BIOMARKERS OF CHRONIC STRESS

by

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Stress is defined as the internal process that occurs when a person is faced with a demand that is perceived to exceed the resources available to effectively deal with it. It can be either acute or chronic. The current approaches to measure stress include self reports, measures of affect, measures of stressor exposure and use of biomarkers. This paper seeks to act as a review of the various neuroendocrine biomarkers for chronic stress. A brief overview of metabolic and immunological biomarkers is also included. Serum cholesterol, serum albumin, waist-hip ratio and glycosylated hemoglobin are some of the common metabolic biomarkers. IL-6, TNF-α, CRP and IGF-1 are some of the common immunological biomarkers. Neuroendocrine factors are effective as biomarkers because they are the first to respond to a given stressor and coordinate the response of many other biological systems. Cortisol, DHEA, adrenaline, noradrenaline, dopamine and aldosterone are some of the commonly used neuroendocrine biomarkers. Use of any single biomarker for stress is associated with problems of multiple determination. Allostatic load model utilizes a suite of indicators to measure the effects of stress across many physiological systems. Use of novel technologies like metabolomics, determining changes in ultrastructure of mitochondria and quantifying the induction of DRR1 in the brain are some interesting research areas that could throw up novel biomarkers. Chronic stress is associated with many diseases. It can possibly potentiate the health effects of various exposures and thus, it is an important public health concern. In order to conduct research on any given condition, it is important to characterize that condition. Biomarkers can help characterize stress objectively. Stress, unlike any other pathological condition, triggers a non-specific response and influences multiple physiological systems. Ideally, a study would use a set of biomarkers to measure stress response, while using questionnaires to measure stressor exposure and stress appraisal. The challenge lies in coming up with a set of biomarkers that can capture the chronic stress related information and weed out other confounding factors.

TABLE OF CONTENTS

1.0 INTRODUCTION **1**

1.1 FACTORS THAT MAKE A STRESSOR STRESSFUL 8

1.2 SCOPE OF THE PAPER 9

2.0 THE STRESS RESPONSE **10**

3.0 METHOD USED FOR IDENTIFYING BIOMARKERS DISCUSSED IN THIS PAPER **13**

4.0 METABOLIC BIOMARKERS **14**

4.1 SERUM CHOLESTEROL 14

4.2 SERUM ALBUMIN 15

4.3 WAIST-HIP RATIO 16

4.4 GLYCOSYLATED HEMOGLOBIN 17

5.0 IMMUNOLOGICAL BIOMARKERS **19**

5.1 IL-6 19

5.2 TNF-α 20

5.3 CRP 21

5.4 IGF-1 22

6.0 THE NEUROENDOCRINE SYSTEM **23**

6.1 THE HPA AXIS 23

7.0 NEUROENDOCRINE BIOMARKERS **25**

7.1 CORTISOL 25

7.2 DEHYDROEPIANDROSTERONE 27

7.3 ADRENALINE 28

7.4 NORADRENALINE 29

7.5 DOPAMINE 30

7.6 ALDOSTERONE 31

8.0 ALLOSTATIC LOAD MODEL 33

9.0 PROMISING NEW VISTAS FOR CHRONIC STRESS CHARACTERIZATION 38

9.1 METABOLOMICS 38

9.2 ULTRASTRUCTURE MODIFICATIONS IN MITOCHONDRIA 41

9.3 DRR1 INDUCTION IN BRAIN 43

10.0 CONCLUSIONS 45

BIBLIOGRAPHY 47

LIST OF FIGURES

FIGURE 1. PREGNENOLONE STEAL..... 11
FIGURE 2. TYPES OF ALLOSTATIC LOAD 34

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1.0 INTRODUCTION

Stress is an integral part of our lives. Every organism that lives, experiences some form of stress or the other during its lifetime. In recent times, the term “stress” has been frequently used in popular parlance. While stress can be psychosocial, physical or chemical, the focus of this paper is on psychosocial stress. According to Lazarus, stress is defined as an internal process that occurs when a person is faced with a demand that is perceived to exceed the resources available to effectively respond to it, and where failure to effectively deal with the demand has important undesirable consequences [1]. When under stress, the body responds in a way similar to how it responds to danger. Fatigue, being generally ill and feeling jittery are all sensations of stress [2].

Stress causes certain changes in the structure and chemical composition of the body, which can be accurately appraised. Some of these changes are merely signs of damage, while others are manifestations of the body`s defensive adaptive reactions [1]. These changes caused by stress, collectively called the General Adaptation Syndrome, develop in three stages – the alarm reaction, stage of resistance, and stage of exhaustion [3].

The alarm reaction is the initial stage of the general adaptation syndrome, in which the body responds by exhibiting shock. Selye has called it “the bodily expression of the generalized call to arms of the defensive forces of the organism" [1]. It is triggered by the perception of a stressor, which leads to the arousal of physiological systems [4]. This physiological arousal is the basis for the fight-or-flight response [5]. The alarm reaction is characterized by adaptive physiological changes like increased heart rate,

increased hormonal activity, etc. The stage of resistance is the second stage. The body tries to defend itself by fending off the stressor and attaining homeostasis. It is characterized by increased heart rate and muscle tone, production of stress hormones, etc. Levels of endocrine and sympathetic activity are lower than during the alarm reaction, but higher than normal [4]. Energy sources are spent, the organism becomes vulnerable to diseases and usually, reactions are overdone or excessive. If the stress persists, and additional stress in any form is encountered, the third stage – the stage of exhaustion occurs, which results in the breakdown of the body`s defense mechanism [5]. Muscles become fatigued and the body is depleted of resources required to combat stress. Continued exposure to stress during the stage of exhaustion leads to the development of diseases [3]. Over-stress causes a wide range of harmful effects including development of ulcers, heart disorders, predisposition to musculoskeletal injury, etc [5].

According to Lazarus`s cognitive theory of stress, there are three components of stress - Stressor, Stress Appraisal, Stress Response. The stressor is any event, condition or external stimulus that poses a physical or psychological challenge. Stress appraisal is how a person perceives the stressor. Stress responses are the physiological and psychological consequences of stress appraisal [6].

A stressor can be appraised as harm/loss (the harm/loss already suffered by the individual) or threat (expected harm/loss) or challenge (stressors out of which a gain is possible for the individual). The feeling of vulnerability to the stressor (perceiving the stressor as stressful) depends on cognitive appraisal. A person feels vulnerable when he/she feels that they don’t have enough resources for coping with a given stressor.

The perceived significance of the potential harm/loss also shapes vulnerability [1]. Hence, what is stressful for one person may not be stressful for another.

The biopsychosocial model is one of the most comprehensive models of stress. this model incorporates the previous models of stress into its framework. According to the this model proposed by Bernard and Krupat, stress involves three components, an external component, an internal component and an interaction between the external and internal components. The external component of the Biopsychosocial Model of stress involves environmental events that precede the recognition of stress and can elicit a stress response. The internal component of stress involves a set of neurological and physiological reactions to stress. Selye, with his focus on the general adaptation syndrome dealt with the internal component. The third component of the biopsychosocial model of stress is the interaction between the external and internal components, involving the individual's cognitive processes. Lazarus`s cognitive theory of stress addresses this interaction [7].

Dienstbier offers a reformulation of the cognitive theory, which focuses on the emotional consequences of appraising an event as a stressor or as a challenge. He asserts that when an event is appraised as a challenge, it lead to different physiological consequences than when it is appraised as a harm/loss or threat. Dienstbier uses the term stress to refer to transactions that lead only to negative emotions and he uses the term challenge to describe a transaction that could lead both to positive and negative emotions. A series of studies by Marianne Frankenhaeuser and colleagues provide some support for Dienstbier's assertion that a stressor evaluated as a challenge should be viewed more positively than a harm/loss or threat event [8]. According to

Frankenhaeuser, physiological reactions to stressors depend on two factors: effort and distress. She found that there are three categories of physiological responses to stress. Effort with distress leads to increases of both catecholamine and cortisol secretion and result from daily hassles. These stressors are experienced as negative emotions. This category corresponds to Dienstbier's characterization of the negative emotions present in an event appraised as a harm/loss or as a threat. Effort without distress leads to an increase of catecholamine and suppression of cortisol secretion. These stressors are experienced as positive emotions. This category corresponds to Dienstbier's characterization of the positive emotions present in events appraised as challenging. Distress without effort leads to increased cortisol secretion but not necessarily to catecholamine secretion. This is the pattern often found in depressed individuals [9].

