research for man and environment

RIJKSINSTITUUT VOOR VOLKSGEZONDHEID EN MILIEU
NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT

RIVM rapport 650240 001

Stress markers of health status

J.G.C. van Amsterdam, A. Opperhuizen and M. Olff

April 2000

Dit onderzoek werd verricht in opdracht en ten laste van het Directie RIVM in het kader van project 650240.

RIVM, Postbus 1, 3720 BA Bilthoven, Telefoon 030-2749111; Fax 030-2742971

VERZENDLIJST

1-5 College ter Beoordeling van Geneesmiddelen
6 Depot van Nederlandse Publikaties en Nederlandse Bibliografie
7 Directie RIVM
8 Dr.ir.G.de Mik
9 Dr.W.H.Könemann
10 Prof.dr.D.J.de Wildt
11 Prof.dr.J.G.Vos
12-19 Auteurs
20 SBD/Voorlichting & Public Relations
21 Bureau Rapportenregistratie
22 Bibliotheek RIVM
23-50 Reserve exemplaren tbv Bureau Rapportenbeheer
50-53 Reserve exemplaren

INHOUD

SUMMARY

This report reviews a number of potential stress indicators of health status. The immunobiochemical stress markers are all related in some way to cortisol which may be regarded as the golden standard in stress research. Finally, the relation between the reviewed biological markers and the end points as determined via the psychological approach is discussed.

SAMENVATTING

Dit rapport geeft een overzicht van potentiële endocriene en immunologische stress indicatoren van de gezondheidsstatus. Nadruk is gelegd op de indicatoren die op een of andere wijze gerelateerd is aan cortisol, dat als de gouden standaard in stress onderzoek wordt beschouwd. Tot slot wordt nader ingegaan op de relatie tussen de biologische markers en de eindpunten zoals die middels de psychologische benadering worden bepaald.

1. INTRODUCTION

Society is experiencing increased chronic psychological stress that affects human psychological and physical health. Adequate coping strategies are required to resist to and to reverse the adverse effects of stressful events on human health. If stress is not adequately controlled, sustained activation leads to loss of the homeostasis which will not only affect the individual mentally but also physiologically. As such various diseases, notably immune-related diseases, may develop following prolonged exposure to psychological stress.

Both psychological and physiological stress markers have been developed to study the psychological and psychosomatic patterns and consequences of long lasting stress. Psychological stress markers are mainly assessed via questionnaires that characterize the amount of experienced stress as well as of distress, coping styles, mental and physical health problems. The physiological stress markers comprise a large array of endocrine, biochemical and immunological endogenous substances.

The present paper outlines that the HPA-axis (hypothalamus- pituitary-adrenals-axis) plays a prominent role in the psychobiological consequences of stressful events. Cortisol release is a hallmark of this pathway Though a large array of signaling molecules are involved in activation of the HPA-axis,. For this reason, various endocrine and immunological biomarkers related to cortisol are proposed to study the physiological effects of stress. Finally, the relation between psychological and physiological stress markers is discussed.

2. STRESS, STRESSORS AND DISEASE

Numerous definitions have been associated with prolonged psychological stress. Here we will use the term ‘stress’ to describe the consequences of the sources of stress or ‘stressors’ (1). Stressors include major life events, like death of a loved one, divorce, loosing a job, and unwished relocation. Other examples of stressors are: every day minor life events and daily hassles like a high work-load, traffic jams, conflicts, and many other situations that because of their chronicity often have a major impact on the mental and physical well-being. Stressors can lead to stress, with a consequential large array of psychological and psychosomatic complaints, finally developing to illness and disease (2,3). Associated with stress are mental diseases such as surmenage, burn-out syndrome, chronic fatigue syndrome, post-traumatic stress disorder, depression, and anxiety disorder. Despite certain differences, they share the aspect of negative emotional stress. Other diseases that have been associated with long-term negative stress are cardiovascular disease, rheumatic arthritis, diabetes, auto-immune disease, and an increased morbidity and mortality linked to e.g. infections and neoplasms (4-8).

3. ACUTE STRESS VERSUS CHRONIC STRESS

To adequately describe the psychophysiological consequences of stress, it is important to discriminate between acute and chronic stress (9). For instance, research in the interdisciplinary field of psychoneuroimmunology has shown that various forms of *chronic stressors*, such as marital problems, work-related stressors or bereavement, generally have a suppressive effect on parameters of the immune system (10-14). In the case of chronic stress, the down-regulation of components of the immune system may lead to increased susceptibility to infections (colds, herpes, HIV) and worsen existing diseases such as cancer (4,5). The impaired functioning of the immune system is likely to contribute to increased morbidity and mortality (6-8).

In contrast to the down-regulation of the immune system seen with the longer lasting chronic stress, *acute stress*, such as examination stress or experimentally induced stress, is rather associated with up-regulation of the immune functions (15-19). The related reactivity of cardiovascular variables and NK cell numbers to an acute stressor suggests that dynamic short-term changes in both systems are mediated by catecholamines (15). Considering that acute short-term stress does not result in severe health consequences, only markers of prolonged stress will be described here.

