploration behaviors (21, 74).

**Changing light-dark cycle**

The circadian rhythm has a substantial impact on proper physiologic function of all living creatures including humans. Disturbances in the sleep-awake cycle lead to profound hormonal, metabolic, immunologic, and psychological disorders (140). The suprachiasmatic nucleus is the main site for processing information regarding the day and night cycle in humans. Visual information is transferred to the pineal gland via the suprachiasmatic nucleus, which secretes melatonin in the dark or under dim light conditions. On the other hand, bright light increases serotonin concentrations in the brain. The balance in the melatonin-serotonin levels adjusts the circadian cycle (140). Stress induced by disturbances in the circadian rhythm can be achieved by placing nocturnal animals under bright light during the night and vice versa. This method creates acute stress as animals adapt to the new light patterns. The stress provoked-responses using this method are accompanied by somatic and neurologic symptoms (243, 213, 271).
Noise induced stress

Many people living in urban areas are exposed to uncomfortable levels of noise. The adverse cardiovascular effects of noise pollution are probably the most widely non-auditory events studied. Chronic noise pollution causes frequent stimulation of the sympathetic nervous system that leads to increased heart rate, vasoconstriction, and a reduced parasympathetic tone. These incidences can turn temporary episodes of blood pressure surges into more lasting hypertension. In a study of two population groups in Stockholm, the prevalence of hypertension was higher in residents of a village near the airport, who were routinely exposed to frequent aircraft noise, compared to the control group. This effect was more prominent in older individuals (280). In animal models, daily (15 min) exposure of rats to white noise (90 dB) for a period of 3-6 months led to increased heart rate and blood pressure, reduced stimulation of parasympathetic nervous system, and intestinal inflammatory reactions (17, 46).

Noise pollution also causes structural vascular changes, as manifested by augmented permeability of mesenteric microcirculation to albumin and increased mast cell degranulation. Dietary manipulation (15) (including extra-amount of vitamin E) and application of Reiki (transmission of positive energy) (16) negated these changes. Altura et al. showed an association between noise-induced magnesium reduction in serum/vascular tissue and hypertension (9). Increased coagulability might also participate in adverse cardiovascular side effects of noise pollution (335). Noise pollution also affects cardiac cell respiratory organelles (251) and causes oxidative stress (91) in the central nervous system. Altered neurotransmitter level in different regions of the brain is another consequence of acute noise stress (30 min of 100 dB) (299, 298).

F) Chronic unpredictable mild stress (CUMS)

In this method, sets of mild socio-environmental stressors are applied in a random manner for 7-9 weeks to minimize habituation (153). Even though there are some discrepancies between protocols, commonly used stressors include using a combination of different cage beddings, cage tilting, light-dark cycle reversion, solitary confinement, water or food deprivation, restraint, forced swimming, and light flashing (153, 206). The behavioral consequences of this protocol including, decreased consumption of sweet food (or drinks), reduced motivation toward award gaining, diminished reactivity to acute stresses, and reduced motivational and self-care behavior (grooming) all have resemblance to anhedonia in human mood disorders (153, 23). Chronic, but not acute, administration of anti-depressive compounds can reverse these symptoms (262). Furthermore, hyperactivity of the hypothalamic-pituitary-adrenal axis, hypertrophy of the adrenals and reduction of body weight are compatible with major depression syndromes in humans (206). This protocol also elicits neuro-humoral and immunological changes compatible with human depressive disorders, such as raised proinflammatory cytokines (TNF-α, IL-β), increased corticosterone levels, and higher activity of the RAAS (117). Rats subjected to CUMS also experience negative cardiovascular consequences such as an increased endothelium-dependent sensitivity to the vasoconstrictor effects of phenylephrine in association with hypertrophy of the intima and media of the aorta, decreased expression of eNOS (23), and increased blood lipid levels (242). Four weeks of CUMS also elevated the resting heart rate and reduced rate variability in experimental animals. Introduction of a new stress (e.g. air jet) to previously stressed animals exaggerated the pressor and heart rate responses (115, 119). Akin to with human studies (116), removal of the stressful stimuli restored normal behavioral responses while the cardiovascular changes.
persisted. In an attempt to clarify the role of stress-induced leukocytosis in atherosclerosis, Heidt et al. (138) used a modified model of CUMS (295); they showed that stress increased proliferation of hematopoietic stem cells which in turn increase the number of pro-inflammatory leukocytes. In atherosclerosis prone Apo E-/- mice these event caused looser fibrous caps and larger necrotic plaque cores of atherosclerotic plaques, which are characteristic of rupture-prone lesions in patients with myocardial infarction or stroke (138).