Stress can either be acute (short term, generally lasting days to weeks) or chronic (longer term, generally lasting weeks to months). Acute stress is experienced in response to an immediate perceived threat. The autonomic nervous system is activated and there is an increase in cortisol and adrenalin levels, higher heart rate, quickened breathing, etc. The fight-or-flight response kicks in [10]. The term fight-or-flight was coined by Walter Cannon in the 1920s when he first described the acute stress response as a theory where an animal reacts to danger “with a general discharge of the sympathetic nervous system” [5]. Once the danger is averted, the body returns to homeostasis by activating the relaxation response, which is the opposite of the fight-or-flight response, characterized by reduction of heart rate, decreased production of stress hormones, slower breathing, vasodilation, etc [11]. Chronic stress is a state of on-going physiological arousal. This occurs when the body experiences many stressors or a

single stressor continuously, that it does not have the ability or opportunity to activate the relaxation response [12]. It can develop in response to everyday stressors which are ignored or poorly managed, or in response to traumatic events [13]. It has many negative consequences including suppression of the immune system, increased risk of heart attacks and strokes, speeding up of the ageing process, infertility, etc [14]. Additionally, chronic stress can take a severe emotional toll.

The current approaches to measure stress include Self report of stress (for eg., Perceived stress scale – questionnaire with questions designed to provide information about how unpredictable, uncontrollable and overloaded the respondents felt their lives to be. Based on the answers, a score is assigned. A high score on this scale has been correlated with failure to quit smoking, greater susceptibility to colds, etc. [15]) and Measures of affect (for eg., POMS questionnaire – assesses transient, fluctuating affective mood states. Consists of 6 identifiable affective states which are rated by the subjects on a 5 point scale [16]) which are measures of stress appraisal, Measures of stressor exposure (for eg., Major life events stress scale – a list of major events is given and subjects are asked to indicate which events they have experienced. Each event is given a weighted score and an overall score is given to each subject by summing the weighted scores of all events indicated. A high score correlates to high susceptibility to stress related illnesses [17]) which measures the stressor, using Biomarkers (for eg., Cortisol, CRP, IL-6), or using a composite set of parameters (Allostatic load model) which measure either the stressor or the stress response. Self reporting of stress, stressor exposure and measures of affect suffer from recall bias. Biomarkers are objective measurements, but they cannot be used in isolation to measure stress

because, unlike other pathological conditions which induce specific responses from the systems they affect, stress induces a generalized response from all systems of the body and each response can also be triggered by other conditions, i.e., the responses are multiply determined. Hence, when using a biomarker, it is necessary to interpret the results in the context of other existing pathological conditions.

A biomarker is defined as a substance that can be used as an indicator of a biological state. It is a characteristic which can be objectively measured and evaluated as an indicator of that state [18]. Biomarkers are essential in order to effectively quantify stress. They can be used to objectively measure the physiological response to psychosocial stressors. They however, do not provide a direct measurement of the stress appraisal component, which plays a major role in determining if the given stressor is being perceived as harmful, beneficial or irrelevant. Stress appraisal need not always be a conscious process [1]. When a stressor is subconsciously perceived as stressful, in view of the stressor exposure, biomarkers can be used to measure the physiological responses. They also have immense diagnostic and prognostic value. Biomarker discovery is the process by which biomarkers are discovered. Biomarker discovery is based on clear biological insight, from physiology or biochemistry. Detailed knowledge of the mechanism of disease is usually required. A clear mechanistic understanding of stress is still evasive. Biomarkers for mechanistic studies are those based on some previous knowledge of the relationship between the disease and exposure being studied. These mechanistic studies lead to a more comprehensive understanding of the underlying mechanism, leading to the discovery of biomarkers that are closer to the clinical manifestation of the disease state and thus, can be used for

diagnostic purposes. These clinical biomarkers are useful to epidemiological studies. However, it is very difficult to separate out which biomarkers represent the underlying biology and which biomarkers arise as a consequence of stress. Biomarkers currently available to quantify stress, are based on limited mechanistic knowledge. These can be used in studies addressing the mechanisms underlying stress and disease. They have also been used successfully in various epidemiological studies despite the lack of a comprehensive understanding of the biological basis behind the behavior of the biomarker being measured. Biomarkers for stress can be grouped into Neuro-endocrine markers, Immune function markers, Metabolic markers and Morphological markers. Many biomarkers have been identified for both acute and chronic stress that fall into each of the four categories. Due to the problem of multiple determinations, the current challenge is to identify novel biomarkers or a combination of biomarkers and other parameters that can effectively measure stress, independent of other existing conditions in an individual. Also, biomarker measures may undergo variation within a given day or across the individual`s lifespan. Hence, when interpreting functional significance and predicting outcomes, a single measurement at a single time point can be misleading. An ideal biomarker for stress should be able to capture what is happening to the body in a naturalistic social environment. Since stress elicits a multi systemic non-specific response, searching for a single biomarker that can assess and quantify it is not realistic. It would be more meaningful to seek out a panel of biomarkers that can capture changes in various stress response systems to give a holistic picture of the physiological state of the body due to a given exposure to stress.

The allostatic load model, proposed by McEwen and coworkers, is one such effort where a set of indices are measured simultaneously. The indices measured encompass the various physiological systems known to be influenced by chronic stress. It is advantageous to use the allostatic load model in place to standalone biomarkers because, stress affects many physiological systems simultaneously and the allostatic load model can capture changes in various systems and give an overall score which can be related to susceptibility to chronic stress related illness. Also, the problem of multiple determination can be mitigated to an extent because, it is unlikely that any given pathological condition would affect all the indices being measured in the same manner as chronic stress. This model will be been discussed in more detail later.[19]

It has been suggested that males and females respond to stress differently, with a group of researchers hypothesizing that males are more likely to respond with aggression (fight) and females are more likely to respond with fleeing (flight) or turning to others for help and trying to diffuse the situation (tend and befriend) [20]. Though this is an important hypothesis, considering the fact that various hormones which are used as biomarkers for stress, behave differently in the two sexes, this paper will not dwell on the sex differences in stress response or biomarker behavior.

1.1 FACTORS THAT MAKE A STRESSOR STRESSFUL

Evidence suggests that unpredictable and uncontrollable events are more stressful than predictable and controllable events. Experiments have shown that when people believe some action can be taken regarding a given challenge, they perceive it

as less stressful, even if they do not take action [21]. Hence, lack of control is an important variable influencing appraisal of a given stressor. A second variable is suddenness. It is usually easier to cope with an event that one can foresee. Thirdly, ambiguity influences stress appraisal. This is because; it is easier to determine a course of action for a well defined situation, whereas ambiguity entails lot of energy expenditure towards understanding the nature of the stressor and evaluating possible coping mechanisms [22].

1.2 SCOPE OF THE PAPER

This paper seeks to act as a review of various neuro-endocrine indices currently available for measuring chronic stress. These indices can be used as biomarkers to aid mechanistic studies and can lead to a more well rounded understanding of chronic stress and a consequent discovery of biomarkers for epidemiological and diagnostic use. A brief overview of some metabolic and immunological biomarkers is also included and promising new directions of research in chronic stress characterization has also been discussed.