4. ENDOCRINE AND BIOCHEMICAL STRESS MARKERS

The biological effects of stress are generated via an integrated complex network of central-neural and endocrine processes. In addition to sympathetic nervous activation, numerous (hormonal) mediators like catecholamines, corticosteroids, endogenous opioids are released by this network (e.g. (3).

Frequently applied endocrine markers in stress research are circulating cortisol, ACTH and CRH. While the hormones ACTH and CRH are associated with the initial steps of HPA-axis activation, cortisol, released from the adrenals, is a major final product of this route. Other routes activated by CRH, such as the thymus-pituitary-adrenal axis, and the thymus-pituitary-gonadal axis will not be considered here.

4.1. Cortisol

A key mediator of the systemic response to stressors is CRF, released by the hypothalamus. During the stress response CRF regulates ACTH secretion from the pituitary (20) and, via an endocrine action, ACTH controls the release of corticosteroids from the adrenal medulla. CRF also activates the SNS system resulting in catecholamine release into the circulation (21). CRH release after stress has been associated with reduced lymphocyte proliferation and NK-cytotoxicity (22). In addition, CRF slows IgG induction in response to the foreign protein KLH (keyhole limpet hemacyanin).

Negative life events increase plasma cortisol (23) and the level of cortisol is fully accepted in stress research as a valuable measure of stress. Measurement of cortisol in saliva is preferred because it is non-invasive, reliable, and elicits no stress. Salivary values compare well with the serological value. Like most hormones cortisol follows a circadian rhythm so that for proper measurement of cortisol levels the time of sampling should be carefully noticed. The flattening of this rhythm can also be used to characterize stress.

Cortisol (and ACTH) levels are also very frequently increased in severely depressed patients. In most depressive patients the dexamethasone suppression test (DST) is positive though false positive and negative results are regularly obtained. The combined dexamethasone-CRH test

is the best neuroendocrine tool currently available to identify HPA abnormalities in psychiatric patients. Surprisingly, the DST has never been applied in stress research.
Cortisol can be easily assayed by commercial available immunoassays while HPLC-techniques have also been described.

4.2. Dehydroepiandrosterone (DHEA)

Another adrenal steroid hormone released by the adrenal cortex in response to ACTH is dehydroepiandrosterone (DHEA) that is further metabolized to its ester DHEA-sulfate (DHEA-S). Four week training in self management reduced cortisol by 23% and increased DHEA by 100% (24) and both effects were associated with decreased stress, anxiety, burn-out and hostility. This observation was recently confirmed in HIV-patients (25). In a cohort of senior male army officers Labbate et al. also showed that perceived stress was inversely related with DHEA-S levels (26) while Fava et al. (27) showed that Italian managers, who reported more cynicism and hostility, and less enjoyment in leisure activities than Americans managers, had significantly lower levels of serum DHEA.
Major depression is characterized by high evening cortisol and low morning DHEA level (28,29) and a raised cortisol/DHEA ratio (i.e. > 60^{th} percentile) predicts delayed recovery (30,31). Barrett et al. (32) recently showed in a large cohort of older women (N=699) that mood and depression as scored by the Beck Depression Inventory was inversely associated with DHEA-S plasma level.
Dexamethasone and cortisol decrease DHEA level (29,33,34). DHEA can antagonize cortisol (35) and may thus be effective to treat depression (36). Indeed, a recent double blind study showed that treatment of depressive patients with DHEA induced a significant antidepressant effect as assessed via the Hamilton Depression Rating Scale (37).
Commercial immunoassays are available to measure DHEA and DHEA-S in saliva and serum.

4.3. Pteridines (THB, biopterine en neopterine)

Major depression is characterized by low levels of neurotransmitters (dopamine, serotonin and noradrenaline) in the CNS and pharmacotherapy addresses increased availability of these hormones in the synaptic cleft. The neurotransmitters are synthetized by different hydroxylases using tryptophan, tyrosine and phenylalanine as substrate. The essential co-factor

of these hydrolases is tetrahydro-biopterin (THB), that is synthetized from guanosine tri-phosphate (GTP) by GTP-cyclohydrolase. Both biopterin and neopterin are markers of this synthetic pathway.

THB-synthesis in CNS is decreased in depressive patients and increased by electro-shock therapy. Most studies report increased neopterin en biopterin level in plasma and urine of depressive patients (38,39), while plasma THB and ratio THB/biopterin in such patients is decreased. Finally, in patients with chronic fatigue syndrome increased neopterin levels have been reported (40,41).

In analogy with major depression, stressful stimuli affect the level of both pteridines. Two studies describe a decrease of neopterin in urine after examination stress (42,43). Serum neopterin was shown to be associated with high chronic professional stress (44). In rats cold stress (45), immobilisation stress and food deprivation (46) increased THB and adrenal GTP-cyclohydrolase. In adrenals of a genetic strain of depressive rats tyrosine hydroxylase, dopamine beta-hydroxylase and GTP cyclohydrolase I mRNA were markedly elevated compared to the control strain. Immobilization stress in the control strain but not the stressed strain, increased mRNAs of the enzymes (47). Serova et al. (48) recently described that incubation of PC12 cells in vitro with plasma of immobilized rats increased GTP-cyclohydrolase mRNA. Interestingly, incubation of the cells with cortisol induced the same effect. These findings confirm previous data of Abou-Donia et al. (49) showing that the stress hormones ACTH and adrenaline stimulate GTP-cyclohydrolase activity. In rat liver cells dexamethasone increased THB (50) and in the adrenals of the rat cortisol increased GTP-cyclohydrolase mRNA. On the other hand the synthetic glucocorticoid dexamethasone inhibited cytokine-induced stimulation of GTP-cyclohydrolase in cardiac endothelial cells (51), macrophages (52) and mesangial cells (53).

