Introduction

Much health care research involves measurement of biological function. This chapter is concerned with the assessment of biological function in psychosocial research on health. The psychosocial factors studied in health care research include features of the individual’s sociodemographic position (age, gender, ethnicity, socioeconomic status etc.), aspects of social experience (such as social networks and social support), exposure to different types of adversity (e.g. work stress, life events, informal care-giving), family and neighbourhood factors (such as family conflict and neighbourhood social cohesion), and psychological parameters (such as depression, anxiety and hostility). Psychosocial studies in health care research are concerned with the contribution of these factors to disease causation, progression and prognosis, and the impact of psychosocial interventions on disease status and quality of life. The biological measures assessed in psychosocial research fall into three categories:

1 Biological indicators of disease states, such as blood pressure in hypertension, or airways resistance in bronchial asthma. The biological measures in this category are direct markers of the physiological dysfunction constituting the disease state, and are used in research on the role of psychosocial factors in the aetiology or prognosis of disease states, and in studies evaluating the impact of psychosocial interventions.

2 Biological markers of processes involved in the aetiology of disease. Examples include measures of vascular endothelial function, fibrinogen and C-reactive protein in the study of coronary heart disease, the concentration of CD4 lymphocytes in the study of HIV/AIDS, and the measurement of metabolites of corticosteroids and catecholamines in the investigation of obesity and insulin resistance. The biological parameters assessed in such studies are more distal to disease states than those assessed in Category 1, but nevertheless provide objective information concerning the impact of psychosocial factors on precursors of disease or on underlying physiological dysfunctions.

3 Non-specific biological markers of stress-related activation or resistance to disease. Biological measures in this category include heart rate, blood pressure, sweat gland activity, the stress hormones cortisol, adrenaline and noradrenaline, circulating lymphocyte numbers and activity, and concentrations of inflammatory cytokines and immunoglobulins. These biological variables are not assessed as
markers of specific disease states, but as more general indicators of psychobiological activation and resistance.

Biological measures are used extensively in animal research on psychosocial factors, but this chapter will be limited to work on humans. Biological measures are assessed in three types of psychosocial study: epidemiological surveys, naturalistic monitoring studies and experimental studies. Each of these applications will be described, along with their strengths and limitations. I also discuss issues of practical measurement and interpretation for several common measures. This chapter is not exhaustive, since different areas of health care research can involve complex and sophisticated biological assessments related to specific medical conditions (Cacioppo et al. 2000).

I have also not included discussion of biological correlates or indicators of health behaviours in this chapter. Examples include the measurement of cotinine or exhaled carbon monoxide in the study of smoking, the use of accelerometers for assessing physical activity and the monitoring of biomarkers for alcohol intake and specific dietary nutrients. The purpose of such measures is to provide more objective information about health-related activities than is available with self-report assessments. These measures are all important, but give rise to issues of validity and reliability that go beyond psychosocial research.

**Theoretical foundations of psychosocial biology**

Biological processes underpin all actions and behaviours, ensuring appropriate supplies of energy to working muscle, brain and other tissues, and the maintenance of bodily functions. The body’s defence mechanisms are continuously active, providing physical and chemical barriers against pathogens and invading micro-organisms, and responding to potential dangers. The foundations of psychosocial biology lie in the disturbance of these regulatory processes, and in the notion that psychosocial factors influence bodily functions and thereby lead to increased or decreased risk of illness. These influences are mediated through psychobiological pathways: the pathways through which central nervous system function activates the autonomic nervous system, neuroendocrine and immunological responses (Steptoe 1998).

There is a substantial research literature on the relationship between psychosocial factors and biological function, much of which derives from the study of the effects of acute and chronic stress (Weiner 1992; Fink 2000; Ader et al. 2001). Biological stress responses occur both under conditions of extreme psychosocial adversity (such as a natural disaster or assault), and when longer-term but lower-level demands exceed the individual’s capacity to cope (Steptoe and Ayers in press). Physiological stress responses encompass most of the main organ systems and regulatory processes of the body, including respiration and cardiovascular function, water balance, glucose metabolism and energy supply, blood clotting, inflammation and immune defences. These multiple components are controlled through the autonomic nervous system and neuroendocrine circuitry. During stressful encounters, the sympathetic branch of the autonomic nervous system is activated in concert with catecholamines such as adrenaline released from the medulla of the adrenal glands. The opposing parasympathetic branch of the autonomic nervous system becomes more active under conditions of conservation or behavioural withdrawal. The hypothalamic–pituitary–adrenocortical (HPA) axis is the most important neuroendocrine pathway in stress responses, although other neurotransmitters and hormone systems are also involved. Activation of the HPA axis leads to the release of cortisol in humans (corticosterone in rodents) from the adrenal cortex. The
sympathoadrenal and HPA axes are interdependent, and are controlled by complex feedback loops involving the central nervous system (Sapolsky et al. 2000). Even short-term stressors can trigger gene expression of enzymes regulating neuroendocrine function (Sabban and Kvetnansky 2001).

The acute activation of biological variables elicited by stress is adapted for the support of vigorous physical activity and defence (fight or flight). Risk to health arises for two reasons. First, the circumstances in everyday life that provoke psychobiological responses are typically not ones in which vigorous activity is required; having an abrasive interaction with family members, or being frustrated at work by equipment breakdown do not call for high levels of energy expenditure. Consequently, the intense mobilization of biological responses is inappropriate. Second, the psychosocial factors that elicit biological responses are ubiquitous in people’s lives, so repeated activation is common. In many areas of health care research it is low level, repeated, or chronic disturbance of function that is more significant than large reactions to severe but rare stressful events.