2.0 THE STRESS RESPONSE

A number of physiological systems respond to stress. The neuroendocrine system is the first to respond to stress and causes certain changes in the immune system, cardiovascular system, metabolism, etc [1]. Under a normally functioning system, stimulation of hypothalamus results in the secretion of Corticotropin releasing hormone (CRH). CRH stimulates the pituitary gland to secrete Adrenocorticotropic hormone (ACTH) [23]. ACTH in turn stimulates the adrenal glands to produce various hormones. These include aldosterone which has a direct effect on the kidneys [24], cortisol which has an effect on the immune system and brain [25-26], 5-dehydroepiandrosterone (DHEA) which has an effect on the body`s metabolism [27] and adrenaline which has an effect on the cardiovascular system and respiration [28-29]. Stress also results in the activation of the sympathetic nervous system. Sympathetic nervous system activation causes the adrenal glands to secrete noradrenaline, which has an effect on the cardiovascular system and respiration [28-29].

Chronic stress leads to prolonged activation of this stress response. Pregnenolone steal (or cortisol escape) is the body`s hormonal response to chronic stress. Pregnenolone is the precursor to all steroid hormones. Under chronic stress, the body utilizes most of this precursor for the synthesis of the principal stress hormone, cortisol. this results in imbalances and deficiencies in all other steroid hormones [30]. The diagram illustrates the chronic stress response [30].

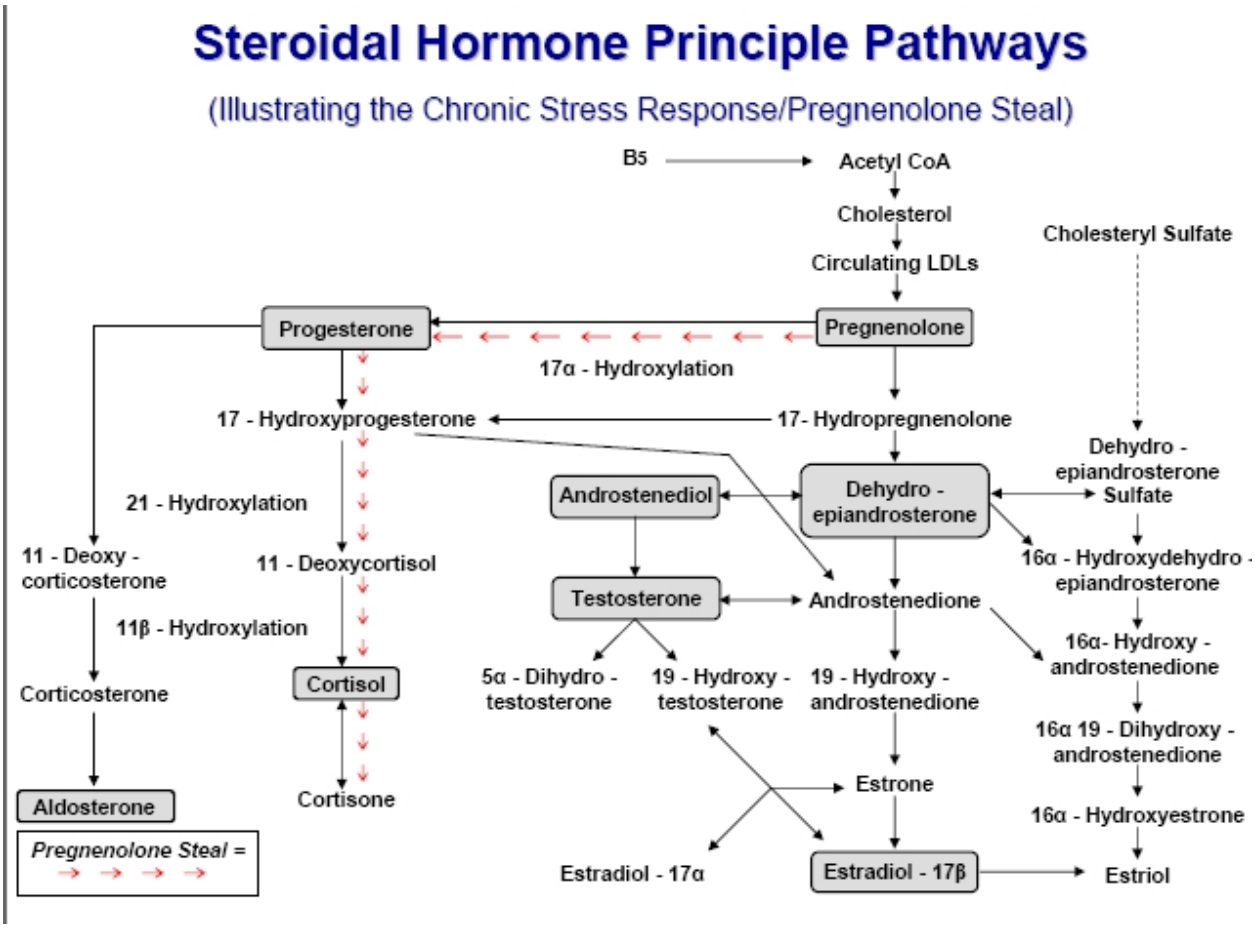

Figure 1. PREGNENOLONE STEAL

Behavioral and morphological changes are also part of the chronic stress response. Negative emotional states are strongly associated with stress. The behavioral changes associated with long term stress exposure are diverse, but may be classed into four major types. The two common kinds of behaviors are based on the biological response of fight or flight. Though fight-or-flight is primarily an acute stress response, under chronic stress, an individual may become withdrawn and depressive, or become aggressive and irritable. The other two types of behaviors are adaptation and habituation [31-32]. At the cellular level, there are changes in the structural integrity of mitochondria, fragmentation of the Golgi complex, aggregation of cytoskeleton

structures, collapse of vimentin containing intermediate filaments around the nucleus, etc. have been observed [33-34]. Cortisol is able to cross the blood brain barrier and bind to receptors in the hippocampus and so, long term production of stress hormones is able to induce memory impairments, shrinking and atrophy of the hippocampus [35].

3.0 METHOD USED FOR IDENTIFYING BIOMARKERS DISCUSSED IN THIS PAPER

The biomarkers discussed in this paper were identified based on literature search. The Pubmed database was searched with the search phrase, “biomarkers of chronic psychosocial stress”. The various biomarkers used in the research articles shown in the results of the search were tabulated. The search was repeated with the search phrases, “metabolic markers of chronic psychosocial stress”, “immunological markers of chronic psychocosial stress”, “neuroendocrine markers of chronic psychosocial stress” and “biomarkers of chronic stress”. Some of the metabolic, immunological and neuroendocrine markers most frequently used in the studies presented as search results have been discussed.

4.0 METABOLIC BIOMARKERS

Changes in metabolism are easily quantifiable and can be used as biomarkers for chronic stress. Cholesterol levels, Albumin, Waist-Hip ratio and Glycosylated hemoglobin are some of the parameters which quantify change in metabolism, currently in use as markers of chronic stress.

4.1 SERUM CHOLESTEROL

Serum cholesterol is the total level of cholesterol in the blood stream. The normal range of serum cholesterol is 120-250 mg/dL. Serum cholesterol levels drop under chronic stress. Various studies have suggested that serum cholesterol levels are decreased under chronic stress. Researchers at Bethesda, MD reported that after severe stress occurring during military training, the serum cholesterol concentration in navy trainees enrolled in the study decreased by 17.2% [36]. In a study conducted on monkeys, it was reported that in the presence of persistently high amounts of adrenaline, serum cholesterol levels drop [37]. Low serum cholesterol levels have also been observed in persons who suffered from accidents, persons showing aggressive behavior and in depression patients [38-40]. This lowering in serum cholesterol can be attributed to elevated endocytosis of lipoproteins by the action of ACTH and the intracellular hydrolysis of cholesterol either by the activation of cholesterol esterases or by the suppression of cholesterol acetyltransferase [41]. However, some studies have provided conflicting evidence. In a chronic stress study conducted on young men by the

United States army research institute of environmental medicine, it was reported that serum cholesterol levels rose progressively throughout the study period. [42]. Another study conducted in elderly persons found no significant difference in the level of serum cholesterol in stressed individuals and controls [43]. Cholesterol captures pathophysiology of liver, metabolism, and risk of cardiovascular disease. It can be used for large epidemiological studies to establish associations between cardiovascular disease and chronic stress because it can be measured easily from blood. It is more suited to toxicology studies because, to understand the significance of chronic stress and the level of cholesterol observed, it is necessary to be able to document cholesterol level before the onset of chronic stress. This information is usually not easy to collect in an epidemiology study. Confounding variables include age, smoking, hypertension, diet, etc.