Kits, based on either radioimmuno assay or enzyme immunoassay are commercially available for neopterin, but not biopterin. Alternatively, HPLC determinations have been described for both pteridines.

4.4. Kynurenine

TDO (tryptophan dioxygenase, tryptophan pyrolase) resides in liver only and specifically degrades tryptophan. Interestingly, cortisol stimulates tryptophan degradation in vitro by TDO via enzyme induction 7-8 fold (54). In addition, cortisol stimulates in the liver the degradation

of tyrosine via induction of tyrosine aminotransferase (TAT). Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in serotonin biosynthesis. Immobilization stress induced a 6-10 fold rise in TPH mRNA but did not affect GTP cyclohydrolase I mRNA (55). This is in apparent contrast with the decrease in TPH mRNA by dexamethasone observed by (56).
IDO (indole-dioxygenase) has a much broader substrate specificity and is localized in multiple tissues incl. macrophages. IDO metabolizes tryptophan, serotonin (5-HT) and melatonin. Cortisol does not induce IDO, but facilitates induction of IDO by γ-interferon (57). Like the formerly mentioned hydrolases, the enzyme IDO uses THB as co-factor for its metabolism of tryptophan to kynurenine. Kynurenine is further metabolized by enzymes which all are more or less dependent of vitamin B_6. When the individual is deficient in vitamin B_6, as depressive patients, increased tryptophan degradation will lead to a specific increase in the level of xanthurenic acid.
Indeed, immobilization-stress in the rat induces TDO en TAT via increased cortisol release. Stress increases the level of kynurenine and decreases the level of tryptophan and tyrosine. Cold-stress (48 h) decreases plasma tyrosine and tryptophan and increases kynurenine and xanthurenic acid in urine (58).
Mental depression coincides with decreased tryptophan level while a decreased kynurenine level was observed as well. The decreased kynurenine level was normalized by pharmacotherapy with antidepressives. Paroxetine and other anti-depressives inhibit TDO and increase tryptophan level. In patients with anxiety disorder kynurenine level was increased while in endogenous depression it was increased (59). The precursor is tryptophan melatonin is also decreased in depressive patients. Kynurenine and tryptophan are routinely assayed by HPLC.

4.5. NO-synthase

NO is synthetised from L-arginine by NO-synthase (NOS). Until now plasma levels of nitric oxide (NO) have not been used in stress research. This is mainly due to analytical limitations because NO is rapidly degraded in the circulation to nitrate and nitrite which are both food constituents and therefor not typical for increased NO-synthesis. Interestingly though, cortisol inhibits the induction of inducible NOS (iNOS) the enzyme responsible for the bulk of NO-synthesis under pathophysiological conditions. Another relevant observation related to stress is that plasma L-arginine level is decreased for up to 6 h after cessation of stress in the rat

(60,61). Decreased L-arginine plasma levels have also been reported in mood disorder (62). It is further remarkable that NK-cells in-vitro are cytotoxic only if L-arginine is available (63,64).

Stress increases neuronal expression of NOS in the adrenals and HPA-axis. Inhibitors of NOS increase corticosteron and ACTH fourfold while L-arginine is inhibitory (65). Inhibitors of NOS also stimulate release of CRF and ACTH (i.e. NO inhibits release). Immobilization stress increases the expression of nNOS mRNA in the adrenals 2.5 fold (66,67) and hippocampus (68). Animal studies further show that stress leads to excess consumption of L-arginine. One of the actions of the antidepressive drug paroxetine (serotonin re-uptake blocker) is inhibition of NO by NO-synthase (69). Finally, NO is involved in increased forearm blood flow during mental stress (70).

A major drawback of this interesting NO-pathway as stress marker is its evaluation which is sensitive to artefacts. In principle measurement of its degradation products nitrate and nitrite can be easily performed but both ions are also food constituents. Under restricted control conditions both NO-metabolites can, however, be used to monitor NO-synthase activity. The same holds for L-arginine levels.

Due to the wide interest in NO-research, various commercial assays are available to determine NO-levels. The colorimetric assay of the main metabolites nitrate and nitrite, based on the Griess reaction, is simple, not expensive, and highly reproducible. The high detection limit of this assay of 0.1-1 µM may, however, be an analytical problem to detect low NO-synthase activity.