In recent years, the concept of allostatic load has become influential in understanding the chronic dysregulation of biological responses caused by psychosocial factors. This construct was introduced by Sterling and subsequently developed by McEwen (1998; McEwen and Wingfield 2003). It refers to the cumulative cost to the body of the process of achieving stability of physiological systems in the face of environmental challenge. Central to allostatic load is alteration in the activity of mediators such as the HPA axis and the sympathoadrenal system, resulting in imbalances that can increase risk to health.

A very simplified schematic description of possible manifestations of disturbed psychobiological function is shown in Figure 20.1. The graph on the left represents the normal pattern of response, with a biological system such as blood pressure or cortisol increasing in the face of challenge, then decreasing back to reference levels following termination of the stimulus. The graphs on the right summarize three types of disturbance to this response pattern (illustrated with the dotted line). In graph A, the response is heightened above normal levels, perhaps as a consequence of concurrent psychosocial adversity. For example, our group has shown that people reporting low control at work produce heightened fibrinogen responses to demanding behavioural tasks (Steptoe et al. 2003a), and relationships between neuroendocrine responses and social support have been described (Uchino et al. 1996). Heightened responsivity may in turn be associated with disease risk. Thus it has been found that high blood pressure stress reactivity, coupled with exposure to elevated life stress or work demands, predicts increased risk of hypertension and progression of subclinical atherosclerosis (Everson et al. 1997; Light et al. 1999).

High levels of cortisol promote increased concentration of lipids in the circulation, accumulation of abdominal fat, impaired fertility and decalcification of bone (Weiner 1992).

In graph B, the magnitude of responses is not altered, but restitution of homoeostasis is impaired. This leads to prolongation of responses and delays in post-stress recovery. An example of this pattern comes from a study of men and women recruited from the Whitehall II epidemiological cohort to investigate the psychobiological concomitants of low socioeconomic position. The magnitude of biological responses to behavioural tasks did not differ with socioeconomic position defined by a grade of employment. However, post-stress recovery in blood pressure and in heart rate variability was impaired in lower socioeconomic status participants (Steptoe et al. 2002). Additionally, lower-status individuals showed prolonged increases in prothrombotic factors (plasma viscosity and Factor VIII) following termination of the behavioural challenge (Steptoe et al. 2003b). Recovery in cortisol and catecholamines can be delayed for several hours after stressful work (Sluiter
Heightened or prolonged cortisol responses may also lead to downregulation of immune function, rendering the individual more vulnerable to infection (Vedhara et al. 1999a).

Graph C illustrates a third manifestation of allostatic load, with reduced responsivity in the biological parameter, due either to changes in receptor sensitivity or depletion of physiological mediators. The best studied example is hypocortisolism, or suppressed cortisol output and responsivity (Heim et al. 2000a). Several clinical conditions appear to be associated with low levels of cortisol including chronic fatigue syndrome, bronchial asthma and rheumatoid arthritis. Since cortisol suppresses inflammatory responses, low levels can lead to overactivity of the immune system in autoimmune disease. The circumstances in which these different disturbances of psychobiological responses are elicited depend on a combination of the nature and duration of the challenge, and the life stage of the individual (McEwen and Wingfield 2003). Low cortisol has also been recorded in several studies of post-traumatic stress disorder (Yehuda 2002), although this pattern has not been replicated in some population-based studies (Young et al. 2004).

**Figure 20.1** Schematic outline of patterns of biological response to behavioural or social challenge. The left panel shows the normal response pattern, and on the right three types of disturbed response are illustrated, as described in the text. The vertical axis scale is arbitrary, and could refer to a variety of responses (blood pressure, cortisol, muscle tension, immune reaction, etc.). The solid bar in each schematic illustrates the period of exposure to challenge.
Research paradigms

There is no single ‘ideal’ feasible study of psychosocial biology that can definitively prove or falsify the involvement of psychobiological dysfunctions in a particular health outcome. Instead, the case depends on the convergence and aggregation of data from different types of study, each with its strengths and limitations.

Epidemiological studies

Epidemiological studies provide the core method of establishing the contribution of psychosocial factors to the development of disease, and are also used to identify the biological mediators of these associations. For example, lower socioeconomic status is associated with heightened fibrinogen, vascular inflammation and incidence of the metabolic syndrome, which in turn increase risk of coronary heart disease (Brunner et al. 1997; Hemingway et al. 2003). High levels of anger and hostility have been related to blood pressure in population studies, and may increase risk of hypertension (Jorgensen et al. 1996; Yan et al. 2003). Biological and psychosocial measures from large samples can be obtained at relatively low cost, prospective study designs can be employed and potential confounders can be taken into account statistically. However, the biological measures in epidemiological studies are generally recorded on a single occasion under resting conditions (in a clinic or medical office) that are not typical of everyday life. Such studies can provide limited information about the dynamics of biological response, or the consequences of biological activation. Factors such as time of day and nutritional state affect many measures used in psychosocial biology, and may need to be recorded to permit accurate interpretation.