4.2 SERUM ALBUMIN

Serum albumin is the most abundant protein in mammals. Albumin helps to transport many hydrophobic substances through the blood stream. The normal range is 3-6 g/dL. The synthesis of albumin occurs only in hepatic cells, and its half life is about 21 days. The rate at which it is synthesized is about 15 g per day in healthy individuals, and the rate of degradation is about 4% per day. These rates can vary significantly with stress. Hypoalbuminemia is linked to acute or chronic inflammatory responses. The levels have been reported to return to normal upon resolution of inflammation. Chronic stress, via various inflammatory or neuroendocrine mediators, could reduce albumin

levels by either increasing the rate of degradation, or by reducing the rate of synthesis. Hypoalbuminemia can facilitate edema, which can result in further reduction in serum albumin, as more albumin can now enter the extra vascular space. In mice, TNF-α has been reported to suppresses the transcription of the *alb* gene [44-45]. Studies conducted on adult males have suggested that serum albumin levels can drop due to chronic stress and increase following resolution [46-47]. Serum albumin captures the pathophysiology of liver and metabolism. It can also capture nutritional state, which can either be an outcome of stress or a confounding exposure. Albumin can be easily measured from blood and hence is useful in animal studies and epidemiology studies, especially those studying associations between stress and nutrition. Many health conditions result in reductions of serum albumin, hence the findings must be analysed in view of such confounding. In animal studies where proper controls are used and baseline albumin levels are known, confounding from existing pathologies can be minimised.

4.3 WAIST-HIP RATIO

Waist-hip ratio tends to be higher for chronically stressed individuals. Bjorntorp [48] and Rebuffe-Scrive [49] have hypothesized that greater vulnerability to stress (perceiving a stressor as stressful) increases exposure to stress-induced cortisol, which in turn fuels central fat deposition. A study was conducted using the retrospective cohort design, with women selected on the basis of their fat distribution phenotypes. The level of life stress and response to stress was studied in all these women. It was found that

central fat deposition is related to greater psychological vulnerability to stress and cortisol reactivity [50]. A mechanistic study conducted on both male and female adults concluded that high levels of cortisol causes fat stores and excess circulating fats to be relocated and deposited near the abdomen, resulting in a high waist-hip ratio [51]. It is used as an indicator of obesity which in turn is a risk factor for more serious health conditions, including heart disease. Hence, waist-hip ratio can capture the general health status of an individual. It is a non-invasive measurement and can be very useful in large epidemiological studies.

4.4 GLYCOSYLATED HEMOGLOBIN

Glycosylated hemoglobin is hemoglobin to which glucose is bound. Glucose stays attached to hemoglobin for the life of the red blood cell, hence the level of glycosylated hemoglobin reflects the average blood glucose level over a period of three months [52]. Chronic stress is linked to hyperglycemia [53] This has been explained to be caused either due to the presence of excessive counter-regulatory hormones like glucagon, glucocorticoids, etc. or due to high circulating or tissue levels of cytokines, particularly TNF-α and IL-1, which interfere with the functioning of insulin [54]. Hyperglycemia results in elevated levels of glycosylated hemoglobin in chronically stressed individuals [55]. Diabetes is a confounding pathological condition, type 2 diabetes may even be an outcome of chronic stress. It can capture abnormal metabolism and risk for cardiovascular disease. It can be useful in epidemiological studies, especially those exploring the associations between chronic stress and

cardiovascular diseases and can be measured from blood. It can also be used in toxicology studies exploring the mechanisms underlying the associations between stress and diabetes where the animals being used do not have the disease at the beginning, and gradually develop diabetes during the course of the study.

5.0 IMMUNOLOGICAL BIOMARKERS

Changes in circulating levels of cytokines are easily quantifiable and hence, can be used as biomarkers. IL-6, TNF-α, CRP, and IGF-1 are some of the immune factors currently in use as biomarkers for chronic stress.

5.1 IL-6

IL-6 production by peripheral blood mononuclear cultures from chronically stressed individuals has been reported to be higher than cultures from control subjects, when stimulated by LPS, in a study conducted on older adults [56]. In a longitudinal study comparing parents of cancer patients (chronically stressed) and parents of healthy children (control), it was reported that chronically stressed persons showed higher resistance to anti-inflammatory signals [57]. IL-6 is a pro-inflammatory cytokine which works synergistically with TNF-α and IL-1 to cause inflammation, a common feature of chronically stressed individuals. It has been used as a biomarker of chronic stress in many mechanistic and epidemiological studies because it can be easily measured from blood and it can capture pathophysiology of the immune system which is an important stress response system [58-59].

Studies by Sheng and coworkers have suggested that chronically stressed individuals are biased toward a humoral immunity oriented cytokine production, for unknown reasons [60]. IL-6 is produced in response to stimuli like IL-1 or TNF-α. Stress causes the release of IL-1. A properly functioning HPA axis prevents the peripheral

release of IL-6 following acute stress [61]. But due to the dysregulation of the HPA axis and a resistance to the immunosuppressant effects of glucocorticoids seen in chronic stress [62], there may be a decrease in the ability of the HPA to prevent peripheral inflammation which accounts for individuals suffering from chronic stress showing increased systemic levels of IL-6. Thus, IL-6 is also able to capture indirectly, dysfunction of the HPA axis.

5.2 TNF-α

TNF-α is a pro-inflammatory cytokine, produced mainly by activated macrophages. Together with IL-1 and IL-6, it is involved in the suppression of appetite, increasing insulin resistance, promotion of expression of adhesion molecules on endothelial cells to aid neutrophil migration, etc. It may also be involved in central nervous system pathology because it has been associated with demyelination [63]. TNF-α levels have been reported to be increased in adults aged 19-55, under chronic stress [64]. TNF-α promotes gene expression by activating NFκB which results in the transcription of inflammatory cytokines [65]. Hence, an elevation in TNF-α is concomitant with elevation in the levels of IL-1 and IL-6. The mRNA of TNF-α is higher during chronic stress, which suggests denovo synthesis rather than the release of preformed inducible protein upon activation of lymphocytes and macrophages [66]. The levels of spontaneously produced TNF-α are also reported to be higher in persons suffering from chronic stress, as compared to controls [67]. This increase in TNF-α production can be explained similarly to the increase in IL-6 levels. A dysregulated HPA

axis, unable to prevent peripheral inflammation, combined with a loss of sensitivity to glucocorticoid inhibition results in high levels of TNF-α. It captures the inflammatory outcomes of HPA axis dysregulation mediated by glucocorticoid signaling. It can be useful in epidemiological studies because sample (blood) collection is relatively easy. However, a study conducted on young adults have shown that the production of TNF-α is inhibited by glucocorticoids under chronic stress [68].

5.3 CRP

C-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation. Its physiological role is to bind to the phosphocholine expressed on the surface of apoptotic cells in order to activate the compliment system [69]. Measuring CRP is a marker for inflammatory diseases. Since chronic stress is known to induce the production of IL-1 and a subsequent chronic low-grade inflammation, CRP levels can be used as a screen for this inflammatory response due to chronic stress. In a study conducted on adolescents, under chronic stress, CRP levels have been suggested to rise [70]. CRP can be measured from blood and has been successfully used in epidemiology studies as a marker of the chronic inflammatory response caused by chronic stress [71]. Some sex differences have been reported with women showing higher CRP levels than men, however, the level of CRP has been reported to rise in both the sexes with exposure to chronic stress [72].