5. IMMUNOLOGICAL MARKERS

It has been now well established that the immune system is affected by psychological stimuli. Some of these effects are mediated by circulating neurotransmitters and hormones, for which the different types of immune cells, including lymphocytes, have receptors. These changes are generally slow, and sometimes even very slow i.e. changes in the B-cells may take weeks or months to develop. Psychoneuroimmune stimuli mediated by neuronal pathways that innervate lymphoid tissues appear to be the fastest reacting system. The number of lymphocytes, in particular natural killer (NK) cells may be increased within minutes after an acute psychological stressor. These changes are so fast that it is difficult to understand what really happens (18,19). It is possible that the changes are due to cellular migration of immune cells from the blood vessel or from the extravascular space into the blood flow (18,71).
Number of immune cells are routinely measured via coulter counter and FACS-analysis, while immunoglobulin levels are assayed via commercially available immuno assays.

5.1. Number of immune cell subtypes

Both meta-analytic (72) and enumerative (5,73) reviews clearly show that chronic stress decreases the number of circulating B cells, T cells, and large granular lymphocytes (NK-cells), to decreased proliferative responses of lymphocytes to several mitogens as well as to decreased natural killer cell activity (NKCA).
In response to acute stress the number of NK cells, i.e. CD57-positive cells, increase by 25 to 40 % (9,18,19). The parameters have returned to baseline values within 15 minutes after termination of the stressor. In general short lasting, relatively mild psychological stressors temporally increase the number of several cell types in peripheral blood. These stressors seem to have in common a predominant but mild effect on arousal and anxiety levels, generally accompanied by increased secretion of catecholamines. Indeed, infusion of adrenaline stimulated the redistribution of lymphocytes into the blood circulation (e.g. (74) indicating that adrenaline is one of the mediators of these effects.
Long lasting effects of changes in the immune system due to (repeated) stress have also been studied. An elegant study, performed by Kiecolt Glaser et al. (75) on persons taking care of

patients with Alzheimer's disease, showed that this long-lasting stressful situation decreased the proliferative response to mitogens and increased the number of days with infectious disease and other health problems.

Decreased NK cell activity has been related to certain human diseases (e.g. progression of cancer, chronic viral infection and auto-immune disease). In the elderly, it has also been shown that, decreased proliferative responses to mitogens were related to increased levels of mortality and more frequent hospitalization (76,77).

5.2. Immunoglobulins

Like other immune cells, B-cell activity is down regulated by cortisol and inhibition of immunoglobulin synthesis by cortisol has been described (78,79). In general, various stressors negatively affect the serum level of IgA, IgG, IgM due to a diminished immune response during stress, which is probably mediated by increased amounts of cortisol released by the adrenals.

On the other hand, due to the diminished immune response during stress, latent virusses multiply and titers of specific antibodies directed against latent virusses such as Herpes, Epstein Barr and CMV are increased by psychological stress. Increased levels of latent virusses have been frequently and successfully applied as measure of experienced negative stress (e.g. examination stress, Alzheimer's caregivers, etc). For review see van Amsterdam and Opperhuizen (80).

A most promising immunoglobulin as marker of stress is salivary IgA which is increased for prolonged time after stress (81). In addition to measuring antibody levels against latent virusses, one may also assay specific antibodies directed against *de-novo* antigens (*hepatitis* B, flue vaccines) and microorganisms (experimental infection studies).

6. RELATION BETWEEN PSYCHOLOGICAL AND BIOLOGICAL MARKERS

It is challenging and intriguing to relate the amount of stress as assessed by psychometric instruments with the levels of the physiological stress markers. Such relations are, however, not always observed and a number of possible causes of dyscongruence are summarized below.

a. Stressors do not lead per definition to physiological dysregulation as it largely depends on coping behavior/ability. Specific ways of dealing with stress may be differentially associated with biological functions. As shown in Figure 1 the stressor first has to be perceived, interpreted and evaluated before a psychoneuroendocrine responses develops. The subsequent emotional and behavioral response is determined by the subject's specific coping and defense strategy and this may result in more or less activation of the HPA-axis (2). Activation of the HPA-axis is strongest where efficient coping is not possible, as with severe prolonged stress and the experience of negative affect and the "conservation-withdrawal" or "distress" reaction occurs. (82-85). On the other hand when the subject has to "fight or flight" or to put an effort to control the situation, the sympathetic adrenal medullary system (SNS) is activated that immediately responds to the threatened homeostasis via the release of catecholamines.
b. A wide range of psychological instruments is available to evaluate stressors, respectively stress. Regarding stressors, daily hassles scales usually predict the physiological stress response more adequately than major life event scales. If stress is measured, the relation to physiology depends very much on the type of stress response measured: for instance affective responses (mood-, depression-, anxiety scales), stress complaints scales, general health status, or more specific stress related disease scales (burnout- and chronic fatigue scales).
c. The time course is extremely important and may frustrate the association between the physiological and psychological markers i.e. the hormonal level may change at a time when the stressor is no longer present (86).
d. At later stages of the response, homeostatic mechanisms are active. The slower reactive hormones, such as cortisol, seem to have an important function to dampen the total stress

response. This tail of the acute reaction may, therefore, be regarded as a homeostatic device to restore the physiological dys-balance (87).

e. The physiological marker under consideration may rather be related to the disease propagated by the stressor(s) than to HPA-activation. For instance, in cancer patients the change in NK-cell activity may be initiated either by the stress the patient experiences due to the presence of this severe disease or by the tumor growth itself.