Naturalistic monitoring studies

The second kind of study in psychosocial biology involves the sampling of biological variables during everyday life. Such studies take many forms, from recordings during challenging tasks such as parachuting or speaking in public, to repeated measures of blood pressure or salivary cortisol over an ordinary day. Some of these techniques are extensions of methods used in clinical investigation, such as ‘Holter’ monitoring of the electrocardiogram in patients with coronary disease, and the use of ambulatory blood pressure monitors for evaluating hypertension. The purpose of these methods in psychosocial research is to assess biological activity under natural conditions and to examine the covariation between everyday activities, emotions and biology. For example, one study of people with bronchial asthma and non-asthmatic controls involved repeated spirometric measurements and mood assessments several times a day for three weeks (Ritz and Steptoe 2000). Negative mood states were associated with reduced forced expiratory volume in asthmatic participants but not in controls. Multiple samples of saliva have been obtained to measure the profile of cortisol release over the day, showing that cortisol increases in response to stressful daily events (van Eck et al. 1996). Measurements of muscle tension from surface electrodes have been made in supermarket cashiers, and have shown heightened trapezius muscle tension during work that is associated with complaints of neck and shoulder pain (Lundberg et al. 1999).

Naturalistic monitoring methods have the advantage of ecological validity, evaluating biological activity in real life rather than the artificial conditions of a laboratory or clinic. Associations between psychosocial factors and biological responses may be observed that are not detectable when single measures are taken under
clinical conditions. But naturalistic methods also have limitations. First, the range of biological markers that can be assessed is relatively small in comparison with the more sophisticated possibilities available in the clinic. Second, the measurement techniques need to be relatively unobtrusive, so as not to interfere with ongoing activities. There have been some heroic developments in naturalistic monitoring, such as the development of portable radioactivity detectors focused over the heart, and mounted in vest-like garments, that have been used to assess the impact of mental stress on cardiac function in patients with coronary artery disease (Burg et al. 1993). Much of the data on circadian rhythms of cortisol secretion have involved venepuncture every two hours for 24 hours, or the periodic withdrawal of blood from an indwelling catheter (Van Cauter et al. 2000). There is a danger that such methods are so stressful in themselves that they will obscure any association between psychosocial factors and biological responses, and certainly they can cause sleep disturbance (Jarrett et al. 1984). Measures of hormones such as cortisol, dehydroepiandrosterone (DHEA), testosterone, prolactin and estrogen in saliva overcome many of these problems. Third, there are several extrinsic factors that influence biological function that need to be taken into account, including cigarette smoking, food and caffeine intake, sleep and physical activity. These factors have to be monitored in naturalistic studies and taken into account statistically in analysis. Multilevel modelling has become the method of choice in analyses of these data (Schwartz and Stone 1998).

**Mental stress testing**

The third research method involves monitoring biological responses to standardized psychological or social stimuli. A wide range of mental stress tests are employed, including cognitive and problem solving tasks, simulated public speaking, upsetting films and interpersonal conflict tasks. Mental stress testing is typically carried out in a laboratory or clinic, but similar methods have been applied in home settings, particularly with children and elderly groups. A mental stress testing session involves a period of rest so that baseline levels of physiological function can be established, followed by a stress or challenge period that may last anything from five minutes to three hours. Further biological measures are obtained during the challenge period and for some time afterwards, depending on the dynamics of the measure under investigation. For example, blood pressure and heart rate respond within 1–2 minutes of onset of stress, while cortisol in saliva and blood may not peak for 30 minutes, and inflammatory cytokines such as interleukin (IL) 6 continued to rise for at least two hours. It is possible to obtain measures from several people simultaneously, if equipment is available. An important series of studies by Kiecolt-Glaser et al. involved measurement of cardiovascular, endocrine and immune function from couples during discussion of areas of conflict. Hostile and negative behaviours elicited heightened biological responses in both men and women, and there were also striking differences in the response patterns of husbands and wives (Kiecolt-Glaser and Newton 2001). Interestingly, the magnitude of adrenaline and noradrenaline responses during the conflict task were found to predict troubled marriages and divorce ten years later (Kiecolt-Glaser et al. 2003a).

The value of mental stress testing is that responses to psychosocial stimuli can be monitored under environmentally controlled conditions, reducing many of the sources of bias and individual difference that might otherwise be present. Experimental designs can be used with randomization to different conditions (such as low and high stress controllability), and sophisticated biological measures are possible. There are two major limitations. The first is that the stimuli used are often arbitrary and divorced from everyday life; few people spend much of their lives carrying out mental arithmetic or problem-solving under time pressure. Studies using more
Intervention studies

One of the principle methods of determining causality in biomedical research is to modify putative causal pathways and assess impact on outcome. Biological measures are used extensively in intervention studies to assess the impact of lifestyle or cognitive-behavioural treatments on disease processes (e.g. Tuomilehto et al. 2001). It is also known that psychological treatments such as relaxation training have effects on neuroendocrine activity, muscle tension and blood pressure that are opposite to those induced by stress (Antoni 2003). However, intervention methods have not been extensively used to test causal models, for instance by evaluating the effects of changes in putative biological mediators of psychosocial influence. Such an approach has been used to test mechanisms in animal research, applying beta-adrenergic pharmacological blockade to demonstrate that sympathetic activation mediates the impact of social stress on atherosclerosis in primates (Kaplan et al. 1991). But intervention approaches have yet to be exploited fully in psychobiological research in many health care settings.

Combinations of methods

I have described the main research methods in psychosocial biology, but there is an increasing trend towards combining the different methods. For example, naturalistic monitoring of salivary cortisol has been included in epidemiological population studies such as the CARDIA (Coronary Artery Risk Development in Young Adults) and Whitehall II surveys. Mental stress testing has been extended into the epidemiological framework, allowing the impact of individual differences in stress responsivity on disease progression to be evaluated (Everson et al. 1997; Carroll et al. 2003). Mental stress testing and naturalistic monitoring have been combined to evaluate the extent to which individual differences in acute blood pressure stress reactivity generalize to everyday life situations (Kamarck et al. 2000). This trend is very welcome, and is likely to grow with advances in instrumentation of technology and biological assay of techniques.