5.4 IGF-1

IGF-1 or Insulin-like growth factor-1, is a hormone with a structure similar to insulin, synthesized by the liver and other cell types. It has anabolic effects in adults and is important for growth during childhood. Growth hormone induces the production of IGF-1. It is a potent inhibitor of programmed cell death and a stimulator of cell growth and proliferation. The normal range of IGF-1 in blood is 10-1000 ng/ml. A decrease in growth hormone levels results in a decrease in IGF-1 levels [73]. ACTH and noradrenaline influence cellular immunity by decreasing mitogen responsiveness of lymphocytes. This results in impaired production of IGF-1 by lymphocytes [73]. Glucocorticoids have catabolic effects [74]. They reduce the responsiveness of various cell types to anabolic hormones. High levels of circulating glucocorticoids result in inadequate anabolic hormone synthesis. This causes a catabolic/anabolic imbalance [75]. Hence, in adults under chronic stress, higher than normal levels of cortisol have been suggested to result in low levels of IGF-1 [76-77].

6.0 THE NEUROENDOCRINE SYSTEM

The neuro-endocrine system consists of neurons and endocrine glands and the interactions between them [78]. The main component of the neuro-endocrine system is the hypothalamus-pituitary-adrenal axis or HPA axis. Apart from controlling reactions to stress, the HPA axis regulates digestion, immune function, mood and emotions [79].

6.1 THE HPA AXIS

The hypothalamus contains neuroendocrine neurons that synthesize and secrete vasopressin and corticotrophin releasing hormone (CRH) which regulate the pituitary gland and stimulate the production of adrenocorticotropic hormone (ACTH) which in turn stimulates the adrenal cortices to produce glucocorticoids, mainly cortisol. The glucocorticoids act on the hypothalamus and pituitary, to suppress the production of CRH and ACTH, thus completing the negative feedback circuit [80]. Adrenaline and Noradrenaline are secreted by the adrenal medulla via sympathetic nervous stimulation and local effects of cortisol. These enzymes positively feedback to the pituitary and increase the production of ACTH. Various neurotransmitters, like dopamine and serotonin are involved in regulating the HPA axis [80].

Real or interpreted threats to homeostasis induce the release of adrenaline and noradrenaline, along with glucocorticoids, and the fight-or-flight response is initiated under acute stress. Under chronic stress, the HPA axis is stimulated for prolonged periods of time. The hyperactivity of the HPA axis causes the related biological systems

to overcompensate and eventually collapse [81]. HPA axis dysfunction, caused by chronic stress may present itself as hypercortisolism, hypocortisolism or some form of diurnal dysrhythmia [82]. ACTH which is elevated under chronic stress, induces an elevation in the levels of cortisol and depletion of other glucocorticoids through the already described pregnenolone steal. This phase of hypercortisolism can last for several years, but eventually leads to the attenuation of stress response due to habituation of the HPA axis, resulting in reduced cortisol production and eventually hypocortisolism [83].

The HPA axis thus shifts from a hyper-responsive state to a hypo-responsive state, and eventually digress to an unresponsive state. This stage is called “adrenal fatigue”. This attenuation of the stress response might be a defensive mechanism to prevent long term suppression of immune function. But it has many negative consequences, like impaired cognition, sleep disturbances, anorexia, etc [84].

7.0 NEUROENDOCRINE BIOMARKERS

Neuroendocrine factors are effective as biomarkers for stress because, the neuroendocrine system is the first to respond to a given stressor, and coordinates the response of many other physiologic systems to the stressor, including cardiovascular and immune systems, as well as energy production and/or utilization and behavior, therefore bringing the body back to homeostasis [2]. Neuroendocrine factors are also very easy to measure. Even a mild challenge can trigger a response from this system and small changes from normal levels of neuroendocrine factors can be effectively detected [85-86].

The various neuroendocrine biomarkers of chronic stress currently in use include Cortisol, Dehydroepiandrosterone, Aldrenaline, Noradrenaline, Dopamine, and Aldosterone.

7.1 CORTISOL

Cortisol is a glucocorticoid synthesized by the adrenal cortex [86]. It has important immunosuppressive and anti-inflammatory effects [87]. It is also involved in the conversion of stored fats and proteins into carbohydrates [88]. It causes an increase in heart rate and blood pressure [89]; and suppression of growth, digestive and reproductive activities [90]. It is capable of traversing the blood brain barrier and modulating the functions of the prefrontal cortex and limbic lobe [91].

The amount of cortisol in the blood undergoes diurnal variation. The level peaks in the early morning and reaches its lowest level three to five hours after the onset of sleep [92]. Studies conducted on healthy adults suggested that Chronic stressors that threaten physical integrity, are uncontrollable, or involve trauma tend to result in a high flat diurnal profile of cortisol release, with lower than normal levels in the morning and higher than normal levels in the evening and controllable chronic stressors tend to produce higher than normal morning levels of cortisol [93]. A study conducted on male rats inferred that the period following cessation of chronic stress is associated with HPA axis hypoactivity [94]. This produces a glucocorticoid deficient state and lead to relative resistance to infection, but greater susceptibility to autoimmune inflammatory diseases. This hypoactivation of the HPA axis is also seen in disease states like chronic fatigue syndrome, where the dysregulation is due to habituation of the HPA axis to the constant stimulation by the chronic stressor, which results in the shutdown of the stress response [95].

Cortisol can be measured from urine [96], saliva [97] or serum [98]. Cortisol measurements directly capture the status of HPA axis functioning which is a mediator of many secondary outcomes, ultimately leading to disease. Studies have suggested that during acute stress, there is a spike in cortisol levels following exposure to the acute stressor and the levels return to normal once the stress has been resolved [99-101].

Due to the large diurnal variation in cortisol levels which make it difficult to ascertain the levels from a single measurement, it is not generally effective as a biomarker for chronic stress – except in studies where temporally dense repeated measures of cortisol can be obtained from the same subjects, to examine diurnal trends

and cortisol responsivity within the subjects.. Also, habituation to chronic stress causes a down-regulation of the glucocorticoid response which leads to dissociation between chronic stress and cortisol levels [102]. Apart from stress, other factors like injury [103], genetic factors, sex, smoking habits, social support, individual sleep cycle [104], obesity [105], etc influence cortisol levels.

7.2 DEHYDROEPIANDROSTERONE

Dehydroepiandrosterone (DHEA) is an androgen synthesized by the adrenal glands [106]. It functions as a HPA axis antagonist [107-108]. It also suppresses inflammatory cytokines [109], improves lipid metabolism and lean muscle mass [110], decreases insulin resistance and reduces oxidative brain damage [111-112].

DHEA is produced from cholesterol through the cytochrome P450 enzymes P450scc and CYP17A1 [113]. Dehydroepiandrosterone sulphate is the sulphated version of dehydroepiandrosterone. The conversion is reversibly catalysed by sulfotransferase SULT2A1, primarily in the adrenals, liver and small intestine. In blood most dehydroepiandrosterone is found in the sulphated form [114]. The levels of both forms peak in the early morning hours [115]. In healthy adults, in response to a chronic stressor, the dehydroepiandrosterone levels have been reported to fall during the hyper-responsive stage of the HPA axis. Even though cortisol production is attenuated once the shift to the hypo-responsive stage of the HPA axis occurs, DHEA levels have been shown to continue to drop [116]. It can be measured from serum [117], saliva [118] and urine [119]. DHEA measurements directly capture the status of HPA axis functioning.

In response to acute stress, DHEA levels have been reported to rise [120]. Apart from chronic stress, drugs like insulin [121], opiates, corticosteroids, etc and other factors like age [122], certain illnesses like skin diseases [123], etc lower DHEA levels. Hence, low levels of DHEA do not always correlate with chronic stress. The absolute levels of cortisol or DHEA may not always point to a dysregulation of the HPA axis, but the ratio of Cortisol:DHEA can be used to determine how an individual`s HPA axis is functioning. Under chronic stress, the ratio is reported to be significantly higher than in normal individuals. This is mainly due to decreased DHEA production [124]. High Cortisol:DHEA ratio has been successfully used to diagnose treatment resistant depression [125] and to predict success of smoking cessation efforts [126]. Diurnal variation exists in the levels of DHEA and DHEA-S. The peak levels occur in the morning. Hence, when measuring DHEA as a marker of chronic stress, it is important to obtain temporally dense, repeated measures from every subject. Since this is a time consuming process, it is not feasible to use DHEA as a marker for chronic stress in large cohort studies. It may be more useful in mechanistic studies or small case-control studies.