7. BEYOND THE ASSAY OF STRESS MARKERS

Correctly, cortisol represents the golden standard in stress research and the markers outlines above are all related in some way to cortisol. By assaying such cortisol-related markers in addition to cortisol, the neuroendocrinological pathways triggered by chronic stress can be characterized in more detail. One should realize that the physiological system of the body is a dynamic system with multiple feed back loops. So, cortisol release may trigger a hormonal or immunological reaction which follows a quite different time scale i.e. cortisol level may have normalized, while the immune response remains impaired for a much longer period. Thus, the measurement of additional markers provides a more complete picture which enables a better understanding of the pathophysiological effects initiated by stressors.

In addition, such assays may serve as an additional diagnostic tool to differentiate between stress related diseases like depression and chronic fatigue syndrome (CFS). Subjects experiencing chronic stress show hyper-cortisolemia and an exaggerated cortisol response to corticotropin, while CFS-patients rather show decreased plasma cortisol levels and reduced responsiveness to corticotropin (88,89).

Finally, it is an intriguing and yet unsolved question whether the body, in response to chronic stress, launches cortisol as a fine-tuned messenger or just as a blunt and crude down-regulator of immune functions. Measurement of markers of different pathways, modulated by stressful stimuli, may help to find the answer to this question.

REFERENCES

1. Ursin H, Olff M. The stress response. In: Stanford-C, Salmon-P, Gray-J, eds. Stress: an integrated approach. Academic Press, 1993:3-22.

2. Olff M, Brosschot JF, Godaert GL, Benschop RJ, Ballieux RE, Heijnen CJ, De Smet MB, Ursin H. Modulatory effects of defense and coping on stress-induced changes in endocrine and immune parameters. Int J Behav Med 1995;2:85-103.(Abstract)

3. Ader R, Felten DL, Cohen N. Psychoneuroimmunolgy. Second Ed. New York: Academic Press, 1991:-1218.

4. Rosen FS, Cooper MD, Wedgwood RJ. The primary immunodeficiencies (1). N Engl J Med 1984;311:235-242.

5. Kiecolt Glaser JK, Glaser R. Psychoneuroimmunology and health consequences: data and shared mechanisms. Psychosom Med 1995;57:269-274.

6. Whiteside TL, Bryant J, Day R, Herberman RB. Natural killer cytotoxicity in the diagnosis of immune dysfunction: criteria for a reproducible assay. J Clin Lab Anal 1990;4:102-114.

7. Shekelle RB, Raynor WJ, Jr., Ostfeld AM, Garron DC, Bieliauskas LA, Liu SC, Maliza C, Paul O. Psychological depression and 17-year risk of death from cancer. Psychosom Med 1981;43:117-125.

8. Weisse CS. Depression and immunocompetence: a review of the literature. Psychol Bull 1992;111:475-489.

9. Olff M. Stress, depression and immunity: the role of defense and coping styles [comment]. Psychiatry Res 1999;85:7-15.

10. Bartrop RW, Luckhurst E, Lazarus L, Kiloh LG, Penny R. Depressed lymphocyte function after bereavement. Lancet 1977;1:834-836.

11. Jemmott JB, 3d, Locke SE. Psychosocial factors, immunologic mediation, and human susceptibility to infectious diseases: how much do we know? Psychol Bull 1984;95:78-108.

12. Ursin H, Mykletun R, Tonder O, Vaernes R, Relling G, Isaksen E, Murison R. Psychological stress-factors and concentrations of immunoglobulins and complement components in humans. Scand J Psychol 1984;25:340-347.

13. O'Leary A. Stress, emotion, and human immune function. Psychol Bull 1990;108:363-382.

14. Glaser R, Kiecolt Glaser JK, Speicher CE, Holliday JE. Stress, loneliness, and changes in herpesvirus latency. J Behav Med 1985;8:249-260.

15. Benschop RJ, Godaert GL, Geenen R, Brosschot JF, De Smet MB, Olff M, Heijnen CJ, Ballieux RE. Relationships between cardiovascular and immunological changes in an experimental stress model. Psychol Med 1995;25:323-327.

16. Benschop RJ, Brosschot JF, Godaert GL, De Smet MB, Geenen R, Olff M, Heijnen CJ, Ballieux RE. Chronic stress affects immunologic but not cardiovascular responsiveness to acute psychological stress in humans. Am J Physiol 1994;266:R75-80.

17. Brosschot JF, Benschop RJ, Godaert GL, Olff M, De Smet M, Heijnen CJ, Ballieux RE. Influence of life stress on immunological reactivity to mild psychological stress. Psychosom Med 1994;56:216-224.

18. Brosschot JF, Benschop RJ, Godaert GL, De Smet MB, Olff M, Heijnen CJ, Ballieux RE. Effects of experimental psychological stress on distribution and function of peripheral blood cells. Psychosom Med 1992;54:394-406.