Measurement and interpretation of biological variables

This section provides an overview of some of the principle biological variables measured in psychosocial studies. Space prevents detailed evaluation of all the methods available, so the aim is to focus on general measures of neuroendocrine function cardiovascular activity, inflammatory processes, immune function and musculoskeletal activity. For each set of measures, I outline why they are assessed, how they are collected and processed, and what factors need to be taken in account in health care research settings to ensure accurate interpretation.

Neuroendocrine factors

Cortisol

Cortisol is a steroid glucocorticoid hormone produced by the adrenal cortex under the control of adrenocorticotropic hormone (ACTH) and the HPA axis. It acts on
almost all the nucleated cells in the body. It is implicated in a variety of conditions studied in health care research including depression, disturbances of cognitive function, obesity (particularly abdominal or central obesity), inhibition of growth and fertility, hypertension, Type II diabetes, inflammatory, and autoimmune conditions (McEwen et al. 1997; Lupien et al. 1999; Bjorntorp 2001; Wolf 2003). There are changes in cortisol with age which may have implications for the maintenance of memory function, particularly in old age (Lupien and Lepage 2001). Low socio-economic status has been associated with heightened cortisol levels both in adults and children (Lupien et al. 2000; Steptoe et al. 2003c). Many aspects of the ways in which cortisol dysfunction affects disease risk are still poorly understood. The biological actions of cortisol depend not only on output, but on the ability of cortisol to bind to glucocorticoid receptors. The relative importance of changes in secretion as opposed to alterations in uptake and tissue clearance is unclear, and impaired signalling may prove critical in many situations (Raison and Miller 2003).

Cortisol can be assessed in blood, urine and saliva. For many years, urine was the preferred vehicle for non-invasive assessments, and it provides an integrated measure of the secretion of cortisol and its metabolites over several hours. For example, increased secretion of urinary cortisol metabolites collected over a 24-hour period was recently observed in individuals with the metabolic syndrome (Brunner et al. 2002). But salivary cortisol has become the method of choice in much psychobiological research for a number of reasons (Kirschbaum and Hellhammer 2000):

- First, the method of collection is less complicated and embarrassing than for urinary measurement. The individual spits into a test tube, or gently chews a dental roll for a couple of minutes until it is saturated with saliva.
- Second, samples are stable over several days at room temperature and can be sent through the post. This means that samples can be collected by people in their own homes or at work over several days without an investigator being involved.
- Third, levels in saliva respond quickly, and are more sensitive to psychosocial experience than are integrated urinary assessments. For example, several studies have failed to show that urinary cortisol is higher on work than leisure days, and this has cast doubt on the impact of work stress on neuroendocrine function (e.g. Pollard et al. 1996). But salivary measures indicate higher levels on work days, with differences in the magnitude of secretions associated with work stress factors such as low job control (Kunz-Ebrecht et al. 2004a, 2004b).
- Fourth, methods of analysis are relatively straightforward and inexpensive, with established techniques using enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, and immunofluorescence being available in many laboratories. The level of free cortisol in saliva is only a fraction of that in blood, but it nevertheless correlates highly with plasma and serum concentrations.

Cortisol shows a pronounced circadian rhythm. Figure 20.2 summarizes data averaged from 163 middle-aged men and women over a working day. Cortisol levels are high early in the morning, and decline over the day, with a small peak associated with eating lunch. Data can be analysed using point comparisons, averages, or by calculating the slope of decline across the day (Stone et al. 2001). Additionally, there is typically an increase in cortisol over the first 20–30 minutes after waking. The cortisol waking response is of great interest in itself, since it has been associated with several psychosocial factors (Clow et al. 2004). In particular, it has been shown to be larger in more depressed individuals, those experiencing chronic life stress, and in more lonely people (Pruessner et al. 2003; Steptoe et al. 2004). It is inversely associated with socioeconomic position, and positively with work stress (Kunz-Ebrecht et al. 2004a). The evidence is inconsistent about whether it is associated with time of waking or quality of sleep. Cortisol levels can be affected by
smoking, medication, use of oral contraceptives, food consumption and body mass index, so these factors need to be controlled. The assessment of cortisol is compromised in people taking corticosteroid medication.

Experimental studies of acute stress responses have shown increases in cortisol following demanding tasks such as simulated public speaking (Biondi and Picardi 1999). However, it is striking that cortisol increases are frequently not observed with behavioural challenges that elicit responses in blood pressure and other variables. The reason may be that stimulation needs to be quite intense to produce reliable cortisol responses. The time course of cortisol increases is also longer than that of many biological variables, so that peak increases may not emerge until 20–40 minutes following the onset of stimulation. Increased cortisol responsivity to laboratory stress has been positively correlated with waist/hip ratio (Epel et al. 2000) and with the experience of chronic stress (Biondi and Picardi 1999), though some studies have described attenuated responses in stressed groups (Kristenson et al. 1998).

Evidence from animal research indicates that adversity in early life promotes corticosteroid stress responsivity in adult animals (Meaney 2001), but evidence in humans is limited to date (Heim et al. 2000b). In contrast, lower than normal salivary cortisol responses to challenging tasks have been recorded from children with autoimmune conditions such as atopic dermatitis (Buske-Kirschbaum et al. 1997).