7.3 ADRENALINE

Adrenaline is a catecholamine synthesized by the adrenal glands. It increases heart rate [127], glucose levels [128], decreases digestion, and reduces the activity of the parasympathetic nervous system as part of the fight-or-flight response [103].

Adrenaline is released as a result of sympathetic nervous stimulation of the adrenal medulla, usually in response to stress [129]. Chronically stressed individuals have been reported to show low adrenaline responsivity due to habituation to constant adrenaline induced signaling [130]. Chronic stress in adult male rats has been shown to elevate the absolute concentration of adrenaline by decreasing its turnover rate [131-132]. Also, there is a sustained increased production of adrenaline by the adrenal glands due to sympathetic nervous stimulation. It can be measured from plasma [133] and urine [134]. By measuring adrenaline levels, the state of sympathetic nervous system function can be captured. Recently, chronically elevated levels of adrenaline have been linked to DNA damage caused by lowering of p53 levels [135].

Acute stress in healthy adults has been reported to cause an elevation of adrenaline levels, but this is due to increase in production [136]. Due to this reason, it is difficult to attribute the rise in adrenaline levels to acute or chronic stressors. Even though adrenaline is the hormone which most consistently responds to stress, it also responds to any experience that causes mental arousal or requires effort, regardless of whether it is perceived as a threat or as an opportunity for gain. Hence, it is not as useful as a biomarker of stress.

7.4 NORADRENALINE

Noradrenaline is a catecholamine synthesized by the adrenal medulla, the peripheral sympathetic nerves and the central nervous system [137]. It increases blood

pressure and heart rate [138-139], causes vasoconstriction [139], and modulates brain functions as part of the fight-or-flight response [140].

Noradrenaline is a neurotransmitter that has widespread effects across multiple brain areas. Studies in rats have suggested that Plasma levels of noradrenaline are elevated [141] and studies in adult humans have suggested that there is a decrease in the release of brain noradrenaline under chronic stress [142]. A study conducted in adult humans and monkeys have reported that there is also an upregulation of post-synaptic receptor, atrophy of noradrenaline axon projections and reduced transmission [143]. It can be useful in epidemiological studies since it can be easily measured from plasma [133], cerebrospinal fluid [144] or urine [134]. By measuring adrenaline levels, the state of sympathetic nervous system function can be captured.

Acute stress has been reported to cause an elevation in both plasma [145] and brain [146] noradrenaline levels. Essential hypertension [147], diet [148], recreational drug use [149], exercise [150], sex [151], etc influence noradrenaline levels, making it an unreliable biomarker when used on its own. When used in conjunction with other markers of chronic stress in an allostatic load model, it can be one of the useful pointers of sympathetic nervous system functioning.

7.5 DOPAMINE

Dopamine is a precursor of noradrenaline, synthesized in the brain [137]. It increases the overall renal perfusion [152] and has a diuretic effect [153]. It increases heart rate and blood pressure [128] and also functions as a neurotransmitter which is

involved in many activities like voluntary movement [154], motivation [155-156] and cognition [157-158].

Chronic stress is linked to impaired working memory due to reduced transmission of dopamine, combined with an increase in dopamine D1 receptor density in the prefrontal cortex region of the brain of rats [159]. In rats, extraneuronal dopamine is reported to be reduced during chronic stress [160] due to reduced activity of dopaminergic neurons and not due to reduced synthesis of dopamine or enhanced reuptake [161]. Dopamine can be measured from urine [162], plasma [163] and cerebrospinal fluid [144]. The D1 receptor binding can be measured using PET [164].

Acute stress is reported to cause an increase in dopamine release in rats [160, 165]. Recreational drug use and later abstinence [166-167], exposure to chemicals like PCBs [168], exercise [169], obesity [170], feeding habits [171], etc influence dopamine levels. Hence, the use of dopamine alone as a biomarker of chronic stress is not reliable. When used in conjunction with other markers as part of the allostatic load model, it can be one of the useful biomarkers for quantifying stress response. It is a mediator of behavioral and to a lesser extent, cardiovascular outcomes of stress.

7.6 ALDOSTERONE

Aldosterone is a mineralocorticoid synthesized by the adrenal glands [172]. It acts on the CNS via the posterior pituitary gland and causes the release of anti-diuretic hormone. It also influences the cardiovascular system by causing localized vasodilation

at the site of action [173]. It is involved in reabsorbing sodium, water retention and potassium excretion in order to maintain blood acidity [174].

Studies in rats have suggested that aldosterone secretion is reduced due to chronic stress [175]. This can result in hyperkalemia and hyponatremia [176]. Aldosterone can be measured from urine [177], plasma [178] and serum [179].

Acute stress is reported to cause an elevation in aldosterone secretion [180]. The standalone use of aldosterone as a biomarker for chronic stress is unreliable because smoking [181], diet [182], etc. influence aldosterone levels. The effect of these confounding factors can be minimized when it is used in conjunction with other markers as part of the allostatic load model. As part of a consortium of markers measuring allostatic load, aldosterone can be a useful measure of functioning of the adrenal gland.

8.0 ALLOSTATIC LOAD MODEL

The use of any single biomarker for measuring stress is associated with problems. The current approach is to use multiple markers to measure the effects of stress on various bodily systems. Allostasis is defined as the process by which an organism maintains physiological stability by changing parameters of its internal milieu by matching them appropriately to environmental demands [183]. According to McEwen, “Allostatic load refers to the price the body pays for being forced to adapt to adverse psychological or physical situations, and it represents either the presence of too much stress, or the inefficient operation of the stress hormone response system” [184]. Normally, once allostasis is initiated by a stressor, it is sustained for an appropriate period of time and then turned off. Frequent initiation of allostasis by multiple stressors may lead to either the body not turning off the response or not responding sufficiently. Lack of adaptation to a recurring stressor, failure to turn off adaptive responses upon initiation by a stressor, failure to sufficiently respond to a given stressor are other types of abnormal allostatic responses. These can be better explained by the following graph by McEwen [185].