19. Brosschot JF, Smelt D, De Smet M, Heijnen CJ, Olff M, Ballieux RE, Godaert GL. Effects of experimental psychological stress on T-lymphocytes and NK-cell in man: an exploratory study. J Psychphys 1991;5:59-67.(Abstract)

20. Owens MJ, Nemeroff CB. Physiology and pharmacology of corticotropin-releasing factor. Pharmacol Rev 1991;43:425-473.

21. Irwin M, Hauger RL, Brown M, Britton KT. CRF activates autonomic nervous system and reduces natural killer cytotoxicity. Am J Physiol 1988;255:R744-7.

22. Saperstein A, Brand H, Audhya T, Nabriski D, Hutchinson B, Rosenzweig S, Hollander CS. Interleukin 1 beta mediates stress-induced immunosuppression via corticotropin-releasing factor. Endocrinology 1992;130:152-158.

23. Willis L, Thomas P, Garry PJ, Goodwin JS. A prospective study of response to stressful life events in initially healthy elders. J Gerontol 1987;42:627-630.

24. McCraty R, Barrios Choplin B, Rozman D, Atkinson M, Watkins AD. The impact of a new emotional self-management program on stress, emotions, heart rate variability, DHEA and cortisol. Integr Physiol Behav Sci 1998;33:151-170.

25. Cruess DG, Antoni MH, Kumar M, Ironson G, McCabe P, Fernandez JB, Fletcher M, Schneider N. Cognitive-behavioral stress management buffers decreases in dehydroepiandrosterone sulfate (DHEA-S) and increases in the cortisol/DHEA-S ratio and reduces mood disturbance and perceived stress among HIV-seropositive men. Psychoneuroendocrinology 1999;24:537-549.

26. Labbate LA, Fava M, Oleshansky M, Zoltec J, Littman A, Harig P. Physical fitness and perceived stress. Relationships with coronary artery disease risk factors. Psychosomatics 1995;36:555-560.

27. Fava M, Littman A, Lamon Fava S, Milani R, Shera D, MacLaughlin R, Cassem E, Leaf A, Marchio B, Bolognesi E, et al. Psychological, behavioral and biochemical risk factors for coronary artery disease among American and Italian male corporate managers. Am J Cardiol 1992;70:1412-1416.

28. Goodyer IM, Herbert J, Altham PME, Pearson J, Secher SM, Shiers HM. Adrenal secretion during major depression in 8- to 16-year-olds .1. Altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. Psychol Med 1996;26:245-256.

29. Osran H, Reist C, Chen CC, Lifrak ET, Chicz DeMet A, Parker LN. Adrenal androgens and cortisol in major depression. Am J Psychiatry 1993;150:806-809.

30. Herbert J. Neurosteroids, brain damage, and mental illness. Exp Gerontol 1998;33:713-727.

31. Goodyer IM, Herbert J, Altham PM. Adrenal steroid secretion and major depression in 8- to 16-year-olds, III. Influence of cortisol/DHEA ratio at presentation on subsequent rates of disappointing life events and persistent major depression [see comments]. Psychol Med 1998;28:265-273.

32. Barrett Connor E, von Muhlen D, Laughlin GA, Kripke A. Endogenous levels of dehydroepiandrosterone sulfate, but not other sex hormones, are associated with depressed mood in older women: The Rancho Bernardo study. J Am Geriatr Soc 1999;47:685-691.

33. Parker LN, Odell WD. Control of adrenal androgen secretion. Endocr Rev 1980;1:392-410.

34. Abraham GE. Ovarian and adrenal contribuion to peripheral steroids during the menstrual cycle in two hirsute women. Obstet Gynecol 1975;46:29-36.

35. Blauer KL, Poth M, Rogers WM, Bernton EW. Dehydroepiandrosterone antagonizes the suppressive effects of dexamethasone on lymphocyte proliferation. Endocrinology 1991;129:3174-3179.

36. O'Dwyer AM, Lightman SL, Marks MN, Checkley SA. Treatment of major depression with metyrapone and hydrocortisone. J Affect Disord 1995;33:123-128.

37. Wolkowitz OM, Reus VI, Keebler A, Nelson N, Friedland M, Brizendine L, Roberts E. Double-blind treatment of major depression with dehydroepiandrosterone. Am J Psychiatry 1999;156:646-649.

38. Maes M, Meltzer HY, Bosmans E, Bergmans R, Vandoolaeghe E, Ranjan R, Desnyder R. Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. J Affect Disord 1995;34:301-309.

39. Garbutt JC, Duch DS, Nichol CA, Woolf JH. Urinary biopterin and neopterin excretion and pituitary-adrenal activity in psychiatric patients. Psychiatry Res 1985;16:181-187.

40. Buchwald D, Wener MH, Pearlman T, Kith P. Markers of inflammation and immune activation in chronic fatigue and chronic fatigue syndrome. J Rheumatol 1997;24:372-376.

41. Chao CC, Gallagher M, Phair J, Peterson PK. Serum neopterin and interleukin-6 levels in chronic fatigue syndrome [letter]. J Infect Dis 1990;162:1412-1413.

42. Dunbar PR, Hill J, Neale TJ. Urinary neopterin quantification indicates altered cell-mediated immunity in healthy subjects under psychological stress. Aust N Z J Psychiatry 1993;27:495-501.