**DHEA and DHEA sulphate**

DHEA and its sulphate are the most abundant steroid hormones in the human circulation. They have marked effects on the central nervous system, having an antiglucocorticoid function, and an impact on memory and emotional behaviour in animal studies (Wolf and Kirschbaum, 1999). Animal studies also indicate that
DHEA administration reduces body fat and risk of diabetes mellitus and coronary heart disease, while enhancing immune function (Herbert 1995). The concentration of DHEA and DHEA sulphate increases during adolescence, reaching a peak between ages 20 and 30. It then declines markedly with age, such that levels in people aged 70–80 years are less than 20 per cent of those in young adults. There are, however, important individual differences in this age-related decline, and people with smaller decrements have less deterioration in memory and functional capacity in old age (Berkman et al. 1993). Low DHEA predicted increased mortality in the Rancho Bernardo study and in a French cohort of men, but not women (Mazat et al. 2001). Some investigators argue that the ratio of cortisol to DHEA is crucial. Low DHEA and an elevated cortisol/DHEA ratio has been reported in depressed patients (Young et al. 2002). Research by Goodyer et al. (2001) has shown that depressive disorder in adolescence is associated with high cortisol and low DHEA, and that these hormones predict subsequent depression in high-risk individuals independently of life events, long-term difficulties and premorbid symptom levels. Low DHEA is a component of allostatic load, which has been shown to predict mortality and functional decline in the MacArthur studies of successful ageing (Seeman et al. 2001). All these findings might suggest that DHEA administration would reverse age-related decline in function. Unfortunately, trails of DHEA supplementation in old age have yet to demonstrate that effects of low levels can be reversed (Huppert and Van Niekerk 2001).

DHEA and its sulphate can be measured in blood and saliva. There is a diurnal rhythm in output, but this is less marked than for cortisol. Many of the same factors that are relevant for cortisol collection and interpretation apply to DHEA.

**Catecholamines**

The catecholamines noradrenaline (norepinephrine), adrenaline (epinephrine) and dopamine are released from nerve terminals and the adrenal glands into the blood, urine and cerebrospinal fluid. Most noradrenaline is produced by sympathetic nerves and activates adrenergic receptors locally, so only a small fraction enters the bloodstream. Adrenaline by contrast is produced by the adrenal medulla and acts as a hormone, being distributed by the bloodstream to adrenergic receptors in many areas. Catecholamines are measured as indices of sympathoadrenal activity in psychosocial biology, and can be assessed at present in blood and urine but not in saliva.

Urinary measures of catecholamines and their metabolites can be used to provide an indication of sympathoadrenal activity, and have demonstrated associations with psychosocial factors such as work stress (Frankenhaeuser et al. 1989), and with precursors of disease such as the metabolic syndrome (Brunner et al. 2002). Urinary concentrations of adrenaline and noradrenaline collected over 12 hours were components of the index of allostatic load used to predict functional decline in the MacArthur studies of successful ageing (Karlamangla et al. 2002). But studies of responsivity to psychosocial stimuli require measures that change over shorter time periods.

Plasma noradrenaline and adrenaline are often measured for this purpose, yet face several technical and conceptual problems. The most accurate assessments are carried out with high-pressure liquid chromatography, but reproducibility and sensitivity is far from perfect. A more important point is that the noradrenaline concentration measured in venous blood samples is strongly determined by overflow from local tissues (Hjemdahl 1993). Thus about half of the catecholamine extracted from blood samples taken from the forearm is derived from the muscles of the arm itself. Sympathetic nervous system activity is highly differentiated, particularly under stressful conditions, with levels of activity in different tissues varying widely. This
means that venous samples are not good indicators of general ‘sympathetic tone’. This problem has yet to be resolved. More precise measures of sympathetic activity include direct microneurographic assessments from nerves, and measures of radioactively-labelled noradrenaline. For example, Esler et al. (1989) showed that mental stress elicited no change in venous noradrenaline, while radiotracer techniques demonstrated large increases in noradrenergic activity in the heart and kidney. Unfortunately, these are technically demanding methods that cannot be widely used in health care research at present (Grassi and Esler 1999).

**Other hormones**

Insulin-like growth factor-1 (IGF-1) is produced in response to growth hormone released by the pituitary gland. IGF-1 has a variety of effects in the central nervous system, being involved in the differentiation of neurons, the release of neurotransmitters and stimulation of dendritic growth (Schneider et al. 2003). The decrease in hippocampal neurogenesis with advancing age is mediated by reduced IGF-1 among other factors. Both cross-sectional and prospective studies have shown positive associations between IGF-1 and cognitive function. Age-related declines in IGF-1 are associated with deleterious changes in body composition, while studies of growth hormone administration in older adults have documented rises in IGF-1 together with increased bone mineral density content and reduced fat mass (Lamberts et al. 1997). But effects on disease are poorly understood. Numerous studies have shown adverse effects of IGF-1 on atherosclerosis, glucose metabolism and diabetic vascular lesions, and high IGF-1 is associated with increased risk of some cancers (Hankinson et al. 1998; Sandhu et al. 2002). On the other hand, low rather than high circulating IGF-1 has been related prospectively with the incidence of coronary heart disease (Juul et al. 2002).

The gonadal hormones testosterone and oestrogen have also been studied in human psychosocial research. There is ample evidence from animal studies that stressful conditions and social adversity impair gonadal function and reproductive viability. Testosterone levels have been positively related to aggression and violent behaviour in some but not all investigations, and sustained production of testosterone in older men is associated with maintenance of muscle strength and functional well-being (Lamberts et al. 1997; Charney 2004). There is evidence that oestrogen blunts HPA axis and catecholamine stress responses in perimenopausal women (Komesaroff et al. 1999), and effects on mood have been described (McEwen 2002). Additionally, Taylor et al. (2000) have proposed that oxytocin is released as part of the stress response in women, and may mediate sex-specific coping behaviours in the face of adversity.