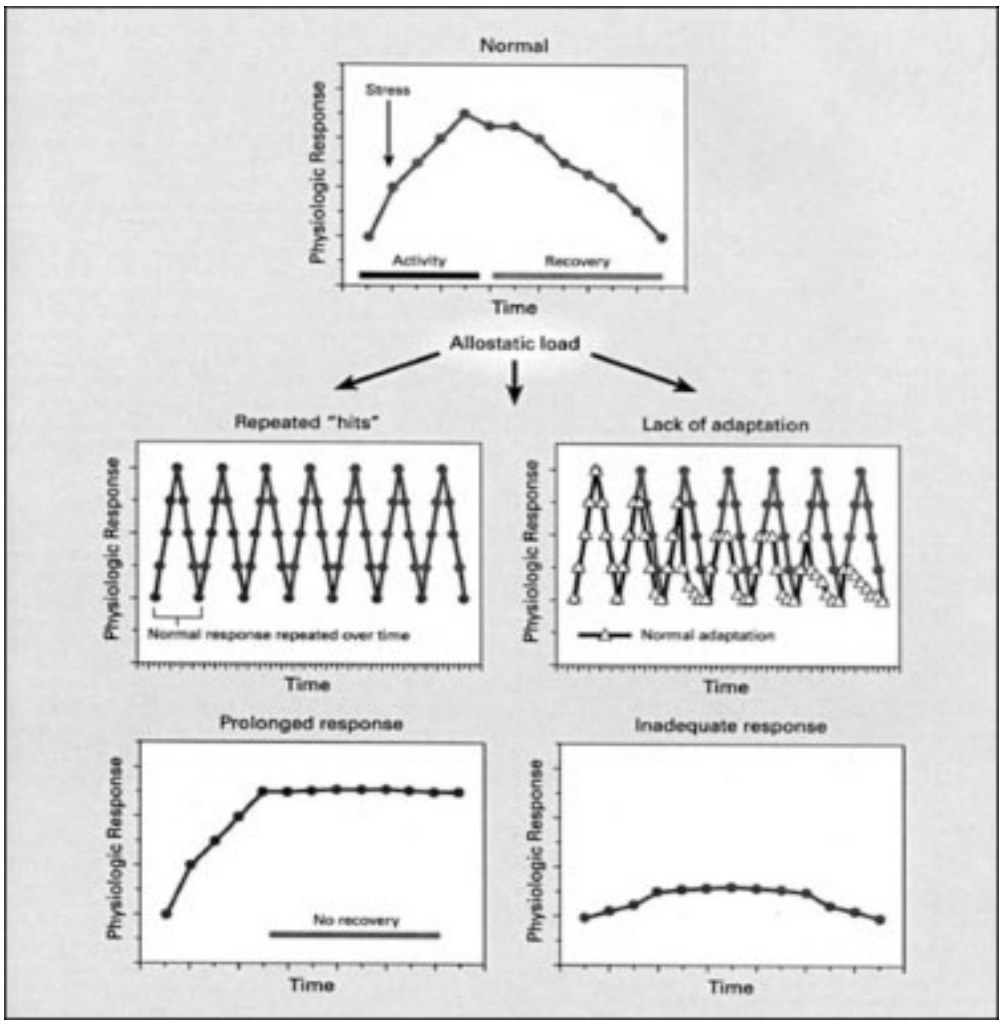

Figure 2. TYPES OF ALLOSTATIC LOAD

Allostatic load is calculated by including information about the status of all major physiological regulatory systems. The allostatic load model expands the theory of allostasis by applying it to study the effects of chronic stress. In the initial studies done to validate the allostatic load model, McArthur Successful Aging Study data was used and this data contained information about the physiological status of the HPA axis, sympathetic nervous system, metabolic processes and cardiovascular system of the study subjects [186]. In total, 10 parameters were included in the study and of these, 4 parameters, namely DHEA, cortisol, adrenaline and noradrenaline were primary

mediators of the stress response, and the remaining 6 parameters were indices of outcomes. Many studies have utilized the allostatic load model to quantify stress. In these studies, biomarkers encompassing neuroendocrine, cardiovascular, immune and metabolic parameters are measured as a suite. Ideally, both resting and changes in resting levels of these biomarkers are measured, and an allostatic load score is given to each study subject by summing the number of indices for which the subject fell into the highest risk group. A high allostatic load score would mean that the subject is experiencing high levels of chronic stress and is at a greater risk of morbidity due to chronic stress. Many studies have utilized the allostatic load model to make predictions about the health outcomes following exposure to chronic stress [19, 59, 187-188].

An individual`s behavior can either increase or decrease the risk for disease. For example, if an individual takes up smoking as a result of psychosocial stress, the risk of several chronic disorders is increased, but if an individual`s behavior helps in increasing stress buffering systems, say social connections, the risk of developing an undesired health outcome due to stress exposure can be decreased. Genetics and family history of a given disease also modifies an individual`s risk for developing the disease. Sociological factors also modify risk of disease. For example, an individual with low socioeconomic status may not have the same kind of access to healthcare services as an individual with higher socioeconomic status and this may increase the risk of developing a more serious form of the disease due to lack of early diagnosis and treatment in the individual with lower socioeconomic status. Since behavioral, sociological and genetic factors modify the risk of disease in an individual, the allostatic load model can be made more robust by incorporating indices of these risk factors for

the health outcome being measured. The strength of the allostatic load model is that it captures variation across multiple physiological domains impacted by stress, and by virtue of being multi-dimensional, can better isolate effects of stress from other exposures because, it is unlikely that any given pathological condition would affect all the indices being measured in the same manner as chronic stress. Using factor analysis, suppose it is hypothesized that the same set of biomarkers (variables) are the primary mediators and indicators of secondary outcomes of chronic stress and a given pathological state (the factors), an exploratory factor analysis can be carried out to unearth the underlying structure of relationships of the variables to the factors. A high allostatic load score usually translates into higher risk of morbidity. Any new information emerging about either the stress response or the health outcomes of interest is also very easy to incorporate into the model.

The set of indicators to be used in a given study are arbitrarily chosen by the researcher. It would be ideal to use those biomarkers which are indicative of health status not revealed by self reports of stress. Also, the weighted scores given to each indicator that is measured is arbitrary. There is no standardization with regards to which indicators must be given a higher weighted score and which indicators must be given a lower weighted score. Depending on what data is available, researchers have adapted the methodology used to arrive at the allostatic load score. An important question to be answered is, what do the levels of biomarkers mean at different points in the life of an individual and is the functional significance of a given level of biomarker different for different ethnicities and sex? Another important question to be answered is if various kinds of psychosocial stress causes the same kind of allostatic load profile. Most of the

biomarkers measured to arrive at the allostatic load score are biologically interconnected. Change in one parameter will consequently result in changes in all other physiologically interconnected parameters due to positive and negative feedback mechanisms, hence making it difficult to ascertain which parameter is a primary mediator and which parameter is a secondary outcome. Though allostatic load reflects cumulative exposures to stress over many years, most allostatic load studies are of a cross sectional nature when it comes to throwing light on changes to the allostatic profile over time. Longitudinal measurement of allostatic load can give information about the allostatic profile of an individual at various stages of developing stress related health outcomes. This can give some insight into the pathways of pathophysiology leading to the development of disease.

9.0 PROMISING NEW VISTAS FOR CHRONIC STRESS CHARACTERIZATION

9.1 METABOLOMICS

Metabolomics is defined as “the systematic study of the unique chemical fingerprints that specific cellular processes leave behind” [189]. For many years physicians and scientists have known that metabolites play an important role in pathology and that by identifying the metabolites, clues to the underlying disease can be obtained. But the “omics” approach to studying metabolites is relatively new. It is said that “small changes in fluxes through pathways can lead to large changes in the concentration of metabolites” [190], hence metabolomics can identify any small discrepancies in normal functioning of enzymes and thus point to a possible pathology where other techniques might not be able to do so. Since stress influences digestion and digestive enzymes [204], it is natural that a comparison of stressed vs. unstressed metabolomes would yield interesting results. A recent study has used the metabolomics approach to identify biomarkers of chronic stress in rats. Blood collected under anesthesia was analysed using NMR. Differences were observed in the spectra between control and stressed rats. The stressed phenotype showed increases in plasma lactic acid, choline, NAC and decreases in HDL, unsaturated fatty acids and unknown components [191]. If the spectra for acute stress can be obtained and compared to the spectra of chronically stressed rats, and if these two spectra are considerably different from each other; and if the spectrum of chronic stress is considerably different from the spectra obtained from known pathological states

commonly affecting stress response systems, such a spectrum may be used as a biomarker in rats and a similar approach can be pursued to identify the differences in spectra of human control and chronically stressed individuals. Using human samples, metabolome of acute stress has already been studied and is available for comparison with chronic stress cases. The metabolites that are significantly different from control groups have been identified as lactate, pyruvate, alanine, and hypoxanthine [192].

Another study used liver metabolic gene profiling in mice to characterize shifts in energy metabolism that result from acute and chronic stress. In this study, hypercatabolic activity is presented as a hallmark of chronic stress [193]. If the pattern of gene regulation is significantly different from those for known pathological conditions affecting the stress response systems, the gene profile can be used as an indicator of chronic stress in mice and the study can be repeated for human metabolic genes.

The metabolomics approach to characterization of stress can be used mainly to make judgments about the stress response component of the stress chain. It remains to be seen if various types of chronic stressors give rise to similar metabolite signatures. If so, more research needs to be done in order to associate a particular level of stressor exposure to a particular metabolic signature. If the various kinds of chronic stressors do not have a common metabolic signature, further research to link a particular level of a given stressor to a particular metabolic signature needs to be carried out. If this is done, metabolomics of chronic stress can be used to make quantitative judgments about the level of chronic stress experienced by a person, after taking into account, the appraisal of the stressor. In toxicology, it is said that the dose makes the poison. Quantifying the level of chronic stress is important to determine the “dose” of chronic stress which is

detrimental to health and if there is a proverbial NOEL (No observed effect level). Objective quantification of chronic stress can make this technique very useful for mechanistic studies. Even though sample (blood) collection is fairly easy, the high cost and skill level involved in performing this technique would make it difficult to use in large epidemiology studies.