43. Fittschen B, Schulz KH, Schulz H, Raedler A, von Kerekjarto M. Changes of immunological parameters in healthy subjects under examination stress. Int J Neurosci 1990;51:241-242.

44. De Gucht V, Fischler B, Demanet C. Immune dysfunction associated with chronic professional stress in nurses. Psychiatry Res 1999;85:105-111.

45. Baruchin A, Weisberg EP, Miner LL, Ennis D, Nisenbaum LK, Naylor E, Stricker EM, Zigmond MJ, Kaplan BB. Effects of cold exposure on rat adrenal tyrosine hydroxylase: an analysis of RNA, protein, enzyme activity, and cofactor levels. J Neurochem 1990;54:1769-1775.

46. Koller M, Goldberg M, Schramm G, Merkenschlager M. The influence of nutritional factors on biopterin excretion in laboratory animals. Z Ernahrungswiss 1990;29:169-177.

47. Serova L, Sabban EL, Zangen A, Overstreet DH, Yadid G. Altered gene expression for catecholamine biosynthetic enzymes and stress response in rat genetic model of depression. Brain Res Mol Brain Res 1998;63:133-138.

48. Serova L, Nankova B, Rivkin M, Kvetnansky R, Sabban EL. Glucocorticoids elevate GTP cyclohydrolase I mRNA levels in vivo and in PC12 cells. Brain Res Mol Brain Res 1997;48:251-258.

49. Abou Donia MM, Duch DS, Nichol CA, Viveros OH. Hormonal regulation of guanosine triphosphate cyclohydrolase activity and biopterin levels in the rat adrenal cortex. Endocrinology 1983;112:2088-2094.

50. Parniak MA, Pilkington J. Glucocorticoid stimulation of tetrahydrobiopterin levels and phenylalanine hydroxylase activity in rat hepatoma cells. Biochem Cell Biol 1989;67:293-296.

51. Simmons WW, Ungureanu Longrois D, Smith GK, Smith TW, Kelly RA. Glucocorticoids regulate inducible nitric oxide synthase by inhibiting tetrahydrobiopterin synthesis and L-arginine transport. J Biol Chem 1996;271:23928-23937.

52. Schoedon G, Schneemann M, Hofer S, Guerrero L, Blau N, Schaffner A. Regulation of the L-arginine-dependent and tetrahydrobiopterin-dependent biosynthesis of nitric oxide in murine macrophages. Eur J Biochem 1993;213:833-839.

53. Pluss C, Werner ER, Wachter H, Pfeilschifter J. Differential effect of dexamethasone on interleukin 1beta- and cyclic AMP-triggered expression of GTP cyclohydrolase I in rat renal mesangial cells. Br J Pharmacol 1997;122:534-538.

54. Salter M, Pogson CI. The role of tryptophan 2,3-dioxygenase in the hormonal control of tryptophan metabolism in isolated rat liver cells. Effects of glucocorticoids and experimental diabetes. Biochem J 1985;229:499-504.

55. Chamas F, Serova L, Sabban EL. Tryptophan hydroxylase mRNA levels are elevated by repeated immobilization stress in rat raphe nuclei but not in pineal gland. Neurosci Letters 1999;267:157-160.

56. Clark MS, Russo AF. Tissue-specific glucocorticoid regulation of tryptophan hydroxylase mRNA levels. Brain Res Mol Brain Res 1997;48:346-354.

57. Ozaki Y, Edelstein MP, Duch DS. The actions of interferon and antiinflammatory agents of induction of indoleamine 2,3-dioxygenase in human peripheral blood monocytes. Biochem Biophys Res Commun 1987;144:1147-1153.

58. Francesconi RP, Boyd AE, 3d, Mager M. Human tryptophan and tyrosine metabolism: effects of acute exposure to cold stress. J Appl Physiol 1972;33:165-169.

59. Orlikov AB, Prakhye IB, Ryzov IV. Kynurenine in blood plasma and DST in patients with endogenous anxiety and endogenous depression. Biol Psychiatry 1994;36:97-102.

60. Milakofsky L, Harris N, Vogel WH. Effects of repeated stress on plasma arginine levels in young and old rats. Physiol Behav 1993;54:725-728.

61. Milakofsky L, Hare TA, Miller JM, Vogel WH. Rat plasma levels of amino acids and related compounds during stress. Life Sci 1985;36:753-761.

62. Altamura CA, Mauri MC, Ferrara A, Moro AR, D'Andrea G, Zamberlan F. Plasma and platelet excitatory amino acids in psychiatric disorders. Am J Psychiatry 1993;150:1731-1733.

63. Park KG, Hayes PD, Garlick PJ, Sewell H, Eremin O. Stimulation of lymphocyte natural cytotoxicity by L-arginine. Lancet 1991;337:645-646.

64. Xiao L, Eneroth PH, Qureshi GA. Nitric oxide synthase pathway may mediate human natural killer cell cytotoxicity. Scand J Immunol 1995;42:505-511.