**Cardiovascular measures**

**Blood pressure**

The need for rigour in the measurement procedure and standardization of conditions when measuring blood pressure cannot be overemphasized. At the least, two readings should be obtained, preferably with a validated electronic blood pressure monitor, after the person has been seated quietly in a chair for five minutes, with feet on the floor and arm supported at heart level. An important issue for psychosocial studies is the ‘white coat’ effect, or the tendency of some people to have higher blood pressure when measured by a physician in the clinic than at home. White coat hypertension, where the blood pressure is sufficiently elevated when measured clinically to warrant a diagnosis, is thought to occur in at least 20 per cent
of patients (Pickering et al. 1988). There is little evidence for white coat effects being associated with particular physiological profiles, and exaggerated responsivity to other stimuli is often not present. It is possible that white coat effects are specific conditioned responses, with the negative emotional concomitants of measurement in the clinic leading to a classically conditioned response in some individuals. One way of overcoming this difficulty in psychosocial studies is to carry out self-monitoring or ambulatory blood pressure monitoring, as described below.

There is an extensive literature concerning blood pressure responses to mental stress testing, and the use of this method to investigate the role of psychological stress in the development of essential hypertension and coronary heart disease (Steptoe 1997). This has stimulated detailed methodological examinations of such issues as the duration of baselines, the blood pressure measurement technique, the control of physical activity and the effects of smoking and hormonal status, that go well beyond the scope of this chapter (Schneiderman et al. 1989). The clinical significance of acute blood pressure stress responses has been a particular concern, and has led to the gradual accumulation of prospective data that tend to confirm that heightened stress responsivity predicts future hypertension and cardiovascular disease risk (Steptoe and Willen 2002). New technologies, such as the Finapres instrument which assesses blood pressure on a beat by beat basis from the finger, are providing more refined insights into the ways in which psychosocial factors affect the cardiovascular system.

**Ambulatory blood pressure and self-measurement**

Ambulatory monitoring devices consist of an arm cuff, a signal detection device and a portable pump. They work in the same way as standard blood pressure monitors, except that the equipment is miniaturized and portable, and can be worn beneath clothing. The cuff is programmed to inflate periodically, with intervals of 15–60 minutes being used in different studies, so that a profile of blood pressure over the day can be built up. Ambulatory monitors are often used at night as well, and provide useful information about blood pressure ‘dipping’ and subsequent morning surges (Kario et al. 2003).

Clinically, the use of ambulatory monitors has increased because of the evidence that they provide better risk assessments than do conventional methods (Pickering 2002). In psychosocial studies, ambulatory devices allow the impact of daily life experience to be measured, and effects are frequently observed that are not detectable with conventional methods. A good example is in the study of work stress. The relationship between clinical blood pressure and work stress is very inconsistent, but it has now been found in several investigations that work stress is associated with small elevations in blood pressure recorded over the working day (Steenland et al. 2000). Figure 20.3 illustrates results from a recent investigation of 200 male and female participants in the Whitehall II study who carried out ambulatory blood pressure monitoring every 20 minutes over a working day. Low control at work was associated with higher systolic and diastolic blood pressure both during the day and in the evening after work, and these effects were independent of age, gender, socioeconomic position, smoking, body mass, and physical activity (Steptoe and Willen in press). Ambulatory blood pressure has been related to many other psychosocial factors as well, including marital conflict, social support, hostility and mood (Schwartz et al. 1994; Baker et al. 1999). Care has to be taken in the interpretation of ambulatory results, since physical activity, smoking, consumption of coffee and alcohol can all affect blood pressure values. Multilevel modelling has been introduced into the analysis of ambulatory blood pressure in order to tease out the independent contribution of psychosocial factors (Schwartz et al. 1994).
Self-measurement of blood pressure is a less expensive method, and has increased greatly in popularity with the availability of reliable instruments. It has been endorsed in the guidelines of the respected Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure as an aid to management (Chobanian et al. 2003), and can equally well be used in psychosocial studies. For example, Evans and Steptoe (2001) carried out a study in which nurses and accountants performed self-measurement of blood pressure and heart rate on work and leisure days. Blood pressure was typically lower on leisure days, while heart rates were reduced in people who had high social support.

**Figure 20.3**  Mean systolic pressure (upper graph) and diastolic pressure (lower graph) in men and women reporting low job control (solid lines) and high job control (dotted lines), over the working day. Data are averaged into four time periods over the day, and are adjusted for gender, employment grade, age, body mass index, smoking status and concomitant physical activity. Error bars are standard error of the mean.

*Source: Steptoe and Willemsen (in press)*

Self-measurement of blood pressure is a less expensive method, and has increased greatly in popularity with the availability of reliable instruments. It has been endorsed in the guidelines of the respected Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure as an aid to management (Chobanian et al. 2003), and can equally well be used in psychosocial studies. For example, Evans and Steptoe (2001) carried out a study in which nurses and accountants performed self-measurement of blood pressure and heart rate on work and leisure days. Blood pressure was typically lower on leisure days, while heart rates were reduced in people who had high social support.
Heart rate variability

Heart rate variability has become an important indicator of autonomic nervous system control over the heart. The heart rate varies naturally with the breathing cycle and other modulators. Variability can be assessed in the time domain by measuring the difference between short and long interbeat intervals, or in the frequency domain with power spectrum analysis. Low heart rate variability is a marker of reduced parasympathetic and high sympathetic control, and is a predictor of all cause mortality, and the prognosis of coronary heart disease and diabetes (Bigger et al. 1993; Dekker et al. 2000). Heart rate variability decreases with age, and the maintenance of higher heart rate variability may be an indicator of successful ageing. Studies in healthy populations have shown that heart rate variability is reduced during acute stress, and is associated with factors such as social isolation and psychological distress (Hemingway et al. 2001). It has been postulated that reduced heart rate variability partly mediates the association between depression and acute myocardial infarction (Carney et al. 2001), and low heart rate variability has also been observed in the metabolic syndrome (Brunner et al. 2002). It should, however, be pointed out that heart rate and heart rate variability are typically inversely correlated. It is unclear in some psychosocial studies whether the more complicated variability measures provide sufficient additional information over and above that obtained with heart rate assessments to warrant the extra work required.