**g) Conditioned fear:** Conditioned fear refers to elicitation of fear responses to a neutral signal after experiencing a traumatic event when previously exposed to that signal. This conditioning has been well described in both animals and humans, with the controversial experiment of Watson (1920), also known as the “Little Albert” Experiments considered an unethical highlight (265). In this cruel experiment, a nine-month infant was exposed to a loud noise whenever he wanted to touch a white rat, which he was not afraid of approaching at first. After several exposures to the loud sounds, the boy not only became afraid of touching the rat but also showed similar fear reactions to other white fluffy objects such as a white rabbit or white puppies. Animals are initially neutral to a harmless stimulus but this harmless stimulus becomes a fearful and provocative signal when it is associated with adverse outcomes in the initial training period. Another type of conditioned fear is contextual conditioned fear in which fear is associated with a specific situation or environment (52). For instance, re-exposure of a rat to an electrical chamber, where it previously received an electrical shock, evokes fear reactions even without applying any shock. Interestingly, behavioral (freezing, grooming) and physiological (increased heart rate and blood pressure) responses are more prolonged in contextual conditioned fear compared to conditioned fear to a specific stimulus, and is comparable with the fight or flight reflex (52). Likewise, Vianna et al. (345) reported that decreased blood circulation in the paws and tail secondary to fight or flight reflex activation lasts longer in contextual conditioned fear animals. Study of animal models of conditioned fear could potentially provide important data on the pathophysiology and therapeutic options for human disorders including post-traumatic stress disorders (PTSD), phobias, conversion, and dissociative reactions. Table 1 shows some of the selected laboratory stress models in human and animals.

**Conclusion:**
Although it is generally accepted that psychological stress can lead to adverse cardiovascular consequences, the mechanisms that may underpin this are not well understood. Recent data from studies in humans and animal models of stress have stressed the critical role for endothelial dysfunction in stress-induced cardiovascular disorders. Different regions of the nervous and humoral systems are activated with variations in the type and intensity of stressors. The personal characteristics and attitudes of stressed also influence pathophysiologic responses to stress. This complexity presents with many challenges in creating suitable animal models of stress, making the interpretation of animal experiments presumptive. A confounding factor is that animals also have a unique ability of adaptation to chronic stresses, so diminishing the magnitude of adverse reactions over time. This has led to the development of animal models of chronic, unpredictable stress models that apply a battery of unexpected stressful stimuli. Identifying specific disease markers is another dilemma that has not been
satisfactorily resolved as these lack pathognomonic features. Ongoing research in animal models of stress and clinical findings in stressed individuals will improve our understanding of this pervasive disease, and present with opportunities to identify biomarkers that will assist in improved diagnosis and monitor therapeutic responses management strategies. On the other hand, recent data suggesting a beneficial heritable aspect of stress make this puzzle even more complicated (101).
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reversible cardiomyopathy provoked by stress in women from the United States. Circulation

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Table 1: Selected human and animal stress studies with their outcomes.

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<th>Stress Marker</th>
<th>Measurements</th>
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<td>Acute stress lowered threshold for arrhythmias and sudden death</td>
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<tr>
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<td>Mice</td>
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<tr>
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<td>Rats</td>
<td>UCMS for 8 weeks ± infliximab (anti-TNFα)</td>
<td>Vascular function</td>
<td>UCMS reduced endothelium-dependent dilation by 50% and eNOS expression. UCMS + infliximab showed no change vs. control</td>
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<tr>
<td>Mice</td>
<td>UCMS for 8 weeks</td>
<td>Vascular function</td>
<td>UCMS reduced endothelium-dependent dilation to acetylcholine in isolated aortic rings. Adrenergic constriction unchanged.</td>
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<tr>
<td>Mice</td>
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<tr>
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Figure 1: Some of the mechanisms involved in stress-induced cardiovascular adverse effects.
Inflammation
- Pro-Inflammatory Cytokines
- Adhesion Molecules

Pro-thrombosis
- Platelet Hyperactivity
- Clotting Factor VII
- β-thromboglobulin

RAAS Activation
- Angiotensin II
- Vasoconstriction

Oxidative Imbalance
- Antioxidant Defense
- ROS Production
- Lipid Oxidation

Sympathetic Overdrive
- Heart Rate Variability
- Blood Pressure
- Heart Rate

Endothelial Dysfunction
- Flow Mediated Dilation
- NO Bioavailability
- eNOS Activity

CHRONIC STRESS
- HPS Axis Activation
  - Cortisol
- Autonomic Nervous Activation
  - Catecholamines

Cardiovascular Disease (CVD)
- Atherosclerosis
- Microvasculopathy
- Perfusion Deficits
- Impaired Tissue/Organ Function