65. Giordano M, Vermeulen M, Trevani AS, Dran G, Andonegui G, Geffner JR. Nitric oxide synthase inhibitors enhance plasma levels of corticosterone and ACTH. Acta Physiol Scand 1996;157:259-264.

66. Tsuchiya T, Kishimoto J, Koyama J, Ozawa T. Modulatory effect of L-NAME, a specific nitric oxide synthase (NOS) inhibitor, on stress-induced changes in plasma adrenocorticotropic hormone (ACTH) and corticosterone levels in rats: physiological significance of stress-induced NOS activation in hypothalamic-pituitary-adrenal axis. Brain Res 1997;776:68-74.

67. Kishimoto J, Tsuchiya T, Emson PC, Nakayama Y. Immobilization-induced stress activates neuronal nitric oxide synthase (nNOS) mRNA and protein in hypothalamic-pituitary-adrenal axis in rats. Brain Res 1996;720:159-171.

68. Leza JC, Salas E, Sawicki G, Russell JC, Radomski MW. The effects of stress on homeostasis in JCR-LA-cp rats: the role of nitric oxide. J Pharmacol Exp Ther 1998;286:1397-1403.

69. Finkel MS, Laghrissi Thode F, Pollock BG, Rong J. Paroxetine is a novel nitric oxide synthase inhibitor. Psychopharmacol Bull 1996;32:653-658.

70. Dietz NM, Rivera JM, Eggener SE, Fix RT, Warner DO, Joyner MJ. Nitric oxide contributes to the rise in forearm blood flow during mental stress in humans. J Physiol Lond 1994;480:361-368.

71. Benschop RJ, Jabaaij L, Oostveen FG, Vingerhoets AJ, Kirschbaum C, Duivenvoorden HJ, Ballieux RE. Psychobiological factors related to human killer cell activity and hormonal modulation of NK cells in vitro. Life Sci 1993;52:1825-1834.(Abstract)

72. Herbert TB, Cohen S. Depression and immunity: a meta-analytic review. Psychol Bull 1993;113:472-486.

73. Kiecolt Glaser JK, Glaser R. Psychoneuroimmunology: can psychological interventions modulate immunity? J Consult Clin Psychol 1992;60:569-575.

74. Toft P, Helbo Hansen HS, Tonnesen E, Lillevang ST, Rasmussen JW, Christensen NJ. Redistribution of granulocytes during adrenaline infusion and following administration of cortisol in healthy volunteers. Acta Anaesthesiol Scand 1994;38:254-258.

75. Kiecolt Glaser JK, Dura JR, Speicher CE, Trask OJ, Glaser R. Spousal caregivers of dementia victims: longitudinal changes in immunity and health. Psychosom Med 1991;53:345-362.

76. Murasko DM, Weiner P, Kaye D. Association of lack of mitogen-induced lymphocyte proliferation with increased mortality in the elderly. Aging: Immunol Infect Dis 1988;1:1-6.(Abstract)

77. Murasko DM, Gold MJ, Hessen MT, Kaye D. Immune reactivity, morbidity and mortality of elderly humans. Aging: Immunol Infect Dis 1990;2:171-179.(Abstract)

78. Khansari DN, Murgo AJ, Faith RE. Effects of stress on the immune system [see comments]. Immunol Today 1990;11:170-175.

79. Parrillo JE, Fauci AS. Mechanisms of glucocorticoid action on immune processes. Annu Rev Pharmacol Toxicol 1979;19:179-201.

80. van Amsterdam JGC, Opperhuizen A. Nitric oxide and biopterin in depression and stress. Psychiatry Res 1999;85:33-38.

81. Deinzer R, Schuller N. Dynamics of stress-related decrease of salivary immunoglobulin A (sIgA): relationship to symptoms of the common cold and studying behavior [published erratum appears in Behav Med 1998 Spring; 24(1):27]. Behav Med 1998;23:161-169.

82. Henry JP, Stephens PM. Stress, health, and the social environment. A sociobiologic approach to medicine. 1977:

83. Frankenhäuser M. Psychobiological aspects of life stress. In: Levine S, Ursin H, eds. Coping and health. New York: Plenum Press, 1980:203-223.

84. Frankenhäuser M. The sympathetic-adrenal and pituitary-adrenal response to challenge: comparison between the sexes. In: Dembroski TM, Schmidt TH, Blümchen G, eds. Biobehavioral bases of coronary heart diseases. Basel: Karger, 1983:

85. Henry JP. Stressors and the adjustment disorders. In: Noshpitz JD, Coddington RD, eds. Wiley series in general and clinical psychiatry. New York: John Wiley and Sons, 1986:477-496.

86. Eriksen HR, Olff M, Murison R, Ursin H. The time dimension in stress responses: relevance for survival and health. Psychiatry Res 1999;85:39-50.

87. Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev 1984;5:25-44.

88. Demitrack MA, Crofford LJ. Evidence for and pathophysiologic implications of hypothalamic-pituitary-adrenal axis dysregulation in fibromyalgia and chronic fatigue syndrome. Ann N Y Acad Sci 1998;840:684-697.

89. Demitrack MA. Neuroendocrine correlates of chronic fatigue syndrome: a brief review. J Psychiatr Res 1997;31:69-82.