Measures of inflammation

Inflammatory markers have been introduced into psychosocial studies only in recent years. The measures assessed include C-reactive protein, fibrinogen and the proinflammatory cytokines IL-6 and tumour necrosis factor α (TNFα). Inflammation is involved in many of the diseases studied in health care research, including coronary heart disease, hypertension, diabetes, some cancers, rheumatoid conditions, osteoporosis, multiple sclerosis and periodontal disease. Inflammatory markers have been associated with all cause mortality and death from cardiovascular disease in several cohort studies (Danesh et al. 1998; Ridker et al. 2000). Proinflammatory cytokines regulate the major drivers of postnatal growth, namely growth hormone and IGF-1. They are also relevant to ageing and overproduction of IL-6 is associated with frailty and functional decline in old age. Cytokines play a major role in inducing sickness behaviour (Dantzer 2001), and may be responsible for the multiple symptoms associated with certain treatments for cancer (Cleeland et al. 2003).

There is growing evidence for an influence of psychosocial factors on inflammatory processes. Fibrinogen concentration is inversely associated with socioeconomic status, and has also been related to low control at work, effort-reward imbalance, social isolation and hostility (Brunner et al. 1996; Wamala et al. 1999). Chronic stressors such as caring for a dementing relative stimulate more rapid increases in IL-6 with age (Kiecolt-Glaser et al. 2003b). Clinical depression and non-clinical depressed mood have been associated with higher IL-6, TNFα and C-reactive protein concentrations (Penninx et al. 2003). The magnitude of IL-6 and C-reactive protein responses to the acute stress of surgery is inversely related to rate of recovery, while chronic stress has been found to predict the magnitude of local inflammation in wounds (Glaser et al. 1999). Several other forms of stress such as academic examination stimulate heightened inflammatory cytokines and C-reactive protein, and the IL-6 response to acute mental stress is greater in people of lower socioeconomic status (Brydon et al. 2004). Inflammatory processes are also affected by body weight, smoking, physical activity and alcohol consumption, and
interactions between adiposity and depression in the prediction of IL-6 have been described (Miller et al. 2003).

Inflammatory markers are assessed with relatively standard biochemical assays of blood samples, and commercial kits are available. In the quantification of C-reactive protein, so-called high sensitivity assays are required. Plasma or serum levels of these substances are typically measured, but in addition, inflammatory cytokines can be quantified by stimulating the mononuclear cells with the mitogen lipopolysaccharide. This procedure requires culturing cells and incubation of samples, so is more complicated than the assessment of plasma levels.

**Immunological measures**

The interdisciplinary field of psychoneuroimmunology has become very prominent over the last 25 years (Ader et al. 2001). Much of this research is focused on basic physiology, the regulation of the immune system, and on the interplay between the brain and immune responses. But psychoneuroimmunology is also relevant to health care research for a number of reasons. First, the evidence indicates that psychosocial factors can modulate immune defences against pathogens such as bacteria, fungi and viruses, and can therefore increase or reduce resistance to disease (Kiecolt-Glaser et al. 2002). Second, there are disorders in which disturbances of immune function are a central feature, notably HIV/AIDS. Psychological characteristics and social factors are thought to be responsible in part for the large variations in the rate of progression of HIV in different individuals, and these influences may be mediated by central nervous system factors (Antoni 2003). Third, variations in clinical status, exacerbations of control, or flares in conditions such as rheumatoid arthritis, diabetes mellitus and systemic lupus erythematosus are associated with psychosocial factors (Da Costa et al. 1999; Zautra et al. 1999). Psychoneuroimmunological pathways may be responsible for these effects.

**Measurement of immune function**

The measurement of immune function in psychosocial research is complicated, and only a brief summary is given here (Vedhara et al. 1999b; Ader et al. 2001). Perhaps the most basic method is to count white blood cells and sub-sets of lymphocytes in the circulation. This is done using flow cytometry, with specific antigens detecting cluster designation (CD) markers on different immune cells. For example, cells marked with CD4+ are T helper cells, CD8+ are cytotoxic T cells, CD19+ is a B cell marker and CD16/CD56 designates natural killer cells. Lymphocyte counts are modified by acute and chronic stress, and in conditions such as depression (Zorrilla et al. 2001). A limitation to this method is that cell numbers do not necessarily correlate with cell function. Various functional assays are therefore used. These include measures of cytotoxicity, in which the ability of natural killer cells or T cells to destroy target cells is measured. Another functional measure is the assessment of lymphocyte proliferation in response to stimuli such as the lectin phytohemagglutinin, where greater proliferation implies that the lymphocytes are more responsive to the presence of potentially dangerous substances. An early finding in psychoneuroimmunology was that proliferative responses were impaired in people who had recently suffered a personal bereavement, a group that is known to be at raised risk for various illnesses (Bartrop et al. 1977). Natural killer cell cytotoxicity has been shown to be impaired in a range of stressful conditions, but to be positively associated with social support (Uchino et al. 1996; Zorrilla et al. 2001).