Mass spectrometry methods currently being employed in metabolomics studies can provide accurate and reproducible qualitative and quantitative assessments of a large complement of the metabolome, but the time required to analyze large sample numbers can be very lengthy. Hence, parallel processing of samples to allow high throughput screening would complement the comprehensive metabolome-wide screening. But, the disadvantage of such a high throughput assay would be the predetermination of metabolites to be screened, which can present a bias in the metabolite patterns observed. A method combining the advantages of the high throughput screening and the comprehensive metabolome screening can be used. Multiple correlations of metabolites are commonly observed in metabolomics studies [6]. If a set of representatives could be identified such that the metabolites can capture much of the variance within a metabolome which is potentially diagnostic of changes due to stress, if the metabolites are relatively independent of each other and are not commonly found in some known pathological conditions, then the resultant set of biomarker metabolites could be used for screening of metabolite patterns as a result of chronic stress, and a metabolite signature diagnostic for chronic stress could then emerge. The ability of the biomarker set to faithfully represent the pattern of variation among the various tissues (potentially in humans, blood draw and urine sample)

sampled in the body can provide a type of internal validation. The biomarker metabolite set should be able to discern the same pattern of relationships based on correlations as those correlations derived from a comprehensive metabolite dataset. The biomarker metabolites can be arrived at by subjecting the metabolome profiles of chronic stress and some known pathologies to principal component analysis and subsequent K-means clustering. Representatives from the clusters can be chosen based on their proximity to the center of the cluster and their relative rarity in all the metabolic profiles studied except chronic stress.

The advantage of using metabolomics is that it measures the phenotype of an organism. When an organism becomes diseased or stressed, it triggers specific molecular changes and the phenotype becomes altered. This change can then be measured using metabolomics. Doctors have used metabolites to diagnose various health conditions, for example, glucose measurement has been used to diagnose diabetes, so the concept is not new, but using metabolomics, a comprehensive set of metabolites can be measured and this gives a clearer picture of the health status of the individual.

9.2 ULTRASTRUCTURE MODIFICATIONS IN MITOCHONDRIA

Mitochondrion is a membrane bound organelle found in most eukaryotic cells. Mitochondria are considered the power house of the cell because it is the site of ATP synthesis. The mitochondrion consists of a phospholipid bilayer, an inter-membrane space and the matrix (the space enclosed by the inner membrane, which contains the

mitochondrial DNA and proteins). The inner membrane of the mitochondrion invaginates into the matrix and the invaginations are called cristae. The diameter of mitochondria ranges from 0.5 to 10 microns [194]. Ultrastucture of mitochondria of masticatory muscles was recently shown to be altered under chronic stress in rats. Swollen mitochondria with cristae loss and reduced matrix density was observed after 3 weeks of stimulation and after 5 weeks, severe vacuolar changes were observed by TEM. Anaerobic metabolism was also increased [195]. Oxidative stress is also known to cause reduced matrix density and disorganization of cristae, but in a reversible manner [196]. In humans, chronic stress is known to cause masticatory muscle disorder [197], hence these findings may be significant. In order to use the morphological changes of mitochondria as biomarkers for chronic stress, it would be important to find out if the changes caused by chronic stress are reversible. If the kinetics of reversion are different from the kinetics of reversion of oxidative stress, the change in ultrastructure can be used as an indicator of the stress response component of the chronic stress chain. A buccal swab can give enough cells to observe mitochondrial changes. But buccal epithelium is subject to other stressors like mechanical stress which can also cause the said changes, but these can be accounted for by using appropriate non-stressed controls who also experience the other non-psychosocial stresses affecting the buccal epithelium, in the experimental or epidemiological studies. Also, mitochondrial fission shows characteristics similar to those observed for chronic stress. Mitochondria are constantly undergoing fission and fusion. Hence, it is necessary to differentiate these normal changes from those induced by chronic stress. It is most likely that the fragmentation of mitochondria that is observed under chronic stress is due to

unbalanced fission [198]. Markers of fission (Fis1) and fusion (DLP1, Mfn1, Mfn2, etc) [199] can be measured and if fission proteins are elevated and fusion proteins are decreased, the changes can be attributed to pathophysiology of chronic stress. Buccal swab collection is noninvasive and inexpensive, making ultrasturcture changes in mitochondria easy to use for large epidemiological studies. The intensity of ultrastructure integrity loss can be related to the length of time under stress; hence it can be incorporated easily into the allostatic load model to not only provide information about the secondary outcomes of stress, but potentially also about the duration and intensity of stressor exposure.

9.3 DRR1 INDUCTION IN BRAIN

DRR1 is a tumour suppressor gene which was recently described as inducible by chronic stress in the brain. Theo Rein et. al. reported in a recently published study conducted on rats that DRR1 has the ability to bind to and remodel actin filaments, which are important components of pre-synaptic and post-synaptic structures [200]. Hence, DRR1 can potentially impact neuroplasticity and information processing by altering the interplay between pre- and post-synaptic structures. It was reported by the same group that DRR1 is prominently expressed and induced by chronic stress in the hippocampus [201]. It is widely regarded that chronic stress mediates changes in neuroplasticity [202-203]. It is possible that DRR1 plays a role in this mediation. A possible link between elevation of DRR1 expression in the brain and behavioral changes in the rats has to be explored. If DRR1 can be mechanistically linked to

behavioral changes, it can be used as a biomarker of chronic stress response in rats to be used in other mechanistic studies exploring the link between chronic stress exposure and health outcomes. This cannot be applied to humans as it is induced only in the hippocampus region of the brain and therefore, obtaining a tissue sample for measuring DRR1 levels in live subjects is not possible. If there are paralogs of the protein, being expressed in other cell types which can be sampled from humans, it might be useful to see if those proteins are also stress inducible in a manner consistent with DRR1. A BLAST search of DRR1 (Accession number NP_001070246.1) with search criteria set as all non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples from WGS projects, search algorithm set as blastp [205], yielded several hypothetical proteins, proteins of unknown functions and proteins normally expressed by cells of the nervous system. This makes it difficult to come up with any proxy measures that could be captured in humans.

10.0 CONCLUSIONS

Stress, unlike any other pathological condition, triggers a non-specific response and influences multiple physiological systems [2]. In order to conduct research on a given condition, it is very important to characterize that condition and be able to objectively measure its indices. Chronic stress was traditionally measured using questionnaires which measured stressor exposure and affect, like the perceived stress scale, POMS questionnaire and major life events scale. Even though high scores on these questionnaires correlated with greater susceptibility to stress related illness, the measurements obtained using these instruments were arbitrary and subjective. The use of biomarkers can solve the problems of arbitrariness and subjectivity. But biomarkers suffer from problems with multiple determinations. Also, biomarkers cannot capture information regarding stress appraisal, which plays a major role in determining of a given stressor is stressful or not. Hence, ideally a study would use a set of biomarkers (allostatic load model) to objectively measure the stress response, while using the questionnaires to measure stressor exposure and stress appraisal. Using statistical methods, the allostatic load score and the scores on the questionnaires can be combined to give one composite score which would be more effective in predicting susceptibility to stress related diseases. The challenge lies in fine tuning the allostatic load model so as to capture only chronic psychosocial stress related information and weed out other confounding factors like exposure to biochemical and physical stressors as much as possible. Some of the recent research has thrown up some interesting possibilities, which if followed up on, could lead to novel methods of chronic stress

measurement which could be added to the allostatic load model to make it more robust. With the help of objective measurements, studies exploring the physiological and pathophysiological effects of chronic stress can be conducted and the mystery of being generally ill, as Selye put it, can be solved.

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