A different set of assays are used to quantify humoral immunity and levels of immunoglobulin. Antibodies are produced by B cells in response to exposure to
antigens, and are grouped in major classes such as immunoglobulin A (IgA), which is secreted in saliva and tears and protects mucosal surfaces, and immunoglobulin E (IgE) which is involved in the release of histamine and is implicated in conditions such as bronchial asthma. Immunoglobulins can be assayed from blood or saliva using ELISA techniques, and levels have been found to vary with psychosocial factors such as mood and daily stressors. However, the interpretation of responses can be problematic. Some investigators assess total immunoglobulin levels, but these values are only moderately associated with specific antibody responses. Antibody titres in response to specific antigens are more precise, but depend on recent exposure to the antigen.

Other methods assess immune function in vivo, rather than through processing of blood or saliva samples in the laboratory. Administration of live virus has been used in experimental research on upper respiratory infection. In a series of studies, Cohen et al. (1991, 1997) demonstrated that stress increases the likelihood that a moderate dose of experimentally administered coronavirus will lead to infection and a clinical cold, while social networks have a protective effect. Another model involves measurement of antibody responses to attenuated viruses such as those used in influenza vaccination. It has been shown in some studies that antibody responses to vaccination are impaired in individuals experiencing chronic life stress, and this may be indicative of weakened immune defences (Vedhara et al. 1999a).

Musculoskeletal measures

There is substantial evidence linking psychosocial aspects of work with upper extremity musculoskeletal problems (Sauter and Moon 1996). Factors such as low autonomy, lack of role clarity, low job satisfaction and high work pressure have been associated with pain in the neck and shoulder regions, and with hand or wrist problems. A recent systematic review confirmed these observations, but also pointed to evidence relating upper extremity problems with psychosocial factors outside work such as low social support and general stress (Bongers et al. 2002). Work-related upper extremity disorders are particularly common in jobs with a static load involving monotonous and repetitive tasks, even when physical demands are only low or moderate. Computer data entry, cashier work in supermarkets and other outlets, routine scientific bench work and traditional assembly line work all have these characteristics, and musculoskeletal problems are particularly common among women (Klumb and Lampert 2004).

Much research on musculoskeletal disorders is based on self-report or physical examination. However, direct measurement of muscle tension using surface electromyography (EMG) provides valuable additional information. Miniaturized transducers and telemetric equipment are available that allow readings to be obtained from free-moving individuals. Positive correlations have been reported between objectively assessed muscle tension and feelings of stress and exhaustion during work, but correlations with pain are often not obtained (e.g. Rissen et al. 2000). Studies of this kind indicate that the perception and appraisal of muscle tension may be important as well as objective differences in tension. Work with chronic back pain patients has identified problems in the accuracy of discrimination of EMG levels recorded from back muscles, and a tendency to overestimate muscle tension by some individuals (Flor et al. 1999). Surface electromyography is also used extensively in headache research, with monitoring of muscles of the neck, back and forehead. A meta-analysis of studies of frontal (forehead) EMG indicated that patients with tension-type headache do have higher muscle tension than controls on average, but with wide variability (Wittrock 1997). This suggests that a
combined assessment of subjective and objective measures may be valuable in the investigation of these problems.

Conclusions

There are many applications of biological measures in health care research. Their use in psychosocial studies is particularly appealing, since they provide objective evidence for the influence of psychological and social processes on pathophysiology and the biological mechanisms underlying disease states. There is sometimes scepticism about whether psychosocial factors are associated with genuine health outcomes, or only with symptom complaints and illness behaviour (Watson and Pennebaker 1989). The evidence that alterations in neuroendocrine function, inflammatory responses, viral antibodies or cardiovascular activity take place allows effects to be verified objectively. The field of psychosocial biology is continually expanding, with recent research on phenomena such as vascular endothelial dysfunction, cytokine gene expression and glucocorticoid receptor sensitivity supplementing the more established measures described here. Great care needs to be taken in the measurement and interpretation of biological measures, and their inclusion in a health care research project may call for additional standardization both of the setting and timing of assessments. However, the gain in understanding of psychosocial influences can be considerable, so the extra costs and effort required for including biological measures is well worthwhile.

Key points

- The biological measures assessed in psychosocial research are: biological indicators of disease states; biological markers of processes involved in the aetiology of disease; and non-specific biological markers of stress-related activation or resistance to disease.

- There is a substantial amount of research on the relationship between psychosocial factors and biological function.

- Epidemiological studies provide the core method of establishing the contribution of psychosocial factors to the development of disease, and are also used to identify the biological mediators of these associations. However, biological measures in epidemiological studies have the limitation that they are generally recorded on a single occasion, under resting conditions that are not typical of everyday life.

- Naturalistic monitoring studies involve sampling biological variables during everyday life, from recordings during challenging tasks to repeated measures (e.g. of blood pressure over an ordinary day).

- Naturalistic monitoring methods have the advantage of ecological validity, although the range of biological markers that can be assessed is relatively small, and need to be relatively unobtrusive.

- Another method is mental stress testing, involving monitoring biological responses to standardized psychological or social stimuli. A wide range of mental stress tests are employed, and experimental designs can be used. Limitations are that the stimuli in mental stress testing are often divorced from everyday life and are brief, so only acute biological responses are recorded.
Biological measures are frequently used in intervention studies to assess the impact of lifestyle or cognitive-behavioural treatments on disease processes, although intervention methods have not been extensively used to test causal models.

Some of the principle biological variables measured in psychosocial studies include neuroendocrine function, cardiovascular activity, inflammatory processes, immune function and musculoskeletal activity.

Care needs to be taken in the measurement and interpretation of biological measures, and in the design of the overall study.

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